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Prevalence of *sea*, *seb*, *tsst*, and *mecA* Genes in *Staphylococcus aureus* Isolated from Shrimps Sold in Seafood Retailers in Tehran, Iran

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HIGHLIGHTS

- Totally, 84 out of 300 (28%) shrimp samples were contaminated with *Staphylococcus aureus*.
- The contamination rate in fresh samples was significantly lower than that in frozen samples.
- The most and least prevalent genes found in the *S. aureus* isolates were *sea* and *tsst*, respectively.

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Acronyms and abbreviations
SEA=Staphylococcal Enterotoxin A
SEB=Staphylococcal Enterotoxin B
TSST=Toxic Shock Syndrome Toxin
MRSA=Methicillin-Resistant *Staphylococcus aureus*
PCR=Polymerase Chain Reaction

ABSTRACT

Background: *Staphylococcus aureus*, a Gram-positive bacterium, is the most prevalent food-borne pathogen in most regions of the world. The current study was carried out with the aim of *S. aureus* isolation from shrimps sold in Tehran, Iran. Furthermore, the genes of *mecA* as indicator of methicillin-resistant *S. aureus*, *sea*, *seb*, and *tsst* encoding enterotoxins were studied in the *S. aureus* isolates.

Methods: Totally, 150 fresh and 150 frozen shrimp samples were collected from seafood retailers in Tehran. Isolation of *S. aureus* from the samples was carried out using conventional methods. The target genes were identified using polymerase chain reaction technique. Data were statistically analyzed using SPSS v. 11.5 software.

Results: Out of 150 fresh and 150 frozen samples, 84 samples (28%) were contaminated with *S. aureus*. The contamination rate in fresh samples (22%) was significantly ($p<0.05$) lower than in frozen samples (34%). Totally, prevalence rates of *sea*, *seb*, *tsst*, and *mecA* genes in the isolates were 39.3, 15.5, 4.8, and 28.6%, respectively showing significant ($p<0.05$) differences.

Conclusion: High prevalence rates of enterotoxigenic and also antibiotic resistance genes in *S. aureus* isolated from shrimp samples in the current study highlighted worries about risk of staphylococcal food poisoning in Iranian shrimp consumers.

Introduction

Staphylococcus aureus, as a prevalent Gram-positive food-borne bacterium, produces heat-resistant staphylococcal enterotoxins such as Staphylococcal Enterotoxin A (SEA) and Staphylococcal Enterotoxin B (SEB) which result in gastroenteritis, fever, nausea, diarrhea, dysentery, and also colitis (Hennekinne et al., 2011). These highly stable enterotoxins are the reasons of a majority of food poisonings caused by *S. aureus*. Another *S. aureus* enterotoxin, Toxic Shock Syndrome Toxin (TSST), causes shock and anaphylactic reactions in patients via the gastrointestinal tract absorption and blood transfusion (Aydin et al., 2011; Soltan Dallal et al.,

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2010a). Resistance to methicillin and other penicillinase-resistant beta-lactams induces severe staphylococcal infections. Children, elder people, pregnant women, and immunocompromised patients are further at risk of being infected by *S. aureus* (Aung et al., 2017; Hammad et al., 2012; Noor et al., 2014).

The consumption of shrimp, a decapod crustacean, has been specifically increased in several countries. There is strong consumers demand for both fresh and frozen shrimp in international market. However, shrimp safety and risk of food-borne diseases are among important challenges in shrimp market and industry (Norhana et al., 2010). *S. aureus* contamination of seafood such as shrimp may occur throughout contaminated packages and worker hands *(*Hammad et al., 2012). Therefore, standard procedures must be carried out during fishing, transportation, and storage to prevent contamination of shrimps with pathogenic bacteria and bacterial toxins. So, the current study was carried out with the aim of *S. aureus* isolation from shrimps sold in Tehran, Iran. Furthermore, the genes of *mecA* as indicator of Methicillin-Resistant *S. aureus* (MRSA), *sea*, *seb*, and *tsst* were studied in the *S. aureus* isolates.

Materials and methods

Sample collection

In this cross-sectional survey carried out during 2013-2014, a total number of 300 samples, including 150 fresh and 150 frozen white-leg shrimps (*Litopenaeus vannamei*) were collected with healthy appearance from seafood retailers in Tehran, Iran. Samples were sterilely transferred to Food Microbiology Laboratory of School of Public Health, Tehran University of Medical Sciences, Tehran, in cold condition and stored at 4 °C until next analysis. The preparation of the samples for microbiological analysis was carried out according to Iran National Standard (ISIRI, 2007).

Bacterial isolation

One g of the shrimp meat was cut using sterile blade, suspended in 25 ml of sterile distilled water and incubated at 37 °C for 24-48 h. Then, one ml of the suspension was cultured on Baird-Parker agar (Merck, Germany) containing 0.1% potassium tellurite solution and egg emulsion; and incubated at 37 °C for 24-48 h. After incubation, small, black shiny colonies were tested for the coagulase activity based on Iran National Standards (ISIRI, 2006, 2014). Briefly, a colony was dissolved in one droplet of citrated rabbit plasma on a clean glass slide in which the *Staphylococcus'* colonies formed agglutination. Suspicious results were confirmed using tube test. *S. aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were used as positive and negative controls.

Gene detection

Conventional Polymerase Chain Reaction (PCR) was used for the detection of enterotoxin genes in *S. aureus* isolates, using specific primers (Table 1). A modified protocol originally described by Mazaheri Nezhad Fard et al. (2011) was used for PCR method as follows: *Staphylococcus* isolates were cultured on Baird-Parker selective agar (Merck, Germany), a positive colony was suspended in 200 µl of sterile distilled water, and then DNA was extracted using DNA Extraction Kit (Viogene-Biotek, Taiwan) based on the manufacturer's instruction. The extracted DNA was stored at -20 °C until use. Four rounds of PCRs (for detection of *sea*, *seb*, *tsst*, and *mecA* genes) were carried out for each sample. To prepare a 25 µl PCR reaction for amplification using HotStarTaq Plus Master Mix Kit (Qiagen, Germany), 12.5 µl of 2×PCR master mix and 2 µl of each forward and reverse primers from 10 pmol/µl working solution (end concentration of 0.8 µM) were mixed in a sterile microtube. Then, 5.5 µl of sterile distilled water were added to the mixture to reach the total volume of 22 µl, and then 3 µl of the extracted DNA (1–100 ng) were added to the mixture. Templates were amplified using Peqlab Primus 96 thermal cycler (Peqlab, Germany) with the protocol modified by Soltan Dallal et al. (2010b). After an initial denaturation step at 94 °C for 5 min, *sea*, *seb*, *tsst*, and *mecA* genes were separately amplified by 35 cycles of denaturation at 94 °C for 45 s, annealing at various temperatures (56, 55, 52, and 51 °C for *mecA*, *sea*, *seb*, and *tsst*, respectively) for 45 s and extension at 72 °C for 90 s. Amplification was finalized by a final elongation step at 72 °C for 7 min. PCR amplicons were detected by electrophoresis in ethidium bromide stained 1–3% agarose gels and visualization under UV light (UVP, France).

Statistical analysis

Data were statistically analyzed using SPSS v. 11.5 software (IBM Analytics, USA). Chi-square test and Fisher's exact two-tailed test were used. *P* values less than 0.05 were considered as significant.

Results

Out of 150 fresh and 150 frozen samples, 84 samples (28%) were contaminated with *S. aureus*. The contamination rate in fresh samples (22%) was significantly ($p<0.05$) lower than in frozen samples (34%). Totally, prevalence rates of *sea*, *seb*, and *tsst*, and *mecA* genes in the isolates were 39.3, 15.5, 4.8, and 28.6%, respectively showing significant ($p<0.05$) differences (Table 2).

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Table 1: PCR primer pairs used in the current study

Gene	Sequence (5'→3')	Base pair	Reference
mecA	F: GAAATGACTGAACGTCCGAT R: CTGGAACTTGTTGAGCAGAG	399	Su and Lee Wong (1995)
sea	F: CCTTTGGAAACGGTTAAAACG R: TCTGAACCTTCCCATCAAAAAC	127	Betley and Mekalanos (1988)
Seb	F: TCGCATCAAACTGACAAACG R: GCAGGTACTCTATAAGTGCCTGC	477	Jones and Khan (1986)
Tsst	F: CATCTACAAACGATAATATAAAGG R: CATTGTTATTTTCCAATAACCACCC	481	Fueyo *et al*. (2005)

Table 2: Distribution of *mecA*, *sea*, *seb*, and *tsst* genes in *Staphylococcus aureus* isolated from shrimps

Sample	No. of *Staphylococcus aureus* isolates	*sea* No. (%)	*seb* No. (%)	*tsst* No. (%)	*mecA* No. (%)
Fresh shrimp	33	15 (45.5)	6 (18.2)	2 (6.1)	10 (30.3)
Frozen shrimp	51	18 (35.3)	7 (13.7)	2 (3.9)	14 (27.5)
Total	84	33 (39.3)	13 (15.5)	4 (4.8)	24 (28.6)

Discussion

In the current study, a considerable rate of contamination with *S. aureus* was detected in Iranian shrimp samples indicating public health concern. Previously, it was indicated by Soltan Dallal et al. (2015) that 30% of marine as well as 20% of farmed shrimps sold in Iran were contaminated with *S. aureus* which is similar with our findings. However, other studies have shown relatively lower contamination rates. For example, Mohamed Hatha et al. (2003) studied microbial quality of the Indian shrimps and reported that only 1% (14 out of 1282) frozen raw shrimps were contaminated with coagulase-positive staphylococci. In another study conducted by Simon and Sanjeev (2007) on the prevalence of enterotoxigenic *S. aureus* in Indian fishery products, 26.7% of the frozen prawns were found to be infected with *S. aureus*. Also, a survey by Di Giannatale et al. (2011) showed that only 6% of the seafood mussels (3 out of 50) in Italy were contaminated with *S. aureus* strains. Zarei et al. (2012) investigated the prevalence of *S. aureus* in seafood products in Ahwaz, Southwestern Iran. They found *S. aureus* isolates in 5.7% (4 out of 70) fresh and 20% (5 out of 20) frozen shrimp samples. Mus et al. (2014) reported that 20% of retail fresh shrimps in Turkey were infected to *S. aureus*. As seen in the previously indicated similar researches, there are some varieties among prevalence rates of *S. aureus* in shrimp samples that may be affected by several factors. These factors could be included contamination of fishing tools, surfaces, freezers, vendors, and vehicles with bacteria; environmental factors such as temperature, humidity, and pH; type of production system (traditional or industrial); food processing types (minced, sliced, skinned, cut, chopped, or whole shrimp) and use of antimicrobials in shrimp farming (Nychas et al., 2008).

In this study, we found four different staphylococcal *sea*, *seb*, *tsst*, and *mecA* genes in Iranian shrimp samples. Similarly, Kim et al. (2011) studied prevalence of toxigenic *S. aureus* in refrigerated ready-to-eat sushi in Korea and stated that 22 out of 110 samples (19.1%) included *sea* gene alone or in combination with other virulence genes. Wongboot et al. (2015) phenotypically and genotypically detected *sea* (47%) and *seb* (5.9%) genes in *S. aureus* isolated from retail ready-to-eat foods in Northeastern Thailand. In agreement with the present work, Ahani and Alipour-Eskandani (2014) detected enterotoxigenic *S. aureus* in snow trout (*Schizothorax zarudnyi*), a native fish in Sistan district, Southeastern Iran and reported that 14.3 and 8.5% of *S. aureus* isolates possessed *sea* and *seb* genes, respectively.

In the present study, we found 5.1 and 7.3% of *mecA* gene in the fresh and frozen isolates, respectively. The *mecA* gene is a part of *mec* operon in Staphylococcal Cassette Chromosome *mec* and encodes resistance to beta-lactams including methicillin. Since these antibiotics are used as the first-line medicine against infections caused by *S. aureus*, monitoring of *mecA* in food and

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dairy products of various sources is necessary. Recently, Aung et al. (2017) have reported that 2.2% of *S. aureus* isolated from retail foods and food handlers' gloves samples in Singapore had been MRSA. In agreement with our results, Normanno et al. (2007) detected *mecA* in 3.75% of *S. aureus* isolated from Italian food samples. Also, it was stated that Sashimi, a popular Japanese ready-to-eat raw fish, is a probable vehicle for transmission of MRSA (Hammad et al., 2012) which is in similar with the findings obtained from the present survey.

In the current investigation, we found higher rate of *S. aureus* contamination in frozen shrimp samples than those of fresh ones which was somewhat unexpectable; because generally, freezing may result in loss of the bacterial count due to the destruction of DNA (Krajden et al., 1999; Schaudien et al., 2007). However, it is assumed that hygienic condition during handling, freezing, and storage steps of frozen shrimp samples in this study was not suitable.

Conclusion

In general, high prevalence rates of enterotoxigenic and also antibiotic resistance genes in *S. aureus* isolated from shrimp samples of the current study highlighted worries about the risk of staphylococcal food poisoning in Iranian shrimp consumers. Since seafood dishes are popular in some regions of Iran, further restrict hygienic regulations are needed to prevent staphylococcal food poisoning. In this regards, it is necessary to maintain the cold chain during catching, transportation, storage, and distribution of these products. Also, the consumers should be trained by local authorities for avoiding consumption of undercooked shrimp.

Author contributions

M.M.S.D. and M.K.S.Y. designed and conducted the study and also supervised the analysis. R.M.N.F. wrote and edited the paper.

Conflicts of interest

No conflict of interest was declared by the authors.

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