Characterization of Toxin Genes and Antimicrobial Susceptibility of *Staphylococcus aureus* Isolates in Fishery Products in Iran

Noushin Arfatahery¹, Abolfazl Davoodabadi‡¹,² & Taranehpeimaneh Abedimohtasab¹

*Staphylococcus aureus* is one of the most common causes of seafood-borne diseases worldwide, which are attributable to the contamination of food by preformed enterotoxins. In this study, a total of 206 (34.3%) *Staphylococcus aureus* strains were obtained from 600 fish and shrimp samples and were tested for their antimicrobial susceptibility. We assessed the prevalence of the genes responsible for the staphylococcal enterotoxins (SEA, SEB) and toxic shock syndrome toxin 1 (TSST-1) genes. The results indicated that 34% of aqua food samples were contaminated with *S. aureus*, and 23.8% of these isolates were mec-A-positive. Sixty-four percent of the strains isolated from contaminated seafood was enterotoxigenic *S. aureus*, and 28.2% of SEs were MRSA-positive. The most prevalent genotype was characterized by the presence of the *sea* gene (45.2%), followed by the *seb* gene (18.5%), and the *tst* gene encoding TSST-1 was found in eight strains (3.9%). Of the 206 *S. aureus* isolates, 189 strains (84.9%) were resistant to at least one antibiotic. Given the frequent outbreaks of enterotoxigenic MRSA, it is necessary to make revisions to mandatory programmes to facilitate improved hygiene practices during fishing, aquaculture, processing, and sales to prevent the contamination of fishery products in Iran.

A major global cause of food poisoning, is caused by heat-stable staphylococcal enterotoxins (SEs) produced by enterotoxigenic strains of *Staphylococcus aureus*. TSST-1 was the first toxin shown to be involved in toxic shock syndrome. Enterotoxins and toxic shock syndrome toxin 1 (TSST-1) are members of the pyrogenic toxin super antigen (PTSAg) family. *Staphylococcus aureus* is considered one of the most common pathogens responsible for outbreaks of food poisoning. The widespread use of antibiotics has led to the emergence of multidrug resistant strains, which makes it more difficult to eradicate the diseases they cause and increases their incidence. The increasing prevalence of antimicrobial-resistant *S. aureus* plays an important role in food safety and is a threat to healthcare systems. Specifically, the emerging antimicrobial resistance of *S. aureus* has become a major public health concern. The number of resistant MRSA strains is increasing, and there have been reports of MRSA in aquatic animals. These studies have raised additional food safety concerns regarding *S. aureus* beyond its role as an agent that causes food poisoning. Previous food safety research on *S. aureus* has focused on characterizing SE production; however, relatively little is known about the antimicrobial susceptibility profiles of enterotoxigenic *S. aureus* strains or the prevalence of SE and other virulence factors among MRSA isolates. Given the frequent outbreaks of enterotoxigenic MRSA, new hygiene policies and management practices should be adopted to increase food safety and avoid extra treatment costs. A number of researchers in other countries have investigated the potential transmission of this dangerous strain to food products by human carriers, the environment, activities such as transport and packaging, the contaminated hands of workers, or infected respiratory secretions. Illness caused by enterotoxigenic MRSA is usually self-limiting but occasionally can be sufficiently severe to cause hospitalization. The real incidence of staphylococcal food poisoning (SFP) is underestimated, primarily due to misdiagnosis, its sporadic nature, or minor outbreaks that go unreported. For example, 29 cases of poisoning were underestimated in the European Union in 2008, primarily due to misdiagnosis; however, ⁰⁹

¹Dev of Microbiology, Dept of Pathobiology, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran. ²Microbiology Department, Medical School, Babol University of Medical Science, Babol, Iran. Correspondence and requests for materials should be addressed to N.A. (email: arfa3133@gmail.com)
outbreaks were indeed reported, affecting 414 persons, of which 26 patients required hospitalization\(^2\). In the United States, SFP is estimated to account for 185,060 illnesses and 1,753 cases requiring hospitalization annually\(^3\). Some studies have reported MRSA in aquatic animals and identified antimicrobial-resistant \(S. \) aureus as the causative agent in cases of fish handlers' disease\(^1\)–\(^4\). Accordingly, a variety of techni\(n\)e\(r\)\(s\)hine\(s\)e\(r\) marine shrimp sd be employed to survey for the presence of MRSA and related toxins in foodstuff.\(^5\) Currently, molecular biology tech\(n\)iques are considered important tools in microbiological studies\(^9\)–\(^11\). Molecular typing of \(S. \) aureus strains plays a crucial role in epidemiological studies examining disease origins and can be used to monitor major incidents of contamination\(^9\)–\(^11\). The aim of the present study was to determine the antimicrobial susceptibilities of \(S. \) aureus isolates derived from various fish and shrimp samples in Iran from 2013 to 2014. It also aimed to characterize methicillin-resistant, toxigenic \(S. \) aureus isolates by evaluating their ability to produce the mec-A, \(sea\), \(seb\), and \(tst\) genes with polymerase chain reaction analysis (PCR). The final aim of this study was to investigate the contamination of fishery products before their purchase and consumption.

**Materials and Methods**

**Full ethics statement.** The authors are reporting experiments carried out on fish and shrimp fishery products; experiments were not performed with live vertebrates or humans.

The experiments were conducted in accordance with the guidelines of the National Committee for Clinical Laboratory Standards (CLSI 2011). \(19\)-Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI document M100-S20. Clinical and Laboratory Standards Institute, Wayne, PA 2011 (date of access: January 2011).

All experimental protocols were approved by the Institute of Standards & Industrial Research of Iran (ISIRI). NO: 6806-1 Microbiology of food and animal feeding stuffs – Enumeration of coagulase – Positive \(Staphylococcus\) (\(Staphylococcus aureus\) and species) technique using Baird–Parker agar medium 2006 (19/09/2006).

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The experiments were conducted as recommended by the Institute of Standards and Industrial Research of Iran\(^19,20\).

**Sampling.** In this cross-sectional study, a total of 600 samples (fresh and frozen, farm and marine), including 150 marine shrimps, 150 farmed shrimps, 150 farmed fish, and 150 marine fish with a healthy appearance were selected from September 2013 to September 2014.

The shrimp samples were caught from the south seas of Iran (Persian Gulf, Oman Sea, Indian Ocean) and, together with aquaculture-farmed shrimps, were brought to the Tehran fishery.

The fish samples were caught from the north seas of Iran (Caspian Sea) and the south seas of Iran (Persian Gulf, Oman Sea, Indian Ocean) and, together with aquaculture-farmed fish, were brought to the Tehran fishery. All seafood samples were prepared in the Central Fish Market at the Tehran fishery. Each week, 12 samples were obtained for this study. Samples were transferred under standard conditions (in sterile container carriers at a temperature of \(4 \) °C for fresh samples and \(-18 \) °C for frozen samples) from the fishery to the laboratory at Tehran University of Medical Sciences, generally within approximately 2 hours. After receiving the samples, diagnostic tests were immediately performed.

**Isolation and identification of \(S. \) aureus.** One gram of each sample was mixed with 9 CCs of Gulty (\(S. \) aureus selective enrichment medium; Merck, Darmstadt, Germany) containing 0.1% potassium tellurite in suspension. Tubes were incubated at 37 °C for 24–48 hours. Tubes that contained deposits or were black were cultured on Baird-Parker agar (Merck, Darmstadt, Germany) containing a egg-yolk tellurate emulsion (Merck, Darmstadt, Germany), which was used for isolation\(^22,23\). Isolates were identified employing the following criteria: production of coagulase, DNase, catalase, mannnitol fermentation, and a haemolytic zone on \(5\% \) sheep's blood agar (Merck); additionally, a VP test and Gram staining were performed. \(S. \) aureus ATCC 25923 and \(S. \) aureus ATCC 29213 were used in tests as a negative control and a positive control, respectively.

**Antimicrobial susceptibility testing.** The resistance of staphylococcal isolates to methicillin was tested via disk diffusion assay using \(\mu\)ller-Hinton agar as recommended by the guidelines of the National Committee for Clinical Laboratory Standards (CLSI 2010)\(^24\). Methicillin-resistant isolates were recognized via the Kirby-Bauer disk diffusion method\(^a\) using cefoxitin-impregnated (MAST, UK) disks on \(\mu\)ller-Hinton agar (Merck, Darmstadt, Germany) containing an egg-yolk tellurate emulsion (Merck, Darmstadt, Germany), which was used for isolation\(^21,22\). Isolates were identified employing the following criteria: production of coagulase, DNase, catalase, mannitol fermentation, and a haemolytic zone on \(5\% \) sheep's blood agar (Merck); additionally, a VP test and Gram staining were performed. \(S. \) aureus ATCC 25923 and \(S. \) aureus ATCC 29213 were used in tests as a negative control and a positive control, respectively.

**DNA Extraction.** DNA was isolated using a Viogene kit (Taiwan) as recommended by the manufacturer; however, 25 \(\mu\)l/ml lysostaphin was added to the bacterial suspensions. The extracted DNA was resolved via the electrophoresis of 5 ml of product on a 1% (w/v) agarose gel stained with ethidium bromide in TAE buffer at 100 V for 25 min and visualized with a gel documentation system (Bio-Rad).

**Primer Design.** To subtype MRSA and the toxins produced by each strain, PCR was carried out employing several primer sets. The oligonucleotide primers used in this study were described by Johnson \(et\) \(al\).\(^24,25\) and the protocol for MRSA subtyping was reported by Vannuffel \(et\) \(al\).\(^26\). Table 1 lists the primer sets used to detect two SE genes (sea, seb), the TSST-1 gene (tst), and MRSA (mec-A).
A total of 206 isolates demonstrated different antimicrobial resistance profiles, and 17 (14.1%) out of 64% of isolates (n = 300) carried the 
\textit{mec-A} gene. As shown in Table 3, the majority of the isolates were resistant to three or more classes (here considered multidrug resistant) (104, 56.3%) or two classes (54, 28.6%) of antimicrobials. The frequencies of resistance to individual agents were 49 (23.8%) for oxacillin, 7 (3.4%) for erythromycin, 5 (2.4%) for clindamycin, 58 (28.1%) for tetracycline, and 10 (4.8%) for gentamicin. Chi-square test, p < 0.05 (Table 4).

Over 64% of 
\textit{S. aureus} isolates (n = 133) carried enterotoxin genes. As shown in Table 5, the majority of the strains included in this study carried the 
\textit{sea} gene (95, 45.2%) and the 
\textit{seb} gene (38, 18.5%); a small number of strains carried 
\textit{tst} (8, 3.9%). Moreover, only nineteen (9.2%) isolates carried both the 
\textit{sea} and 
\textit{seb} enterotoxin genes. In two (0.97%) isolates, the 
\textit{sea}, 
\textit{seb}, 
\textit{tst} and 
\textit{mec-A} genes were detected.

**Table 1.** PCR primers and fragment lengths of the studied genes.

| Target gene | Primer | Oligonucleotide sequence (5′ → 3′) | Size (bp) |
|-------------|--------|----------------------------------------|-----------|
| 
\textit{tst} | F      | CAYCTACAACAGATAATAAAGG               | 481       |
|             | R      | CATGTATTCCAGGTTACCC                  |           |
| 
\textit{sea} | F      | CCTTGGAGACGTTGAACG                   | 127       |
|             | R      | TCTGACCCTTCCCACGAA                   |           |
| 
\textit{seb} | F      | TCGCATCAAACGTGACACG                  | 477       |
|             | R      | GCAGGTACTCTATAAGTCGCC                |           |
| 
\textit{mec-A} | F     | GAA ATG ACT GAA CGT CGT AT           | 399       |
|              | R      | CTG GAA CTT GTT GAG CAG AG          |           |

**Table 2.** Distribution of 
\textit{S. aureus} in the shrimp samples analyzed.

| Studied samples           | Number of non-contaminated samples | Number of contaminated samples | Number of samples |
|---------------------------|------------------------------------|--------------------------------|------------------|
| Fresh marine shrimp       | 57 (76.0%)                         | 18 (24.0%)                     | 75 (100%)        |
| Frozen marine shrimp      | 45 (60.0%)                         | 30 (40.0%)                     | 75 (100%)        |
| Fresh farmed shrimp       | 60 (80.0%)                         | 15 (20.0%)                     | 75 (100%)        |
| Frozen farmed shrimp      | 54 (72.0%)                         | 21 (28.0%)                     | 75 (100%)        |
| Total                     | 216 (72.0%)                        | 84 (28.0%)                     | 300 (100%)       |

**PCR amplification.** PCR for 
\textit{Staphylococcal Enterotoxins} (\textit{SEA}, \textit{SEB}) and \textit{TSST-1}. PCR reaction mixtures contained 20 ng of template DNA, 1 U \textit{Taq} DNA polymerase, 250 mM of each dNTP, 10 mM Tris-\textit{HCl} (pH 9.0), 40 mM KCl, and 1.5 mM MgCl2 (Bioneer, Korea). Amplifications were carried out in a thermal cycler (Primus 96 advanced, USA) with the following thermal program: initial denaturation for 5 min at 95 °C; annealing for 30 cycles of 1 min at 95 °C, 1 min at 53 °C, and 2 min at 72 °C; final extension for 5 min at 72 °C.

PCR for MRSA. The isolated strains were also subjected to a PCR assay to detect the 
\textit{mec-A} gene using a primer pair (Table 1) that was previously reported by Vannuffel et al. PCR amplification was carried out in a thermal cycler (Primus 96 advanced, USA) with initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. A final extension was performed at 72 °C for 5 min.

All PCR products were resolved by the electrophoresis of 5 ml of product on a 1% (w/v) agarose gel stained with ethidium bromide in TAE buffer at 100 V for 25 min and visualized with a gel documentation system (Bio-Rad).

**Statistical analysis.** A chi-square test was used to compare the prevalence of each gene profile among 
\textit{S. aureus} isolates between categories (SPSS 19). Differences between the prevalence rates were considered significant when p < 0.05.
Table 3. Distribution of S. aureus in the fish samples analyzed.

| Samples (no. of isolates) | Studied samples | Number of non-contaminated samples | Number of contaminated samples | Number of samples |
|---------------------------|-----------------|------------------------------------|-------------------------------|------------------|
| Fresh marine fish         | 18              | 16 (88.9%)                         | 2 (11.1%)                     | 18               |
| Frozen marine fish        | 30              | 19 (63.3%)                         | 11 (36.7%)                    | 30               |
| Fresh farmed fish         | 15              | 10 (66.7%)                         | 5 (33.3%)                     | 15               |
| Frozen farmed fish        | 20              | 17 (85%)                           | 3 (15%)                       | 20               |
| Fresh marine shrimp       | 16              | 14 (87.5%)                         | 2 (12.5%)                     | 16               |
| Frozen marine shrimp      | 15              | 10 (66.7%)                         | 5 (33.3%)                     | 15               |
| Fresh farmed shrimp       | 21              | 14 (66.6%)                         | 7 (33.3%)                     | 21               |
| Frozen farmed fish        | 12              | 12 (100)                           | 0 (0%)                        | 12               |
| Fresh marine fish         | 37              | 34 (91.9%)                         | 3 (8.1%)                      | 37               |
| Frozen marine fish        | 43              | 34 (79.1%)                         | 9 (20.9%)                     | 43               |
| Fresh farmed fish         | 20              | 17 (85%)                           | 3 (15%)                       | 20               |
| Frozen farmed fish        | 20              | 17 (85%)                           | 3 (15%)                       | 20               |
| Total Avg (206)           |                 | 163 (79.1%)                        | 43 (20.9%)                    | 206              |

Table 4. Antimicrobial resistance of foodborne S. aureus isolates. *P: penicillin, OX: oxacillin, AM: ampicillin, GM: gentamicin, CIP: ciprofloxacin, E: erythromycin, CC: clindamycin, VA: vancomycin, C: chloramphenicol, TE: tetracycline, RA: rifampin. The numbers in parentheses indicate the percentages.

| Samples (no. of isolates) | No. (%) of isolates resistant to: |
|---------------------------|----------------------------------|
|                           | P | OX | AM | GM | CIP | E | C | VA | CC | TE | RA |
| Fresh marine shrimp       | 16 (88.9%) | 3 (16.7%) | 16 (89.9%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Frozen marine shrimp      | 19 (63.3%) | 5 (16.7%) | 19 (63.3%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fresh farmed shrimp       | 10 (66.7%) | 7 (46.6%) | 11 (73.3%) | 1 (6.7%) | 0 | 0 | 0 | 0 | 0 | 0 | 4 (26.6%) |
| Frozen farmed fish        | 14 (66.6%) | 8 (38%) | 14 (66.6%) | 1 (4.8%) | 0 | 0 | 0 | 0 | 0 | 4 (19) |
| Fresh marine fish         | 34 (91.9%) | 6 (16.2%) | 33 (89.1%) | 0 | 0 | 0 | 0 | 0 | 0 | 17 (46) |
| Frozen marine fish        | 41 (77.3%) | 7 (13.2%) | 41 (77.3%) | 0 | 0 | 0 | 2 (3.7%) | 0 | 2 (3.7) | 9 (17) |
| Fresh farmed fish         | 12 (100) | 5 (41.7%) | 12 (100) | 3 (25) | 0 | 2 (16.7) | 0 | 2 (16.7) | 10 (83) |
| Frozen farmed fish        | 17 (85%) | 8 (40.0%) | 17 (85%) | 5 (25) | 0 | 3 (15) | 0 | 1 (5) | 14 (70) |
| Total Avg (206)           | 163 (79.1%) | 49 (23.8%) | 163 (79.1%) | 10 (4.8) | 0 | 7 (3.4) | 0 | 5 (2.4) | 58 (28.1) |

A methicillin susceptibility test indicated that forty-nine (23.8%) S. aureus isolates were resistant to this antibiotic. All isolates carrying the mec-A gene demonstrated positive MRSA phenotypes, specifically 3 (16.7%) fresh marine shrimp samples, 5 (16.7%) frozen marine shrimp samples, 7 (46.6%) fresh farm shrimp samples, and 8 (38%) frozen farm shrimp samples. Furthermore, S. aureus isolates were MRSA-positive in 6 (16.2%) fresh marine fish samples, 7 (13.2%) frozen marine fish samples, 5 (41.7%) fresh farm fish samples, and 8 (40%) frozen farm fish samples, all carrying the mec-A gene (chi-square test, p < 0.05).

In total, one hundred and thirty-three (64%) of the strains isolated from contaminated fishery products were enterotoxigenic S. aureus, and 5.2% of SEs were MRSA-positive (p < 0.05).

Discussion

It is difficult to detect pathogens; thus, good hygiene is very important. The quality and safety of fish and shrimp can be directly influenced by the lack of hygienic habits of fish handlers and contact with contaminated work surfaces, including benches, tables and unwashed knives. Various factors impinge upon seafood safety, ranging from contamination originating from the environment where it is caught to contamination caused by the consumer prior to eating. The most frequently contaminated types of seafood in our study were frozen marine fish and frozen marine shrimps. This contamination might have originated from contaminated freezing systems or the improper transfer of aqua products from their origin to their destination. Delays in product transport, worker non-compliance with food hygiene standards, improper storage conditions such as incompatible temperature, and the accumulation of fish or shrimp can result in greater amounts of contamination than fresh products. Furthermore, farmed seafood products were less contaminated compared with other types of products that were improperly frozen and packed. Microbial growth is affected by environmental factors such as pH, temperature, and water activity. In this study, resistance to penicillin, amoxicillin, oxacillin, and tetracycline between fresh and frozen products was not equivalent (Table 4). In addition, a statistically significant relationship was observed between the sea and mec-A genotypes and fresh and frozen seafood as follows: fresh products were more frequently positive for the Staphylococcus aureus gene sea, while the mec-A genotype was observed more frequently in frozen products.

In addition, S. aureus was detected in fish at levels as high as those observed in staphylococcal food poisoning cases derived from raw fish reported in many countries.

In Japan, the foods that are most frequently involved in staphylococcal food poisoning are sushi (raw fish) and lunch-box meals that contain multi-ingredient foods. In Italy, the foods that are most frequently involved in staphylococcal food poisoning are multi-ingredient foods, and lunch-box meals. When RTE (ready-to-eat) food contamination was monitored in Japan, it was determined that 19.8% of raw fish was contaminated with S. aureus.
respectively, among isolates collected from food handlers 34. A study in Brazil reported high rates of
Among them, SEA is the most common in staphylococcus-related food poisoning cases 39,40,41, likely due to its
staphylococcal enterotoxins (SEA–SEE) have been reported to cause 95% of staphylococcal food poisoning.
sea
hand, the investigation of strains for enterotoxin production is important in pathogenesis studies38. In our study,
transmission of resistant bacteria from fish and shrimp to humans is a consequence of antibiotic overuse.
non-standard use of antibiotics in fish and shrimp aquaculture can lead to
undergo cross-contamination between humans and the environment. Our results showed that the increasingly
and raw shrimp and fish. Moreover, this study provides further support for the hypothesis that MRSA can
Twenty-nine strains carried one or several of the eight SE genes tested; the
strains were resistant to at least two classes of antibiotics, and among them, two were resistant to methicillin.

| Studied genes of Staphylococcus aureus | Genes: positive | Genes: negative | Total |
|--------------------------------------|-----------------|-----------------|-------|
| sea                                  | 95              | 111             | 206   |
| seb                                  | 38              | 168             | 206   |
| tst-1                                 | 8               | 198             | 206   |
| mec-A                                 | 49              | 175             | 206   |
| sea + seb                             | 19              | 187             | 206   |
| sea + seb + mec-A                     | 7               | 199             | 206   |
| sea + seb + mec-A + tst-1             | 2               | 204             | 206   |

Table 5. Presence of the studied Staphylococcus aureus genes.

primary reasons for variations in these results may be the impact of environmental pollution as well as differences
in the processing of fishery products due to maintenance.
Antibiotic resistance can be spread via residual antibiotics in food products, through the transfer of resist-
ant foodborne pathogens, or through the ingestion of resistant strains among original food microflora and the
transfer of resistance to pathogenic microorganisms32. In our study, the rate of resistant strains was high (91.8%).
Resistance to members of the penicillin family was 79% for penicillin and 78.6% for ampicillin. Among S. aureus
food isolates in Portugal, resistance to erythromycin and tetracycline was 5% and 7%, respectively33, whereas
in a study in Botswana (Loeto et al.)34, resistance rates to erythromycin and tetracycline were 16.8% and 50.5%,
respectively, among isolates collected from food handlers34. A study in Brazil reported high rates of S. aureus
contamination in marine products. In that study, isolates were resistant to ampicillin, and 44% were multidrug
resistant14.
In our study, frozen farmed fish were found to be more frequently contaminated than other types of fish.
Moreover, fresh farmed shrimp were found to be more frequently contaminated than other shrimps. This might
be attributable to the inappropriate use of antibiotics in aquaculture.
Zhang et al.35 showed that multidrug resistance is common among MRSA isolates, which is not surprising
due to their ability to transfer staphylococcal cassette chromosome mec-A elements and additional resistance
determinants on plasmids35. Among S. aureus seafood isolates in Spain31, samples were analysed for the presence
of Staphylococcus aureus16. S. aureus was detected in a significant proportion of products (~25%). All isolates were
resistant to penicillin, chloramphenicol and ciprofloxacin, and most were resistant to tetracycline (82.4%), but
none was methicillin-resistant.
In our study, the mec-A gene was detected in 23.8% of S. aureus isolates (49 out of 206). The high prevalence of
resistance genes should be considered a potential health risk for humans and seafood37. Consequently, mandatory
precautions should be taken by governments and individuals to prevent the further spread of MRSA. Hygiene
promotion and avoiding the unsupervised use of antibiotics are elementary steps in this regard1,37. On the other
hand, the investigation of strains for enterotoxin production is important in pathogenesis studies38. In our study,
5.2% of MRSA isolates were positive owing to the presence of SEs.
With the exception of ninety-five (45.2%) isolates, all SE-carrying isolates identified in this study carried the
sea gene and thus could produce SEA, but thirty-eight (18.5%) isolates were found to be seb-positive. Classical
staphylococcal enterotoxins (SEA–SEE) have been reported to cause 95% of staphylococcal food poisoning.
Among them, SEA is the most common in staphylococcus-related food poisoning cases39,40,41, likely due to its
very high resistance to proteolytic enzymes1. In a study performed by Sanchez et al. in Spain31, strains isolated
from 23.5% of samples were enterotoxigenic. A lower proportion of SEs and tst-1-positive strains were previously
found among isolates collected from fishery products in other geographical regions31,40,41. The sea-only genotype
and the sea genotype in combination with other genes are the most prevalent genotypes in Japan (80% to 90%)
and in the United States (50%)30,41. In 2011, an outbreak of gastroenteritis at the Athletic Club in Barcelona, Spain
was studied, and the results showed that 91 people had been infected via the ingestion of contaminated fish. The
infectious Staphylococcus aureus strains produced enterotoxins types A and D28.
In 2007, Kerouanton studied a total of 178 coagulase-positive staphylococcal isolates recovered from 31
staphylococcal food-poisoning outbreaks (SFPO) 1981–2002 that were screened through biotyping32. Eleven
strains were resistant to at least two classes of antibiotics, and among them, two were resistant to methicillin.
Twenty-nine strains carried one or several of the eight SE genes tested; the sea gene was the most common gene
(n = 23) and was often linked to sed (n = 12) or seh (n = 5).

The results of this study show that enterotoxigenic S. aureus that is resistant to antibiotics can cause dangerous
demics and outbreaks. In brief, detection of the SE genes using molecular techniques may help to understand
the pathogenic potential of this microorganism37.

Conclusions
The results of our study highlight the high prevalence of enterotoxigenic MRSA in fishery products, and
the risk of its transmission through the food chain cannot be disregarded31,37, particularly in uncooked meat
and raw shrimp and fish. Moreover, this study provides further support for the hypothesis that MRSA can
undergo cross-contamination between humans and the environment. Our results showed that the increasingly
non-standard use of antibiotics in fish and shrimp aquaculture can lead to S. aureus resistance. As a result, the
transmission of resistant bacteria from fish and shrimp to humans is a consequence of antibiotic overuse.
Despite advances in food research, contamination remains a problem and a major cause of morbidity and mortality worldwide. Our results showed that seafood monitoring requires urgent attention, although more research is needed to verify these results.

References
1. LeLoir, Y., Baron, F. & Gauthier, M. *Staphylococcus aureus* and food poisoning. *J. Genetics and Molecular Research* 2, 63–76 (2003).
2. Schlevert, P. M. et al. Identification and characterization of a enterotoxin from *Staphylococcus aureus* associated with toxic-shock syndrome. *J Infect Dis.* 143, 509–516 (1981).
3. Normanno, G. A. et al. Coagulase positive *staphylococci* and *Staphylococcus aureus* in food products marketed in Italy. *Int. J. Food Microbiol.* 98, 73–79 (2005).
4. Vassiliades, N. G. Staphylococcal enterotoxins: molecular aspects and detection methods. *Journal of Public Health and Epidemiology* 2, 29–42 (2010).
5. Hammad, A. M., Watanabe, W., Fujii, T. & Shimamoto, T. Occurrence and characteristics of methicillin-resistant and -susceptible *Staphylococcus aureus* and methicillin-resistant coagulase-negative *staphylococci* from Japanese ready-to-eat raw fish. *J Food Microbiol.* 156, 286–289 (2012).
6. Argudina, M. A. et al. Exotoxin Gene Content, and Antimicrobial Resistance of *Staphylococcus aureus* Strains Recovered from Foods and Food Handlers. *J Appl Environ Microbiol.* 78, 2930–2931, doi: 10.1128/AEM.07487-11 (2012).
7. Lozano, C. et al. Detection of methicillin-resistant *Staphylococcus aureus* ST398 in food samples of animal origin in Spain. *J. Antimicrob. Chemother.* 64, 1325–1346, doi: 10.1093/jac/dkp378 (2009).
8. Rhee, C. H. & Woo, G. J. Emergence and characterization of foodborne methicillin-resistant *staphylococcus aureus* in Korea. *J Food Prot.* 73, 2285–2290 (2010).
9. Pu, S., Wang, F. & Ge, B. Characterization of toxin genes and antimicrobial susceptibility of *Staphylococcus aureus* isolates from Louisiana retail meats. *Foodborne Pathog Dis.* 8, 299–306, doi: 10.1089/2010(2011).
10. Simon, S. & Sanjeev, S. Prevalence of enterotoxigenic *staphylococcus aureus* in fishery products and fishprocessing factory workers. *J Food control.* 18, 1565–1568 (2007).
11. Mason, R. J. Recognition and management of *Staphylococcus aureus* toxin-mediated disease. *Intern. Med.* 35, 106–119, doi: 10.1111/j.1444-0903 (2005).
12. European Food Safety Authority. 2010. The community summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. *EFSA J.* 8, 1496 (2008).
13. Mead P. S. et al. Food-related illness and death in the United States. *Emerg Infect Dis* 5, 667–673 (1999).
14. Albuquerque, W. E. A., Macre, O. V., Sousa, G. H. F. & Vieira, R. H. S. F. Multiple drug resistant *Staphylococcus aureus* strains d isolated from a fish market and from fish handlers. *Braz. J. Microbiol.* 38, 131–134 (2007).
15. Atyah, M. A. S., Zamri-Saad, M. & Siti-Zahrah, A. First report of methicillin-resistant *Staphylococcus aureus* from cagecultured tilapia (*Oreochromis niloticus*). *Vet. Microbiol.* 144, 502–504, doi: 10.1016/j.vetmic (2010).
16. Mohammed Hatha, A., Maqbool, T. K. & Suresh Kumar, S. Microbial quality of shrimp products of export trade produced from aquacultured shrimp. *J Food Microbiol.* 15, 82, 213–221 (2003).
17. Mee-Marquet, N. V. et al. Virulence and antibiotic susceptibility of *Staphylococcus aureus* strains isolated from various origins. *Pathol. Biol.* 52, 579–583 (2004).
18. Nejma, M. B. et al. Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated in Tunisia. *Diagn. Microbiol. Infect. Dis.* 55, 21–26, doi: 10.1016/S1208-8122-2 (2006).
19. "Institute of Standards and Industrial Research of Iran (ISIRI). No: 6806-1 Microbiology of food and animal feeding stuffs – Enumeration of coagulase – Positive staphylococci (*staphylococcus aureus* and species) Technique using baird – parker agar mediuminsert,. http://www.isiri.org/portal/files/std/6806-1.pdf. (Date of access: 19/09/2006)"
20. "Institute of Standards and Industrial Research of Iran, (ISIRI). NO: 6806-3. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of *staphylococcus aureus* coagulase posetiv Colony count technique (MPN), http://www.isiri.org/portal/files/std/6806-3.pdf. (Date of access:19/09/2006)".
21. Sánchez, D. V., Cabo, M. L., Ibusquiza, P. S. & Herrera, J. J. Incidence and characterization of *Staphylococcus aureus* in fishery products marketed in Galicia (Northwest Spain) *International J Food Microbiology*. 157, 286–296, doi: 10.1016/j.ifm (2012).
22. Gutierrez, D. et al. Incidence of *staphylococcus aureus* of Associated Bacterial Communities on food Industry surfaces. *J. ASM.* 78, 8547–8554, doi: 11.128/AEM.02045-12 (2012).
23. "Clinical and Laboratory Standards Institute. *Identification of *Staphylococcus aureus* of Associated Bacterial Communities on food Industry surfaces*. *J. ASM.* 78, doi: 11.128/AEM.02045-12 (2012).
24. "Clinical and Laboratory Standards Institute. *Identification of *Staphylococcus aureus* of Associated Bacterial Communities on food Industry surfaces*. *J. ASM.* 78, doi: 11.128/AEM.02045-12 (2012).
25. Johnson, W. M. et al. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. *J. Clin. Microbiol.* 29, 426–430 (1991).
26. Oh, S. K. et al. Occurrence toxigenic *S. aureus* in ready to eat in Korea. *J Food safety Research* 70, 1153–1158 (2007).
27. Van, nufeld. et al. Specific detection of methicillinresistant *Staphylococcus aureus* species by multiplex PCR, *J. Clin. Microbiol.* 33, 2864–2867 (1995).
28. Ayulo, A. M. R., Machado, R. A. & Scussel, V. M. Enterotoxigenic *Escherichia coli* and *Staphylococcus aureus* in fish and seafood from the southern region of Brazil. *Int. J. Food Microbiol.* 14, 687–695 (1994).
29. Solano, R. et al. Enterotoxin production by *staphylococcus aureus*: An outbreak at a Barcelona sports club in july 2011. *J Food control.* 33, 114–118 (2011).
30. Shimizu, A. et al. Characterization of *Staphylococcus aureus* coagulase type VII isolates from staphylococcal food poisoning outbreaks (1980–1995) in Tokyo, Japan, by pulsed-field gel electrophoresis. *J. Clin. Microbiol.* 38, 3746–3749 (2000).
31. Atanassova, V. & ReichKlein, G. Microbiological quality of sushi from sushi bars and retails. *J Food Prot.* 71, 860–864 (2008).
32. Tirado, C. & Schmidt, K. WHO surveillance program forcontrol of foodborne infections and intoxication: preliminary result s and trends across greater Europe. *J Infect.* 43, 80–84 (2001).
33. Pesavento, G., Ducci, B., Comodo, N. & Lo Nostro, A. Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat, a research fo methicillin resistant *Staphylococcus aureus* (MRSA). *J Food control.* 18, 196–201 (2007).
34. Pereira, V. et al. Characterization for enterotoxin production, virulence factors and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. *J Food Microbiol.* 26, 278–282, doi: 10.1016/j.ifm (2009).
35. Zhang, K. et al. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. *J Clin. Microbiol.* 43, 5026–5033, doi: 10.1128/JCM.43.10 (2005).
36. Sánchez, D. V., Cabo, M. L., Ibusquiza, P. S. & Herrera, J. J. Incidence and characterization of *Staphylococcus aureus* in fishery products marketed in Galicia (Northwest Spain) *International J Food Microbiol.* 157, 286–296, doi: 10.1016/j.ifm (2012).
37. Shanehbandi, D., Baradaran, B., Sadigh-Etehad, S. & Zarreddah, H. Occurrence of Mecillin Resistant and Enterotoxigenic *Staphylococcus aureus* in Traditional Cheeses in the West North of Iran. *JSCI Microbiol.* 13:2014:129580, doi: 10.1155/ (2014).
38. Cretenet, M., Even, S. & Le Loir, Y. Unveiling Staphylococcus aureus enterotoxin production in dairy products: a review of recent advances to face new challenges. *Dairy Science & Technology* **91**, 127–150, doi: 10.1007/s13594-011-0014-9 (2011).
39. Pinchuk, I. V., Beswick, E. J. & Reyes, V. E. Staphylococcal enterotoxins. *Toxins* **J Food Prot.** 2, 2177–2197, doi: 10.3390/toxins2082177 (2010).
40. Jay, J. M. Modern food microbiology (5th ed. Chapman and Hall) 240–247 (New York, 1997).
41. Su, Y. C. & Wong, A. C. L. Current perspective on detection of staphylococcal enterotoxins. *J. Food Prot.* **60**, 195–202 (1997).
42. Kerouanton, A., Hennekinne, J. A., Letertre, C., Petit, L. & Chesneau, O. Characterization of *staphylococcus aureus* strains associated with food poisoning outbreaks in France. *J Food Microbiology* **115**, 369–375 (2007).

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N.A. and A.D. cooperated to carry out the microbiological and molecular experiments. T.A. prepared Tables 1–4. All authors reviewed the manuscript. This article is from the thesis by N.A. food microbiology student at the School of Health, Tehran University of Medical Sciences.

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