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HIGH FREQUENCY OF ENTEROTOXIN ENCODING GENES OF STAPHYLOCOCCUS AUREUS ISOLATED FROM THE NOSTRILS OF IRANIAN FOOD HANDLERS

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ABSTRACT

Staphylococcal enterotoxins (SEs) are key virulence factors in staphylococcal food poisoning (SFP). This study aimed to assess the frequency of staphylococcal enterotoxin genes among isolates from food handlers in Iran. Nasal swabs were collected from 575 food handlers. PCR using specific primers was used to detect *sea*, *seb*, and *sec* genes as markers for SEA, SEB, and SEC enterotoxins. Out of 575 nasal swabs, 168 (29.2%) were positive for *S. aureus*. Overall, 63.1% (106/168) of *S. aureus* isolates among food handlers were positive for the presence of at least one or more SE genes. Of the 168 isolates, *sea*, *seb*, and *sec* genes were found in 57 (33.9%), 29 (17.2%), and 20 (11.9%) isolates, respectively. A significant percentage of Iranian food handlers carry enterotoxigenic *S. aureus* in their noses. Therefore, strict hygiene measures, screening programs, and control of these carriers are necessary to prevent food contamination with *S. aureus* during food handling.

Keywords: *Staphylococcus aureus*, Enterotoxins, Food handlers, Nasal carriage, PCR

INTRODUCTION

Staphylococcus aureus is a major food-borne pathogen and the cause of staphylococcal food poisoning (SFP) worldwide **(Wu *et al*., 2016; Féraudet Tarisse *et al*., 2021; Rall *et al*., 2010)**. For over 140 years, *S. aureus* has been known to be a pathogen **(Ilie *et al*., 2024)**. One of the most common commensal bacteria in humans is *S. aureus*, which is part of the natural flora. This bacterium causes mild to complicated infectious diseases and has thus become a public health problem **(Todd, 2024; Chouaib *et al*., 2024)**. Food handlers' close contact with food increases the risk of food contamination. The risk of food safety is increased by the production of staphylococcal enterotoxins (SEs) by several strains of *S. aureus* **(Atxaerandio *et al*., 2025)**. Food workers in the food industry play an important role in food safety because they can contaminate foods during processing, distribution, and manipulation **(Angelillo *et al*., 2000)**. Reports estimated that approximately 20% of the human population are persistent carriers and 70–90% of the general population are at least intermittent (transient) carriers of *S. aureus* **(Hasse-Cieślińska, 2007; Chambers, 2001; Nouwen *et al*., 2004)**. Enterotoxins are a significant virulence factor for *S. aureus*. The consumption of enterotoxin-contaminated food is the cause of SFP, which is one of the most common foodborne diseases in the world **(Kadariya *et al*., 2014)**.
Staphylococcal enterotoxins (SEs) are classified as superantigens due to their molecular weight range of 22-29 kDa. To date, 27 types of SEs (SEA-SEE, SEF, SEG-SEI, SEK-SET, SEY, and SE-like toxins (SE*l*J, SE*l*U, SE*l*V, SE*l*X, SE*l*Z, SE*l*26 and SE*l*27) have been recognized. SE genes are encoded by accessory genetic elements, including plasmids, prophages, *S. aureus* pathogenicity islands (SaPIs), and *v*Sa genomic islands **(Féraudet Tarisse *et al*., 2021; Argudín *et al*., 2010; Hu and Nakane 2014; Pinchuk *et al*., 2010; Ono *et al*., 2019; Wilson *et al*., 2011; Ono *et al*., 2015; Zhang *et al*., 2018)**.
SEA is responsible for over 75% of all SFP outbreaks worldwide **(Pinchuk *et al*., 2010)**. SEB not only plays a role in food poisoning (10% of cases) but has also been historically attributed to being a potential biological weapon used in bioterrorism **(Pinchuk et al., 2010; Greenfield *et al*., 2002)**. Depending on the type of enterotoxin, the amount of toxin that causes SFP symptoms (nausea, hypersalivation, vomiting, muscular pain, abdominal cramps, diarrhea, and even death) is reported to range from 20 to 100 ng **(Argudín *et al*., 2010; Pinchuk *et al*., 2010; Hennekinne *et al*., 2012; Balaban and Rasooly 2000)**. SFP occurs in approximately 214,000 cases each year in the United States **(Scallan *et al*., 2011)**. Although there are no precise statistics regarding individuals in Iran who are admitted to hospitals or die from SFP, there are numerous reports that individuals are *S. aureus* carriers **(Khashei *et al*., 2016; Khorvash *et al*., 2012)**. However, there is a lack of information on the occurrence of enterotoxin-producing *S. aureus* in food workers in Iran. Due to the importance of SEs and their role in food safety and public health, this study aimed to assess the frequency of SE genes among isolates from food handlers in Iran.

MATERIAL AND METHODS

Sampling, isolation and identification of *S. aureus*

In this descriptive-cross-sectional study, 575 nasal swabs from adult people working in dairy shops, Ice cream shops, Confectionery, restaurants, and Butcher shops in Tehran (capital of Iran), Tabriz (East Azerbaijan), Ardabil (northwestern Iran), and Zanjan (northwestern Iran) were randomly collected from April 2019 to early June 2021. Informed consent was obtained from all individual participants included in the study. Inclusion criteria for the study were not taking antibiotics in the last 2 weeks. Nasal samples were taken from the anterior part of the nostrils using sterile dry cotton-wool swabs and transferred to Tryptic Soy Broth (TSB) medium (Merck, Germany). To determine hemolytic activity, they were cultured on blood agar, and to determine mannitol fermentation activity, they were inoculated on mannitol salt agar (Merck, Germany) for 24 hours at 37°C. Colonies suspected of being *S. aureus* were tested using Gram stain, catalase, oxidase, coagulase, and the Voges Proskauer (VP) test. The isolates were confirmed by PCR targeting the *S. aureus*-specific *femA* gene (*S. aureus* species- specific).

Genomic DNA extraction

Genomic DNA extraction of *S. aureus* isolates was optimized based on Gadyari et al.'s (2011) method with subsequent changes. *S. aureus* isolates were cultivated in LB broth (Merck, Germany) for 24 h at 37°C. A total of 1.5 mL of the microbial suspension was centrifuged at 8000 rpm for 4 min. The supernatant was discarded and 100 µL of autoclaved TE buffer was added to the bacterial pellet. Centrifugation was again performed at 8000 rpm for 4 minutes. The washing process was repeated three times. After washing, the supernatant was discarded, and 100 µL of autoclaved TE buffer, and 5 µL of lysozyme were added to the

bacterial pellet. The microtubes were incubated at 37°C for 1 hour. Then, suspensions were transferred to a boiling bain-marie for 10 min and then to ice for 5 min. This process was repeated three times. The above samples were centrifuged at 12000 rpm for 5 min. The supernatant containing bacterial genomic DNA was stored at −20°C for later use. Next, DNA was extracted from *S. aureus* reference strains ATCC 13565, ATCC 14458, and ATCC 19095 and used as a positive control for each of the *sea*, *seb*, and *sec* genes in the PCR method.

PCR detection of Staphylococcal enterotoxin genes

The *sea* (210 bp), *seb* (478 bp), and *sec* (257 bp) genes were used to detect the enterotoxins SEA, SEB, and SEC. The primers used in the detection of SE genes are listed in Table 1. PCR reactions were performed in a total volume of 25 µL, including PCR master mix (Taq polymerase, dNTPs, $MgCl_2$, and 10X buffer) (Fermentase, USA), 20 pmol of each primer, DNA sample, and deionized distilled water. The volume and amount of materials needed for PCR and the temperature program of the Thermal Cycler device (Analytik Jena, Germany) are shown in Table 2. PCR products were subjected to electrophoresis using 1.5% agarose gels stained with ethidium bromide and visualized under UV transillumination. The amplified DNA was controlled by the *S. aureus* reference strains mentioned above.

Table 1 Primers used in this study

primer	Primer sequence (5′→3′)	target gene	Amplicon size (bp)	References
femA-F	AAAAAAGCACATAACAAGCG	*femA*	132	**Mehrotra *et al*., 2000**
femA-R	GATAAAGAAGAAACCAGCAG			
Sea-F	AAAGTCCCGATCAATTTATGGCTA	*sea*	210	**Tsen and Chen 1992**
Sea-R	GTAATTAACCGAAGGTTCTGTAGA			
Seb-F	TCGCATCAAACTGACAAACG	*seb*	478	**Johnson *et al*., 1991**
Seb-R	GCAGGTACTCTATAAGTCCC			
Sec-F	GACATAAAAGCTAGGAATTT	*sec*	257	**Johnson *et al*., 1991**
Sec-R	AAATCGGATTAACATTATCC			

Table 2 Thermal cycler temperature program and quantity of material needed for PCR

PCR materials				**Temperature program**					
Ingredients	***femA***	***sea, seb, and sec***	**Number of cycles**	**Time (minute)**		**Temp (°C)**		**stages**	**program**
	Volume (µL)			A	B	A	B		
Master mix	12.5	12.5	1	5	5	94	94	Primary denaturation	1
Forward primer	1	1.5	30	1	1.5	94	94	Secondary denaturation	2
				1	2	57	55	Annealing	3
				1	1.5	72	72	Extension	4
Reverse primer	1	1.5	1	8	8	72	72	Final extension	5
DNA	3	3							
DD water	7.5	6.5							
Total	25	25							

[A]: Temperature program of *femA*
[B]: Temperature program of *sea, seb,* and *sec*

Statistical analysis

Data were analyzed using SPSS version 18.0 software (SPSS, Inc., Chicago, IL) and Microsoft Excel Worksheet version 2016 with chi-square tests. A *P* value of < 0.05 was considered significant.

RESULTS AND DISCUSSION

Frequency of *S. aureus* in food handlers

Overall, 168 (29.2%) *S. aureus* isolates were identified in the 575 nasal swab samples of food handlers: 44 (36.6%) isolates from dairy workers, 26 (29.5%) isolates from ice cream shop workers, 18 (26.1%) isolates from confectionery workers, 40 (28.7%) isolates from restaurant workers, and 40 (25.1%) isolates from butchery workers.

Table 3 Frequency of *S. aureus* in food handlers

Food handlers	**Number of samples**				**No. (%) Nasal carriage of *S. aureus* among food handlers**			
	Tehran	Tabriz	Ardabil	Zanjan	Tehran	Tabriz	Ardabil	Zanjan
Dairy workers	32	28	30	30	11 (34.3)	10 (35.7)	14 (46.6)	9 (30)
Ice cream shop workers	20	20	25	23	4 (20)	7 (35)	8 (32)	7 (30.4)
Confectionery workers	20	16	17	16	5 (25)	4 (25)	5 (29.4)	4 (25)
Restaurant workers	30	37	36	36	8 (26.6)	11 (29.7)	11 (30.5)	10 (27.7)
butchery workers	40	39	40	40	7 (17.5)	12 (30.7)	12 (30)	9 (22.5)
Total (n= 575)	142	140	148	145	35 (24.6)	44 (31.4)	50 (33.7)	39 (26.8)

Hemolytic activity was observed in all 168 *S. aureus* isolates. The *femA* gene with a gene size of 132 bp were confirmed to be present in all 168 *S. aureus* isolates, as shown in Figure 1. The frequency of nasal carriage of *S. aureus* among food handlers in Tehran, Tabriz, Ardabil, and Zanjan was 24.6%, 31.4%, 33.7%, and 26.8%, respectively. The frequency of nasal carriage of *S. aureus* among food handlers in surveyed cities of Iran is shown in Table 3.

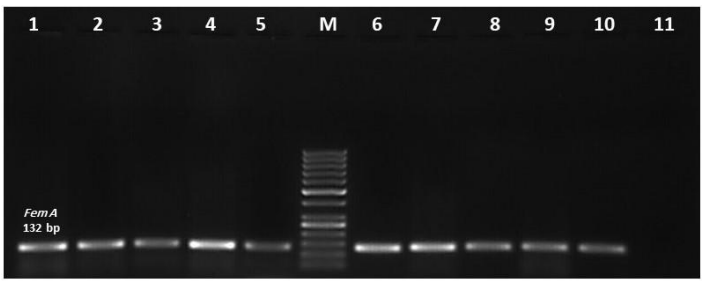

Figure 1 Agarose gel electrophoresis of PCR products for *femA* gene
Lane M: 50 bp DNA ladder, Lane 1: positive control strain, Lane 2- 10: *S. aureus* strains isolates from food handlers, Lane 11: negative control

Distribution of enterotoxin genes in *S. aureus* isolates

Overall, 63.1% (106/168) of *S. aureus* isolates among food handlers were positive for the presence of at least one or more SE genes. Of the 168 isolates, *sea*, *seb*, and *sec* genes were found in 57 (33.9%), 29 (17.2%), and 20 (11.9%) isolates, respectively. The results of PCR for SE genes are shown in Figure 2. The frequency of each SE gene in *S. aureus* isolates is shown in Table 4.
Comparison of SE gene frequency among food handler isolates showed a varying distribution of these genes.
The distribution of SE genes among food handler isolates in Tehran was *sea* (37.1%), *seb* (17.1%), and *sec* (17.1%). The most prevalent SE gene among food handler isolates in Tabriz, Ardabil, and Zanjan was *sea* (36.3%, 30%, and 33.3%), followed by *seb* (22.7%, 14%, and 15.3%), and *sec* (17.1%, 9%, and 12.8%), respectively (Tab 4).

The frequency of SE genes among *S. aureus* isolates from dairy, ice cream, confectionery, restaurant, and butchery workers was 31.8%, 42.3%, 33.3%, 32.5%, and 32.5% for the *sea*, 20.4%, 11.5%, 22.2%, 17.5%, and 15% for the *seb*, and 18.1%, 15.3%, 22.2%, 10%, and 12.5% for the *sec*, respectively (Tab 4).
The most common foodborne pathogen in developing countries is *S. aureus*, which presents a significant public health challenge **(Fetsch *et al*., 2014)**. Poor personal hygiene or cross contamination can be a factor in food handlers spreading foodborne diseases **(Baş *et al*., 2006)**. The PCR method can help identify strains that produce enterotoxin, particularly when enterotoxin genes are not expressed due to suboptimal conditions. Detection of *S. aureus* strains that contain enterotoxin genes becomes crucial when this occurs. Even in small amounts, these enterotoxins have the potential to cause severe food poisoning. This method is currently used by many researchers to identify enterotoxigenic *S. aureus* for this reason **(Johnson *et al*., 1991)**.
Several studies have shown an increasing prevalence of enterotoxigenic *S. aureus* in food handlers **(Acco *et al*., 2003; Loeto *et al*., 2007; Alhashimi *et al*., 2017; Figueroa *et al*., 2002; ÇAKICI *et al*., 2023)**. In our study, among 575 nasal swab samples collected from food handlers, 168 samples (29.2%) tested positive for *S. aureus*. A comparison of the frequency of *S. aureus* among food handlers showed that the distributions in this population varied. The nasal carriage of *S. aureus* among dairy workers (36.6%) was higher than that in other food handlers (P> 0.05). Dairy workers are constantly in contact with natural products. Dairy products provide a conducive environment for the growth of microorganisms, making them ideal for the proliferation of *S. aureus*. As a result, the likelihood of nasal carriage of *S. aureus* is heightened among dairy workers. Furthermore, the prevalence of *S. aureus* among food workers in Ardabil (33.7%) was slightly higher than that in other cities (P> 0.05).

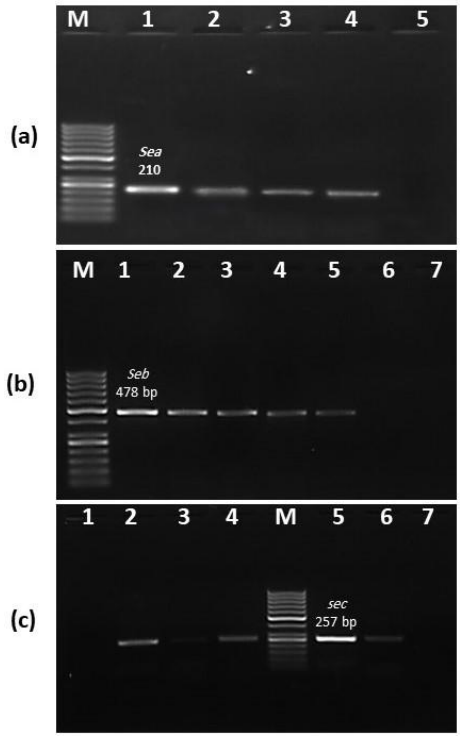

Figure 2 Agarose gel electrophoresis of PCR products for SE genes
For **(a)** Lane M: 50 bp DNA ladder, Lane 1: *S. aureus* reference strain ATCC 13565 (*sea* gene positive control), Lane 2-4: *S. aureus* isolates from food handlers, Lane 5: negative control; **(b)** Lane M: 50 bp DNA ladder, Lane 1: *S. aureus* reference strain ATCC 14458 (*seb* gene positive control), Lane 2-5: *S. aureus* isolates from food handlers, Lane 6: A *S. aureus* isolate that lacks *seb* gene, Lane 7: negative control; **(c)** Lane M: 50 bp DNA ladder, Lane 5: *S. aureus* reference strain ATCC 19095 (*sec* gene positive control), Lane 2, 3, 4 and 6: *S. aureus* isolates from food handlers, Lane 1: A *S. aureus* isolate that lacks *sec* gene, Lane 7: negative control

Table 4 Frequency of SE genes in *S. aureus* isolates from food handlers

Nasal carriers	No. (%) Nasal carrier isolates carrying SE genes											
	Tehran			**Tabriz**			**Ardabil**			**Zanjan**		
	sea	*seb*	*sec*	*sea*	*seb*	*sec*	*sea*	*seb*	*sec*	*sea*	*seb*	*sec*
Dairy workers	4 (36.3)	3 (27.2)	3 (27.2)	3 (30)	2 (20)	1 (10)	4 (28.5)	2 (14.2)	2 (14.2)	3 (33.3)	2 (22.2)	2 (22.2)
Ice cream shop workers	2 (50)	1 (25)	1 (25)	3 (42.8)	1 (14.2)	0 (0)	3 (37.5)	1 (12.5)	2 (25)	3 (42.8)	0 (0)	1 (14.2)
Confectionery workers	2 (40)	1 (12.5)	1 (20)	1 (25)	1 (25)	1 (25)	2 (40)	1 (20)	0	1 (25)	1 (25)	0
Restaurant workers	3 (37.5)	1 (12.5)	0	4 (36.3)	3 (27.2)	1 (9)	3 (27.2)	1 (9)	0	3 (30)	2 (20)	2 (20)
Butchery workers	2 (28.5)	0	1 (14.2)	5 (41.6)	3 (25)	1 (8.3)	3 (25)	2 (16.6)	2 (16.6)	3 (33.3)	1 (11.1)	0
total	13 (37.1)	6 (17.1)	6 (17.1)	16 (36.3)	10 (22.7)	4 (9)	15 (30)	7 (14)	5 (10)	13 (33.3)	6 (15.3)	5 (12.8)

Only a few reports on the frequency of *S. aureus* in food handlers from Iran have been published. According to the previous reports from Iran, 24.5–33.8% of healthy carriers and food handlers were positive for the presence of this pathogen **(Khashei *et al*., 2016; Khorvash *et al*., 2012; Asgarpoor *et al*., 2018)**. The prevalence of *S. aureus* among food handlers in Botswana (south-central Africa) is reported to be 57.5%. *S. aureus* carriage was found to be higher than that in our study **(Loeto *et al*., 2007)**. According to **Acco *et al*. (2003)**, **Al Bustan *et al*. (1996)**, **Figueroa *et al*. (2002)**, and **ÇAKICI *et al*. (2023)**, the frequencies of *S. aureus* in food handlers were 30%, 26.6%, 34%, and 14%, respectively. Furthermore, according to **Fooladvand *et al*. (2019)**, and **Getenet *et al*. (2019)**, the frequency of *S. aureus* among restaurants, hotel workers, and other food handlers were 27.8%, and 20.1%, respectively. This diverse distribution of *S. aureus* among food handlers could be attributed to either having or not possessing sufficient knowledge of food hygiene, observing or not observing personal and environmental hygiene, and handling raw meat or not **(Baş *et al*., 2006; Loeto *et al*., 2007; Ho *et al*., 2014)**.
In our study, SE genes were identified in 106 (63.1%) of 168 nasal swab isolates. According to **Fooladvand *et al*. (2019)**, and **ÇAKICI *et al*. (2023)**, this distribution was reported as 70.1% and 33.6%, respectively. Furthermore, the frequency of *sea* gene (33.9%) was higher than that of other genes (P< 0.05). Similar to our results, *sea* was the most common SE gene in *S. aureus* isolates among food handlers in Turkey, Spain, Brazil and Iraq **(Rall *et al*., 2013; Alhashimi *et al*., 2017; Mehrotra *et al*., 2000; ÇAKICI *et al*., 2023)**.
In a study conducted in Switzerland, the *sea* gene was found in 13 (26%) of 50 isolates from nasal swabs **(Wattinger *et al*., 2012)**. In the Netherlands, *sea* was detected in 20.97 (19.6%) strains of 107 nasal swabs from a healthy population **(Mehrotra *et al*., 2000)**, and *sea* was found in 7 samples (8.75%) from 80 nasal carriers in Poland **(Bania *et al*., 2006)**. Furthermore, in Iraq, the frequency of *sea* gene was detected in 16 (16%) out of 100 strains isolated from food handlers **(Alhashimi *et al*., 2017)**. According to a study by ÇAKICI et al. (2023), n Turkey, Noteworthy *sea* genes were detected in 16% of 125 nasal swabs and hand isolates from food workers. In this study, isolates from ice cream shop workers had a higher prevalence of the *sea* gene, with a frequency of 42.3%, than those from other food handlers (P> 0.05). The frequency of the *sea* gene in *S. aureus* among food handlers in our study was higher than that in the aforementioned studies. This demonstrates that *S. aureus* isolated from food workers in Iran is more enterotoxigenic than other food workers in the world and increases the risk of SFP. One possible explanation for the high frequency of the *sea* gene among *S. aureus* isolates compared to other enterotoxins is that there are strains with high enterotoxin production capacity and high expression of the toxin-related gene. Food handlers seem to not fully observe hygiene tips due to their busy schedule, which is why the frequency of enterotoxigenic *S. aureus*, especially SEA enterotoxin, is high among them.
In the current study, the *seb* gene was detected in 29 (17.2%) out of 168 *S. aureus* isolates. Similar to our results, in Iraq, the *seb* gene was detected in 18 (18%) strains isolated from 100 nasal swabs **(Alhashimi *et al*., 2017)**. In other similar studies, the *seb* gene was also reported in Iran and Kuwait (15.2% and 12.5%, respectively) **(Fooladvand *et al*., 2019; Udo *et al*., 2009)**. Meanwhile, the frequency of the *seb* gene in isolates obtained from food handlers in Poland and healthy populations in the Netherlands was 5% and 5.6%, respectively **(Mehrotra *et al*., 2000; Bania *et al*., 2006)**. Isolates from dairy workers contained the *seb* gene more frequently, with a frequency of 20.4%, than those from other food handlers in this study (P> 0.05). The prevalence of *S. aureus*, which produces enterotoxin

SEB, appears to be higher among food handlers and healthy populations in developing countries than in other countries.

In the current study, *sec* was detected in 20 (11.9%) out of 168 strains of *S. aureus* isolates. the *sec* gene was reported in Turkey in 9.6% of 125 strains isolated from food handlers **(ÇAKICI *et al*., 2023).** In Iraq, *sec* was detected in 8 (8%) out of 100 strains of *S. aureus* isolates from food handlers in Kerbala city **(Alhashimi *et al*., 2017)**. *sec* Genes were more prevalent among isolates from confectionery workers, with a frequency of 22.2%, than those of other food handlers, in this study (P> 0.05).

CONCLUSION

This study found that *S. aureus* was present in the nostrils of a significant number of workers in the food chain. In this investigation, nasal swabs from food handlers who come into contact with food were found to have enterotoxigenic *S. aureus*. The conclusion is that *S. aureus* can be spread by food handlers. In Iran, there have been studies on detecting enterotoxin genes of *S. aureus* in food handlers, but there has been no provision of a suitable standard or solution to prevent bacteria from being transferred to the food chain by carriers. Therefore, based on these findings, it is necessary for epidemiologists, microbiologists, veterinarians, and food hygiene experts to cooperate with each other in order to ensure public health and reduce the risks caused by the transmission of bacteria from carriers to the food chain.

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