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RESEARCH ARTICLE

CARRIAGE OF ENTEROTOXIGENIC *STAPHYLOCOCCUS AUREUS* AND HYGIENE PRACTICES OF FOOD WORKERS

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ABSTRACT

Purpose: To determine the enterotoxigenic *Staphylococcus aureus* carriage rates and personal hygiene practices a total of 300 food workers participated, with 228 working in food businesses and 72 in hospital kitchens in Canakkale, Turkey.
Material and Methods: Participants completed a questionnaire about sociodemographic characteristics, hygiene practices, and food safety. Hand/nasal swabs were collected from the food workers. Inoculums were inoculated on Baird Parker Agar. Multiplex PCR and ELISA methods were used.
Results: The enterotoxigenic *S. aureus* carriage rate was 14% of food workers. Out of the 125 *S. aureus* strains, 42 (33.6%) were positive for one or more SE genes. Furthermore, *sea, seb, sec, sed*, and *sea+sed* were found in 16.0%, 6.4%, 9.6%, 6.4%, and 4.8% respectively. SEA, SEB, SEC, SED, and SEE were found at rates of 14.4%, 7.2%, 12.8%, 11.2%, and 20.8%. It was determined that as the education level of food workers increased, the *S. aureus* carriage rate decreased. The carriage rate was found to be higher in those who use gloves than those who do not. Hand carriers were determined more in nasal carriers ($p<0.05$).
Conclusion: Food workers who are carriers of enterotoxigenic *S. aureus* are a risk factor for food contamination. Training food workers on personal hygiene can be effective in preventing food poisoning.

Keywords: Enterotoxin, food workers, hygiene practices, *Staphylococcus aureus* carriage.

INTRODUCTION

Transference of *Staphylococcus aureus*, which is increasingly reported worldwide, is one of the most important known risk factors for *Staphylococcus* infection (1). Strains present in the nose can generally come into contact with hands, fingers, and the face, and therefore, nasal carriers can easily become skin carriers (2).

Staphylococcal food poisoning (SFP) caused by enterotoxin-producing *Staphylococcus* species is an important foodborne disease in many countries. Enterotoxigenic *S. aureus* can be present on the hands of food handlers and can easily contaminate food during processing (3). Lack of proper hygienic measures during preparation of food is a major risk of contamination, and staphylococcal food poisoning is often associated with manually prepared food (4). The transfer of enterotoxigenic microorganisms that can be present in the nose or skin of food handlers to cooked and especially protein-rich foods, as well as not keeping these foods refrigerated, are factors associated with enterotoxin poisoning (5).

Individuals working in food preparation and food services play an important role in spreading

foodborne diseases and epidemics due to poor personal hygiene, cross contamination, and a lack of food safety practices. This study was performed to determine certain demographic characteristics, personal hygiene practices, food safety practices and enterotoxigenic *S. aureus* carriage rates, of food workers in food businesses and hospitals in Çanakkale, Turkey.

MATERIAL AND METHODS

In the study, three-hundred food workers from 9 hospitals and 17 food businesses (such as diners, restaurants, and food factories) in Canakkale (Turkey) participated during 2014-2015. A multiple-choice questionnaire consisting of 12 questions was completed by 72 kitchen personnel working at the hospital and 228 working at food businesses. The questionnaire asked participants about their demographic characteristics, personal hygiene practices, and food safety behaviors. Participants were asked about age, gender, educational background, job title, and the number of years in the sector to determine their demographic characteristics. The frequency of hand washing and showering as well as glove and mask use were investigated to determine personal hygiene practices. Food workers were also asked if they regularly had nasal culture tests and received food safety training. Enterotoxigenic properties of 125 *S. aureus* isolates obtained from these individuals were tested using the PCR and ELISA methods.

Sampling, Isolation and Identification of *S. aureus*

Samples were collected from both nostrils, left and right hands (palm, interdigital folds, and wrists) using separate sterile swabs (6). Inoculums transferred to 5 ml Brain Heart Infusion Broth (Merck, Germany) were inoculated onto Baird Parker Agar medium (Merck 1.05406) containing egg yolk tellurite emulsion (Merck 1.03785). Isolates were identified using gram staining, catalase, coagulase, biochemical tests, and Latex agglutination tests (Slidex Staph-Kit, Biomerieux, France).

NCTC 10652 FDA 196E (*sea*), NCTC 10654 FDA 243 (*seb*), NCTC 10656 494 (*sed*) (National Collection of Type Cultures Public Health Laboratory Service, London), 1229/93 (*sec*), and FRI 918 (*see*) (National Reference Laboratory for Staphylococci, Robert-Koch-Institute, Wernigerode, Germany) S. aureus reference strains were used to search for staphylococcal enterotoxin genes.

Table 1. Staphylococcal enterotoxin primers

Primers	Oligonucleotide sequence (5'-3')	Product size (bp)	Reference	Multiplex PCR mix no
sea forward	GCA GGG AAC AGC TTT AGG C	520	(8)	1
sea reverse	GTT CTG TAG AAG TAT GAA ACA CG			
seb forward	ACA TGT AAT TTT GAT ATT CGC ACT G	667	(7)	1
seb reverse	TGC AGG CAT CAT GTC ATA CCA			
sec forward	CTT GTA TGT ATG GAG GAA TAA CAA	283	(8)	1
sec reverse	TGC AGG CAT CAT ATC ATA CCA			
sed forward	GTG GTG AAA TAG ATA GGA CTG C	384	(8)	2
sed reverse	ATA TGA AGG TGC TCT GTG G			
see forward	TAC CAA TTA ACT TGT GGA TAG AC	170	(8)	2
see reverse	CTC TTT GCA CCT TAC CGC			

DNA Extraction

Bacteria were boiled in lysis buffer solution and then centrifuged. Lysis buffer solution consisting of 925 µl H20, 25 µl sodium dodecyl sulfate, and 50 µl NaOH (2M) was prepared. Several *S. aureus* colonies were added and mixed in 50 µl Lysis buffer solution. Tubes were maintained in heating blocks at 100°C for 10 minutes to perform bacterial lysis. Fifty microliters of Tris EDTA buffer was added to the tubes, and centrifugation was performed at 13000 xg for 10 minutes. Forty microliters was transferred to 150 µl Tris EDTA buffer.

Detection of Staphylococcal Enterotoxin Genes by Multiplex PCR

A multiplex PCR method was used in the identification of staphylococcal enterotoxin genes (*sea, seb, sec, sed, see*). *Sea, seb*, and *sec* genes were investigated in the first tube, and *sed* and *see* genes in the second tube (Lovseth, et al., 2004) (Table 1).

For the PCR reaction, 20 µl mixtures consisting of 10 µl 2X ExPrime Taq premix (GenetBio, Korea), 1 µl (10 pmol/µl) forward primer, 1 µl (10 pmol/µl) reverse primer, pure water, and 1,5 µl bacterial DNA sample were prepared. Multiplex PCR protocol was set as: initial denaturation at 94°C for 3 minutes, 30 cycles; and final elongation at 94°C for 1 minute, at 53°C for 45 seconds, at 72°C for 1 minute and at 72°C for 5 minutes.

Detection of Staphylococcal Enterotoxins by ELISA

A RIDASCREEN SET A, B, C, D, E (R-Biopharm, Darmstadt, Germany) kit was used to identify SEA, SEB, SEC, SED, and SEE toxins by ELISA method. The testing procedure was carried out in the following order.

100 µl of supernatants and control solution was transferred to the wells. The cassette containing the strips was left for 1 hour incubation at 37 °C. The first washing of the strips was carried out with washing buffer (0.1% Thimerosal) in 5 replicates in an ELISA washing device (Biotek ELx50). 100 µl of conjugate *(1)* solution was added to the washed wells and left for 1 hour incubation at 37 °C. The second washing process was carried out as mentioned above. 100 µl of conjugate *(2)* solution was added to each well and left for 30 minutes incubation at 37 °C. The third washing process was carried out as mentioned above. 100 µl substrate/chromogen solution was added to each well and left for 15 minutes of incubation at 37 °C in the dark environment. The reaction was stopped by adding 100 µl stop solution to each well. The absorbance value at 450 nm wavelength within 5-10 minutes was performed using an ELISA reader (Biotek ELx800).

The cutoff value was calculated for each sample by adding 0.15 to the arithmetic mean of the negative controls on the studied strip. The toxins in the samples with an absorbance value above the cutoff value were determined as "positive".

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) software, version19.0, was used for the statistical analysis of data. Some sociodemographic characteristics, levels of knowledge about hygiene and food safety of food workers participating in the study were determined by applying a face-to-face questionnaire. The relationship between this information and *S. aureus* carriage was evaluated by Chi-square test. The significance test of the difference between the two averages (T-test) was used in comparing the statistical data of food workers employed in food businesses and hospital kitchens. The frequency table was used to evaluate the distribution of *S. aureus* strains that produce enterotoxin and the specified spa types to the workplaces. $p< 0.05$ was considered statistically significant.

Ethical Approval

The study was conducted using a protocol approved by the Local Ethics Committee of Clinical Research (Date: No: 050.99-214), and written informed consent was obtained from all participants.

RESULTS

S. aureus were isolated from the nose and/or hands of 125 (41.7%) food workers out of a total of 300. Ninety (30%) of these individuals had nasal, and 84 (28%) had hand carriage. *S. aureus* were also isolated from the hands of 49 (54.4%) nasal carriers. *S. aureus* hand carriage occurred at an increased rate in nasal carriers ($p < 0.05$). Among 300 food workers, ages ranged from 15 to 65 years, and the mean age was 33.9. Among these individuals, 164 (54.7%) were between the ages of 19-35 years; 193 (64.3%) of the participants were male. Among 294 individuals who answered the question about educational background, 104 (35.4%) were primary

school graduates, and 43 (14.6%) were college graduates. The carriage rates in primary school, secondary school, high school, and college graduates were 46.2%, 50.8%, 37.8%, and 25.6%, respectively. The percentage of carriers was inversely related to educational level ($p < 0.05$). (Table 2).

Among the 299 individuals who answered the question about the frequency of hand washing, 278 (93.0%) indicated that they washed their hands regularly and frequently, and 11 (3.7%) said that they washed their hands when they got dirty. In addition, 259 (86.6%) participants out of 299 said that they took a shower every day, and 31 (10.4%) indicated that they showered three days a week. One-hundred and twenty out of 299 participants (40.1%) said that they always used gloves, while 54 (18.1%) said that they did not use gloves while working. Sixty (50.0%) individuals of the 120 participants who always used gloves were carriers of *S. aureus*, whereas the remaining 60 (50.0%) did not carry *S. aureus*. Fifteen (27.8%) of the 54 individuals who did not use gloves carried *S. aureus*, whereas 39 (72.2%) did not. The rate of carriage in glove users was found to be significantly higher than the rate of carriage in those who did not use gloves ($p < 0.05$).

One-hundred and ninety-eight of 263 individuals (75.3%) stated that they changed their gloves when they got dirty, whereas 19 (7.2%) said that they never changed gloves. The rate of carrier was found to be higher in those who stated that they changed their gloves more frequently ($p <0.05$). Among 295 individuals, 120 (40.7%) indicated that they didn't use masks while working. One hundred and three (34.1%) out of 300 individuals stated that they had nasal culture tests once a year. Among 289 individuals, 153 (52.9%) indicated that they did not receive such training. There was no significant relationship between mask use, having regular nasal culture tests, or receiving food safety training in food workers and carrier rates ($p > 0.05$) (Table 3).

Among 125 *S. aureus* isolates, 20 (16%) had the sea, 8 (6.4%) had seb, 12 (9.6%) had sec, 8 (6.4%) had sed genes, and none of the isolates had the *see* gene. Six of the strains that had the *sed* gene also had the *sea* gene. From a total of 125 *S. aureus* isolates, 42 (33.6%) carried at least one of the enterotoxin genes. The enterotoxigenic *S. aureus* carriage rate was 14% of food workers. Thirty-one (33.3%) of these isolates were obtained from food businesses, and 11 (34.4%) from hospital food workers. There was no statistically significant difference when comparing workplace types in terms of the percentage of isolates that carry a toxin gene ($p > 0.05$). The *sea* (no:3,4,6,8,15), *seb* (no:7), *sec* (no:14) multiplex PCR gel appearance of *S. aureus* isolates is shown in Figure 1. In 42 *S. aureus* isolates that carried an enterotoxin gene, the presence of A, B, C, D, and E staphylococcal enterotoxins was investigated. There was A, B, C, D, and E toxin production in 18, 9, 16, 14, and 26 of the *S. aureus* isolates, respectively. Nine of the 42 isolates were found to be compatible with gene and toxin positivity. The *see* gene was not detected in any of the isolates with the PCR method, whereas the ELISA method revealed that 25 isolates contained the E toxin. Although the *sea* gene (n=4) and the *sed* gene (n=2) were detected in some strains, there was no toxin positivity according to the ELISA method. Twenty-seven isolates were found to carry one or two more different toxins according to the ELISA method in addition to the gene(s) detected by the PCR method (Table 4). The manufacturer reported that there might be antibody/toxin cross-reactivity for the RIDASCREEN SET A, B, C, D, E test kit (A/E, E/A, B/C, and C/B), and cross-reactions might be seen at a rate of 10–20%. In our study, the percentage of cross-reactions (60.4%) of such results was higher than what was reported by manufacturer. Similar results were also obtained in another study on this subject (9). The PCR method used in our study for enterotoxin detection was cheaper, faster, and more reliable than ELISA.

DISCUSSION

The percentage of nasal carriage in 47 individuals working in the food business in Brazil and 64 individuals working at the same job in Malaysia was found to be 30% and 24.3%, respectively. According to the results obtained from these studies, nasal carriage percentages were not higher than in our study (10). Among 200 food workers that were included in a study from Botswana, 115 (57.5%) were found to be *S. aureus* carriers. *S. aureus* carriage was found to be higher than our study (11). Carriage was detected in 93 (40.8%) of 228 individuals working in food businesses, and in 32 (44.4%) of 72 individuals working in hospital kitchens. The difference between the workers in food businesses and hospital kitchens was not statistically significant in terms of *S. aureus* carriage ($p > 0.05$). The relationship between age, gender, task in workplace, years of employment for food workers, and *S. aureus* carriage rates was not

Table 2. Distribution of demographic characteristics by workplace type and the relationship with *S. aureus* carriage rates.

Demographic characteristics		Workplace type		Total n (%)	*S. aureus* carrier rate		Total n (%)	*p* value
		Food business n (%)	Hospital n (%)		Pos. n (%)	Neg. n (%)		
Age	14-18	13 (5.7)	0 (0)	13 (4.3)	4 (30.8)	9 (69.2)	13 (100)	> 0,05
	19-35	140 (61.4)	24 (33.3)	164 (54.7)	69 (42.1)	95 (57.9)	164 (100)	
	36-50	54 (23.7)	44 (61.1)	98 (32.7)	42 (42.9)	56 (57.1)	98 (100)	
	51-65	21 (9.2)	4 (5.6)	25 (8.3)	10 (40.0)	15 (60.0)	25 (100)	
Total n (%)		228 (100)	72 (100)	300 (100)	125 (41.7)	175 (58.3)	300 (100)	
Gender	Female	64 (28.1)	43 (59.7)	107 (35.7)	42 (39.3)	65 (60.7)	107 (100)	> 0.05
	Male	164 (71.9)	29 (40.3)	193 (64.3)	83 (43.0)	110 (57.0)	193 (100)	
Total n (%)		228 (100)	72 (100)	300 (100)	125 (41.7)	175 (58.3)	300 (100)	
Educational background	Primary school	65 (29.3)	39 (54.2)	104 (35.4)	48 (46.2)	56 (53.8)	104 (100)	< 0.05
	Secondary school	44 (19.8)	21 (29.2)	65 (22.1)	33 (50.8)	32 (49.2)	65 (100)	
	High school	70 (31.5)	12 (16.6)	82 (27.9)	31 (37.8)	51 (62.2)	82 (100)	
	College	43 (19.4)	0	43 (14.6)	11 (25.6)	32 (74.4)	43 (100)	
Total n (%)		222 (100)	72 (100)	294 (100)	123 (41.8)	171 (58.2)	294 (100)	
Task in workplace	Cook	61 (27.7)	9 (12.5)	70 (24.0)	33 (47.1)	37 (52.9)	70 (100)	> 0.05
	Assistant cook	24 (10.9)	15 (20.8)	39 (13.3)	22 (56.4)	17 (43.6)	39 (100)	
	Kitchen cleaning personnel	34 (15.5)	13 (18.1)	47 (16.1)	16 (34.0)	31 (66.0)	47 (100)	
	Service personnel	101 (45.9)	35 (48.6)	136 (46.6)	49 (36.0)	87 (64.0)	136 (100)	
Total n (%)		220 (100)	72 (100)	292 (100)	120 (41.1)	172 (58.9)	292 (100)	
Years of employment	0–1 year	58 (25.8)	10 (13.9)	68 (22.9)	24 (35.3)	44 (64.7)	68 (100)	> 0.05
	1–4 years	56 (24.9)	15 (20.8)	71 (23.9)	32 (45.1)	39 (54.9)	71 (100)	
	4–10 years	50 (22.2)	24 (33.3)	74 (24.9)	31 (41.9)	43 (58.1)	74 (100)	
	> 10 years	61 (27.1)	23 (31.9)	84 (28.3)	37 (44.0)	47 (56.0)	84 (100)	
Total n (%)		225 (100)	72 (100)	297 (100)	124 (41.8)	173 (100)	297 (100)	

statistically significant (p>0.05) (Table 2). In other studies conducted in Ethiopia and Egypt, the relationship between sociodemographic characteristics of food handlers and *S. aureus* carriage rates was not statistically significant. In a similar study on hand washing practices, 179 (89.5%) food handlers had a habit of hand washing after toilet, while 21(10.5%) of food handlers had no habit of hand washing after toilet. In this study, as in our study, there was no significant relationship between food workers (5). Using gloves is of utmost importance in preventing the contamination of food. In addition, gloves should be changed at certain intervals, such as while moving on to other work and after touching raw fruits and vegetables. The ambiance under gloves provides a favorable condition for the growth of microorganisms if the glove is punctured or torn. It is well known that foodborne diseases due to contamination will not be reduced unless the habit of using gloves is completely adopted.

If, in fact, they have used gloves for a long time or if they have not changed or changed rarely, it may have been possible. Individuals who always use gloves and never or rarely change them seem to play a greater role in transferring pathogens to food than those who work with clean bare hands. A study conducted in the USA investigated the effect of glove use by food processing personnel in fast food restaurants on the microbial load in foods, and it was thought that the use of the same gloves for an extended period, as well as less frequent hand washing, increased bacterial contamination (12). The percentage of individuals who did not receive food safety training (52.6%) was extremely high in our study and is similar to the percentage 47.8% found (13). Lack of personal hygiene among workers in the food industry is an important factor in the development of foodborne diseases. These individuals may transfer pathogens to food from their hands during the production and distribution processes, which can lead to food poisoning (14).

In a prior study analyzing swab samples obtained from the nose and hands of 82 food workers, it was found that 20 (24.3%) workers had *S. aureus*, and 19 (95%) of those isolates contained one or more enterotoxin genes (6). Forty percent of the *S. aureus* strains (n = 99) obtained from food workers working at 5 different workplaces contained enterotoxin genes. The most frequently detected genes in the study were *sea* (20%) and *seb* (11%). Eight of the

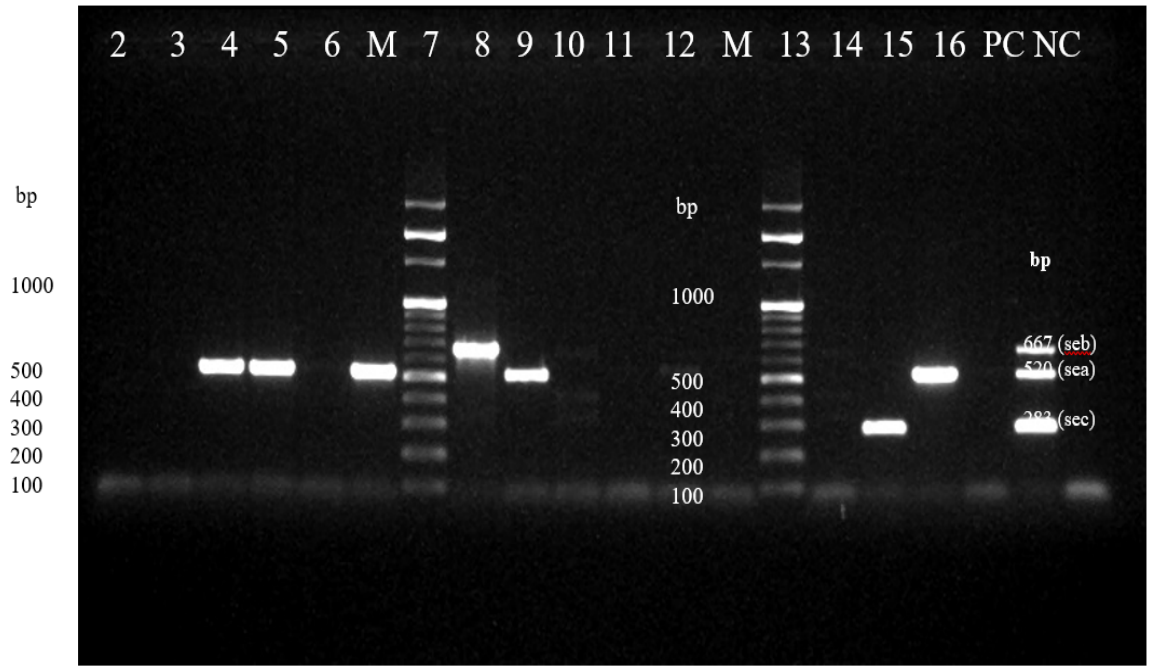

Figure 1. The *sea*+*seb*+*sec* multiplex PCR gel appearance of *S. aureus* isolates.

Table 3. Distribution of personal hygiene/food safety practices and the relationship with *S. aureus* carriage.

Questions	Answers	Total n (%)	*S. aureus* carriage		*p* = 0.05
			Positive n (%)	Negative n (%)	
How often do you wash your hands?	After going to the bathroom	7 (2.3)	2 (28.6)	5 (71.4)	> 0.05
	Before preparing food	3 (1.0)	2 (66.7)	1 (33.3)	
	As my hands get dirty	11 (3.7)	4 (36.4)	7 (63.6)	
	Regularly often	278 (93.0)	116 (41.7)	162 (58.3)	
Total n (%)		299 (100)	124 (41.5)	175 (58.5)	
How often do you take a shower?	Once a week	2 (0.7)	1 (50.0)	1 (50.0)	> 0.05
	Twice a week	7 (2.3)	0 (0)	7 (100)	
	Three times a week	31 (10.4)	13 (41.9)	18 (58.1)	
	Every day	259 (86.6)	111 (42.9)	148 (57.1)	
Total n (%)		299 (100)	125 (41.8)	174 (58.2)	
Do you use gloves?	No, I don't	54 (18.1)	15 (27.8)	39 (72.2)	< 0.05
	Sometimes	69 (23.1)	29 (42.0)	40 (58.0)	
	Frequently	56 (18.7)	20 (35.7)	36 (64.3)	
	Always	120 (40.1)	60 (50.0)	60 (50.0)	
Total n (%)		299 (100)	124 (41.5)	175 (58.5)	
How often do you change gloves?	I don't	19 (7.2)	3 (15.8)	16 (84.2)	< 0.05
	1-2 times a day	19 (7.2)	5 (26.3)	14 (73.7)	
	5-6 times a day	27 (10.3)	11 (40.7)	16 (59.3)	
	As they get dirty	198 (75.3)	95 (48.0)	103 (52.0)	
Total n (%)		263 (100)	114 (43.3)	149 (56.7)	
Do you use a mask?	No, I don't	120 (40.7)	45 (37.5)	75 (62.5)	> 0.05
	Sometimes	62 (21.0)	29 (46.8)	33 (53.2)	
	Frequently	39 (13.2)	16 (41.0)	23 (59.0)	
	Always	74 (25.1)	32 (43.2)	42 (56.8)	
Total n (%)		295 (100)	122 (41.4)	173 (58.6)	
Do you have a nasal culture test regularly (once a year)?	I never had	70 (23.4)	22 (31.4)	48 (68.6)	> 0.05
	I have the test irregularly	27 (9.1)	12 (44.4)	15 (55.6)	
	Yes	103 (34.1)	47 (45.6)	56 (54.4)	
	No	100 (33.4)	44 (44.0)	56 (56.0)	
Total n (%)		300 (100)	125 (41.7)	175 (58.3)	
Did you receive food safety training?	Yes	136 (47.1)	61 (44.9)	75 (58.3)	> 0.05
	No	153 (52.9)	60 (39.2)	93 (55.1)	
Total n (%)		289 (100)	121 (41.9)	168 (58.8)	

Table 4. Staphylococcal enterotoxin types in *S. aureus* isolates obtained from the PCR and ELISA methods.

Isolate number	SE genes	SE
1	*sec*	C,E
2	*sea*	A,D,E
3	*seb*	B,C,E
4	*seb*	B,C,E
5	*seb*	B,C,E
6	*sec*	C
7	*seb*	B,C,E
8	*sec*	C
9	*seb*	B,C,E
10	*seb*	B,E
11	*sec*	B,C,E
12	*sea*	A,D,E
13	*sea*	A,D,E
14	*sea, sed*	A,D,E
15	*seb*	B
16	*sea*	A,D,E
17	*sed*	Neg
18	*sec*	A,C,E
19	*sea, sed*	A,D,E
20	*sea*	A,D,E
21	*sea*	Neg
22	*sea*	A,D,E
23	*sea, sed*	A,D,E
24	*sea, sed*	A,D,E
25	*sec*	A,B,C
26	*seb*	B
27	*sec*	C
28	*sea*	Neg
29	*sea, sed*	A,D,E
30	*sea*	Neg
31	*sea*	Neg
32	*sec*	C
33	*sea*	A,D,E
34	*sea*	A,D,E
35	*sec*	C
36	*sea*	A,E
37	*sea*	A,E
38	*sec*	B,C
39	*sec*	C
40	*sea, sed*	A,D,E
41	*sed*	Neg
42	*sec*	C

A: SEA, B :SEB, C: SEC, D: SED, E: SEE

isolates (8%) carried more than one toxin gene (15). In another study including 332 food workers, 100 (30.1%) were carriers, 38 (38%) carried one or more enterotoxin genes, and *sea, seb, sec, sed*, and *see* genes were detected in 16%, 18%, 8%, 6%, and 8% of cases, respectively (16). In our study, the overall rate of enterotoxigenic *S. aureus* carriage in food workers in hospitals and food businesses was 14%, with a rate of 13.6% in hospital workers and 15.3% in workers from food businesses.

It was reported that 86.6% of *S. aureus* strains obtained from the employees of the city restaurants in Kuwait produced enterotoxin, and their distribution was 28% SEA, 28.5% SEB, 16.4% SEC, and 3.5% SED (17). In another study conducted in Botswana, 43 (21%) of the 204 isolates obtained from the nose, hands, and samples of 200 food workers were found to be enterotoxigenic, and the most common type of enterotoxin was SEA (34.9%) (11). *S. aureus* was isolated in 35 of the 102 food workers (34.3%) of the 19 restaurants serving in Santiago, and 19 (54%) of these strains were found to produce enterotoxin. The most frequently detected enterotoxin was the type A (12/19) (18).

CONCLUSION

The demographic characteristics, education levels, and hygiene practices of people working in food processing, preparation, and service are different. It was determined that as the education level of food workers increased, the *S. aureus* carrier rate decreased. Training food workers in personal hygiene and food safety practices can be effective in preventing food poisoning due to staphylococcal enterotoxins. Effective, periodically repeated trainings given by experts on food safety can reduce the attitudes and behaviors of these people that may pose microbiological risks. Food workers should be taught the importance of hand washing to reduce the transport of microorganisms and prevent contamination of the work environment (tools, equipment, etc.). It is also necessary to increase the knowledge and responsibilities of employers on food safety, workplace, and personnel hygiene.

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REFERENCES

1. Soriano, JM, Font G, Molto JC, Manes J. Enterotoxigenic Staphylococci and Their Toxins in Restaurant Foods. Trends Food Sci Technol 2002;13:60–67.
2. Cakici N, Demirel-Zorba NN, Akçali A. Food industry employees and staphylococcal food poisoning. Turk Bull Hygiene Exp Microbiol 2015;72(4):337-350.
3. Cha JO, Lee JK, Jung YH, Yoo JI, Park YK, Kim BS, et al. Molecular Analysis of Staphylococcus aureus Isolates Associated with Staphylococcal Food Poisoning in South Korea. J Appl Microbiol 2006;101:864–871.
4. Akineden O, Hassan AA, Schneider E, Usleber E. Enterotoxigenic properties of *Staphylococcus aureus* isolated from goats' milk cheese. Int J Food Microbiol 2008;124:211-216
5. Dagnew M, Moges T, Feleke M, Zinaye T. Survey of Nasal Carriage of *Staphylococcus aureus* and Intestinal Parasites among Food Handlers Working at Gondar University, Northwest Ethiopia. BMC Public Health 2012;12(1):837-844.
6. Rall VLM, Sforcin JM, Augustini VCM., Watanabe MT, Fernandes Jr A, Rall R. et al. Detection of Enterotoxin Genes of Staphylococcus spp Isolated from Nasal Cavities and Hands of Food Handlers. Brazilian J Microbiol 2010;41:59-65.
7. Lovseth A, Loncarevic S, Berdal KG. Modified Multiplex PCR Method for Detection of Pyrogenic Exotoxin Genes in Staphylococcal Isolates. J Clin Microbiol 2004;42(8):3869–3872.
8. Monday SR, Bohach GA. Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. J Clinical Microbiol 1999;37:3411–3414.
9. Aydın A, Sudagidan M, Muratoglu K. Prevalence of Staphylococcal Enterotoxins, Toxin Genes and Genetic-Relatedness of Foodborne *Staphylococcus aureus* Strains Isolated in the

Marmara Region of Turkey. Int J Food Microbiol 2011;148(2):99–106.
10. Acco M, Ferreira FS, Henriques JAP, Tondo E. C. Identification of Multiple Strains of *Staphylococcus aureus* Colonizing Nasal Mucosa of Food Handlers. Food Microbiol 2003;20:489–493
11. Loeto D, Matsheka MI, Gashe BA. Enterotoxigenic and Antibiotic Resistance Determination of *Staphylococcus aureus* Strains Isolated from Food Handlers in Gaborone, Botswana. J Food Protect 200;12:2764-2768.
12. Lynch RA, Phillips ML, Elledge BL, Hanumanthaiah S, Boatright, DT. A preliminary evaluation of the effect of glove use by food handlers in fast food restaurants. J. Food Protect 2005;68:187–190.
13. Baş M, Ersun AŞ, Kıvanç G. The Evaluation of Food Hygiene Knowledge, Attitudes, and Practices of Food Handlers in Food Businesses in Turkey. Food Control 2006;17(4):317–322.
14. Campos AKC, Cardonha AMS, Pinheiro LBG, Ferreira NR, Azevedo PRM, Stamford TLM. Assessment of Personal Hygiene and Practices of Food Handlers in Municipal Public Schools of Natal, Brazil. Food Control 2009;20(9):807-810.
15. Ho J, O'Donoghue MM, Boost MV. Occupational Exposure to Raw Meat: A Newly-Recognized Risk Factor for *Staphylococcus aureus* Nasal Colonization Amongst Food Handlers. Int J Hyg Environ Health 2014;217:347-353.
16. Alhashimi HMM, Ahmed MM, Mustafa JM. Nasal carriage of enterotoxigenic *Staphylococcus aureus* among food handlers in Kerbala city. Karbala Int.J. of Modern Science 2017;3:69-74.
17. Al Bustan MA, Udo EE, Chugh TD. Nasal Carriage of Enterotoxin Producing *Staphylococcus aureus* Among Restaurant Workers in Kuwait City. Epidemiol Infect 1996;116(3):319-322.
18. Figueroa G, Navarrete P, Caro M, Troncoso M, Faundez G. Carriage of Enterotoxigenic *Staphylococcus aureus* in Food Handlers. Rev Med Chile 2002;130(8):859-64.