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A Cross-Sectional Survey on Ready-to-Fry Falafel Regarding Its Potential to be Host for *Escherichia coli*, *Staphylococcus aureus*, and *sea*, stx_1, stx_2 Genes Responsible for Toxin-Origin Food Poisoning

Mina BALOUCHI[1], Mohamad RAHNAMA[2], Majid ALIPOUR-ESKANDANI[2], Saeed SALARI[3], Dariush SAADATI[2]

[1]University of Zabol Faculty of Veterinary Medicine, Zabol, Iran
[2]Department of Food Hygiene, University of Zabol Faculty of Veterinary Medicine, Zabol, Iran
[3]Department of Pathobiology, University of Zabol Faculty of Veterinary Medicine, Zabol, Iran

Cite this article as: Balouchi, M., Rahnama, M., Alipour-Eskandani, M., Salari, S., & Saadati, D. (2023). A cross-sectional survey on ready-to-fry falafel regarding its potential to be host for *Escherichia coli, Staphylococcus aureus, and sea, stx1, stx2* genes responsible for toxin-origin food poisoning. [published Early View Article, 2023 December 19] *Acta Veterinaria Eurasia*, 10.5152/actavet.2023.23040, DOI: 10.5152/actavet.2023.23040.

Abstract

Information about the putative foodborne bacteria in falafel is scanty, at least in Iran. We aimed to evaluate the frequency of *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* and the putative genes responsible for toxin-origin food poisoning, including encoding gene of Shiga toxin type 1 protein (stx_1) and Shiga toxin type 2 protein (stx_2) of *E. coli* and encoding gene of staphylococcal enterotoxin A (*sea*) of *S. aureus* in ready-to-fry falafel (RTF) collected from the southeast of Iran. The frequency of stx_1 and stx_2 in *E. coli* and *sea* in *S. aureus* isolates were evaluated by polymerase chain reaction after the isolation of *E. coli* and *S. aureus* using routine procedures in 65 samples of RTF. The prevalence of contamination by *E. coli*, *S. aureus*, and both bacteria was 56.9%, 44.6%, and 32.3%, respectively ($p > .05$). The genes stx_1, stx_2, and both stx_1 and stx_2 were present in 43.2%, 27%, and 13.5% of the falafel containing *E. coli*, respectively ($p > .05$). A significant statistical correlation was found between the presence of stx_1 or stx_2 and the presence of *E. coli* ($p < .05$). The *sea* gene was remarkably found in 44.8% of the falafel containing *S. aureus* ($p < .05$). The RTF can host the dangerous levels of *E. coli* and *S. aureus*, containing stx_1, stx_2, and *sea* genes, accentuating an alarming signal for human consumption and public health.

Keywords: *E. coli*, Iran, PCR, ready-to-fry falafel, *S. aureus*

Introduction

Over the past several decades, fast foods have gained a progressive trend of popularity among consumers in all parts of the world, both in developed and in developing countries. As a result of various changes to lifestyle, the dietary habits of people—mainly teenagers and young adults—have shifted toward fast foods (Askari Majabadi et al., 2016). On average, young adults and middle-aged adults in Tehran eat 161 and 108 g of fast food per week, respectively (Bahadoran et al., 2015). For these reasons, the global tendency to eat fast foods has been associated with increased concerns (Bahadoran et al., 2015) and bacterial contamination by *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) are among the primary concerns (Ananchaipattana et al., 2012). Instructive schedules can be employed, and practical policies can be developed to reclaim the quality of the dietary habits in adolescents and young adults (Akbari & Azadbakht, 2014). Eating food outdoors can be highly susceptible to health risks, especially through bacterial contamination. The first approach to prevent and control of foodborne diseases, effectively, is monitoring through the identification of pathogenic organisms (Harakeh et al., 2005).

Falafel is generally made with fava, chickpeas, or a combination of the two, based on the geographical location of its cooking. The dried legumes are primarily soaked in water, ground, mixed with spices, shaped into small balls, and deep fried (Raviv, 2003). It is a popular snack and has become a common meal in the lives of many individuals (Ismail & Kucukoner, 2017; Raviv, 2003). It can be found in almost every fast-food supermarket and modern fast-food chains. Falafel is a quick meal, with quick no-frills meal, which makes it an affordable and satisfactory dish. Falafels are prepared, cooked, and consumed regularly in falafel stands on the streets of Sistan and Baluchistan province, Iran.

Diarrheal diseases are a big cause of acute illness due to foodborne or waterborne microbial pathogens, sometimes amounting to cases of death in developing countries. The European Food Safety Authority (EFSA) report shows that in 2010, 5262 foodborne outbreaks (FBOs) were reported in Europe (Bianchi et al., 2013). In addition, the World Health Organization estimates the annual burden of foodborne illness to number 600 million cases globally; of this, diarrheal illness accounts for the largest proportion of these cases and results in 230,000 deaths (WHO, 2015).

One relevant bacterium is Shiga toxin-producing *E. coli* (STEC), a pathotype of Enterohaemorrhagic *E. coli*. Shiga toxin-producing *E. coli* pathotypes are among the strains of *E. coli* that cause diarrhea and stand out distinctly because of their ability to cause severe

Corresponding Author: Saeed SALARI • **E-mail:** saeedsalari@uoz.ac.ir
Received: July 8, 2023 • **Accepted:** October 10, 2023 • **Available Online Date:** December 19, 2023 • DOI: 10.5152/actavet.2023.23040
Available online at actavet.org

symptoms of illness (Xing et al., 2014). Shiga toxin-producing *E. coli* is extensively associated with outbreaks of foodborne illness. Shiga toxin type 1 (STX_1) and type 2 (STX_2) are virulence factors encoded by stx_1 and stx_2. Produced by the STEC pathotype, STX_1 and STX_2 are the causes of bloody diarrhea and the hemolytic uremic syndrome (Balagué et al., 2006). These toxins can be transmitted to the central nervous system (CNS) through the bloodstream, thereby inflicting harm on the CNS (Hunt, 2010).

Staphylococcus aureus is a widespread cause of malign infections and continues to be a significant pathogenic species of bacteria (Demirci et al., 2017). If taken into the gastrointestinal tract, it can lead to painful symptoms such as abdominal cramps, vomiting, nausea, and diarrhea (Demirci et al., 2017). Contamination of food is frequently the result of contamination with pathogenic bacteria, some of which can cause illness through the production of enterotoxins. These foodborne diseases often present with self-limiting gastroenteritis symptoms, i.e., nausea, vomiting, diarrhea, abdominal cramps, and fever, but severe complications can also arise, such as kidney and liver failure, brain and neural disorders, and paralysis, which can have fatal consequences (Walker-York-Moore et al., 2017). Various strains of *S. aureus* can produce one or more toxins, including staphylococcal enterotoxins (SE), exfoliative toxins, toxic shock syndrome toxin-1, the SE-like proteins, and leukocidin. The EFSA report shows that in 2010, SE were responsible for 274 FBOs in Europe (Bianchi et al., 2013). The SE are heat-stable enterotoxins comprised of various classes such as SEA to SEE, SEG to SEI, and SEK to SET, of which SEA, SEB, SEC, SED, and SEE are considered classical SE (Argudín et al., 2010; Walker-York-Moore et al., 2017). According to previous researches, classical SE usually causes about 95% of the outbreaks in relation to staphylococcal food poisoning (Le et al., 2021). The class SEA, encoded by *sea,* is known to cause food poisoning frequently (Pinchuk et al., 2010). *sea* was found in 14.3% of *S. aureus* isolates of sheep and goat dairy products in southern Italy (Basanisi et al., 2016). The *sea* gene was found in 10% of *S. aureus* isolates recovered from cow's milk originated from cows with positive California Mastitis Test and subclinical mastitis in Iran (Khoramrooz et al., 2016). Respectively, genes encoding *sea* was detected in 45.2% (95/206) and 47.6% (30/63) of the *S. aureus* isolates of fishery products in Iran (Arfatahery et al., 2016) and shrimp samples in China, respectively (Dai et al., 2023). Ertas Onmaz et al. (2015) investigated the presence of *sea* gene using multiplex polymerase chain reaction (PCR) in 100 fish samples, and none of the *S. aureus* isolates had *sea* gene. This research was performed in Sistan, a district that is situated in the south of Sistan and Baluchistan province in southern Iran (Latitude: 31°01′50″ N; Longitude: 61°29′41″ E). It has a population of nearly 329,317 individuals, among whom the falafel is a popular fast food. Falafel stands are a frequent site on outdoor markets where foodstuffs are sold by individual vendors. Falafels are generally available on falafel stands where pieces of paper are used for wrapping them up at ambient temperatures. In general, other fast foods may also be sold by the same vendors owning falafel stands. These small businesses and their food are prone to microbial contamination. However, there have been little details on the prevalence of foodborne pathogens in falafels that are sold by these vendors.

Information about the specifications of contamination in falafel, in particular bacterial contamination, is scanty, at least in Iran. A relevant study carried out tests against microbial contamination in ready-to-fry Samboosa and ready-to-fry falafel in Hamedan, West Iran (Kahledian et al., 2020). A previous study in Ahvaz, Southwest Iran, aimed at screening Samboosa for the contamination rate of *S. aureus*. The samples were collected in various seasons and from different part of Ahvaz (Fazlara et al., 2005). So far, there have been little amounts of data with reference to the molecular delineation of pathogenic bacterial strains in falafel (in both forms of ready-to-fry and ready-to-eat). One study in Hamedan investigated the prevalence of *sea* in *S. aureus* isolates of falafel alone or in combination with other enterotoxin-encoding genes (Kahledian et al., 2020), while no study was found to investigate the prevalence of stx_1 and stx_2 in bacterial isolates of falafel.

The objective was to assess the prevalence of *E. coli* and *S. aureus* in ready-to-fry falafels and then determine the presence of stx_1 and stx_2 in *E. coli* and the presence of *sea* in *S. aureus*.

Materials and Methods

It is a descriptive, cross-sectional investigation. Ethical approval and/or consent form has not been obtained because this research does not involve animals/human participants.

Sample Size Estimation and Sample Collection

The sample size was calculated based on the assumption that the prevalence of *E. coli*, the prevalence of *S. aureus*, the prevalence of *sea* in *S. aureus*, the prevalence of stx_1 in *E. coli*, and the prevalence of stx_2 in *E. coli* have standard deviations of 50%, separately. The sample size was determined using margins of error (i.e. 15%), paralleled with a CI of 95%. The minimum sample size had to be 45 (Charan & Biswas, 2013). Randomly, 65 ready-to-fry falafels were collected from falafel stands in various parts of Sistan (31°0'N –61°2'E), Sistan and Baluchistan Province, southeast Iran, from November 2017 to March 2018. Random numbers of samples were taken in each falafel stands. Beside ice, the samples were immediately transferred to the Laboratory of Microbiology, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran, for relevant analyses.

Bacterial Isolation

According to previous methods (Quinn et al., 2002; International Organization for Standardization (ISO), 2015, 2021), homogenizing a 25 g sample was assisted by 225 mL of sterile peptone water broth (Merck, München, Germany). After incubation of the mixtures (37°C for 24 hours), as for the isolation of *E. coli* and *S. aureus,* one loopful of broth was streaked onto Eosin methylene blue (EMB) and Baired Parker agars (Merck), respectively, and incubated. The *E. coli* colonies in EMB agar were confirmed by Triple Sugar Iron (TSI) and Indole, Methyl Red, Voges-Proskauer, and Citrate (IMViC) tests. As for *S. aureus*, the colonies formed on Baired Parker agar supplemented with egg yolk tellurite emulsion (Ibersco, Iran) were confirmed by catalase, coagulase, and gram staining tests, besides the mannitol salt agar (Ibersco, Iran) test. The confirmed isolates were stored at –80°C for molecular analysis.

Molecular Analysis

In this study, the main enterotoxin gene of *S. aureus* (i.e., *sea*) was targeted in *S. aureus* isolates. The evaluation involved the use of primers and certain programs through PCR (Table 1). In addition, a duplex-PCR was run to test the prevalence of stx_1 and stx_2 genes in *E. coli* isolates (Table 1).

Extracting the DNA from the isolate was performed according to the boiling method (Reischl et al., 2000). Further procedures involved a

Table 1.

Oligonucleotide Primers and Protocols Used in Polymerase Chain Reaction to Identify sea *in* Staphylococcus aureus *and in Duplex Polymerase Chain Reaction to Identify* stx_1 *and* stx_2 *in* Escherichia coli *Isolated from Falafel*

Gene (Product Size)	Oligonucleotide Primers (5′–3′)	PCR (µL)	PCR Program (35 Cycles)[a]	Reference
sea (102 bp)	Forward: GGTTATCAATGTGCGGGTGG	13 µL 2× master mix-red (Pishgam, Iran); 1 µL forward primer (10 pmol, Pishgam, Iran); 1 µL reverse primer (10 pmol, Pishgam, Iran); 3 µL DNA template; 7 µL DDW	94°C, 120 seconds; 57°C, 35 seconds; 72°C, 35 seconds	(Mehrotra et al., 2000, Sharma et al., 2017)
	Reverse: CGGCACTTTTTTCTCTTCGG			
stx_1 (180 bp)	Forward: ATAAATCGCCATTCGTTGACTAC	12.5 µL 2× master mix-red (Pishgam, Iran); 1 µL of each forward primer (10 pmol, Pishgam, Iran); 1 µL of each reverse primer (10 pmol, Pishgam, Iran); 5.5 µL DNA template; 5 µL DDW	94°C, 60 seconds; 55°C, 30 seconds; 72°C, 60 seconds	(Paton & Paton, 1998)
	Reverse: AGAACGCCCACTGAGATCATC			
stx_2 (255 bp)	Forward: GGCACTGTCTGAAACTGCTCC			
	Reverse: TCGCCAGTTATCTGACATTCTG			

[a]PCR amplification was carried out by an initial denaturation at 94°C for 5 minutes and a final extension step of 72°C for 10 minutes. DNA, deoxyribonucleic acid; DDW, Double Distilled Water; PCR, polymerase chain reaction; stx_1, encoding gene for shiga toxin type 1 protein; stx_2, encoding gene for shiga toxin type 2 protein.

gradient Eppendorf's Master Cycler® Pro (Eppendorf, Germany) for carrying out the amplifications. Then, the PCR products were run with 1.5% (wt/vol) agarose gel through electrophoresis in 1x Tris-borate EDTA buffer. The ultraviolet trans-illuminator (Cambridge, USA) facilitated the visualization of the resultant products.

Control strains harboring genes were from archived bacteria of Laboratory of Microbiology, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran.

Statistical Analysis

The chi-square (one dimensional) test was used to reveal the statistically significant differences among the prevalence of contamination by *E. coli, S. aureus,* and both bacteria in ready-to-fry falafel. In addition, the correlation between the presence of either stx_1 or stx_2 genes and *E. coli* isolates of falafel and correlation between the presence of *sea* gene and *S. aureus* isolates of falafel were statistically tested using chi square test (two dimensional). Also, one dimensional chi square test was used for evaluating how different the samples were in terms of the presence of genes (i. e., stx_1 vs. stx_2) in *E. coli* isolates of ready-to-fry falafel. Each difference was considered as statistically significant if the *p*-value became <.05. The 95% CI for the contamination rates were estimated by the bootstrap method.

Results

As can be seen in Table 2, out of the total 65 samples of the ready-to-fry falafel, 37 samples (56.9%) were identified with *E. coli*, 29 samples (44.6%) were with *S. aureus,* and 21 samples (32.3%) had both bacteria. Statistical analyses showed no significant differences among the prevalence of contamination by *E. coli*, *S. aureus,* or both bacteria ($p_B = .065$), although the concurrent occurrence of both bacteria was more prevalent. The *sea* gene was remarkably found in 44.8% (13/29) of the falafel containing *S. aureus*. This result showed a correlation between the presence of *sea* gene and *S. aureus* isolates of falafel ($p_S = .046$). Respectively, stx_1, stx_2, and both stx_1 and stx_2 were

Table 2.

*Frequency (%) of Bacterial Species and Genes Investigated in Falafel Samples (*n = *65)*

Bacterial Species	Frequency	Genes	Frequency	Statistical Analysis
Escherichia coli$^+$	37/65 (56.9%)	stx_1^+	16/37 (43.2%)	$\chi^2 = 16.062$; $df = 1$; $p_1 = .000$
		stx_2+	10/37 (27%)	$\chi^2 = 8.943$; $df = 1$; $p_2 = .002$
		stx_1^+ and stx_2^+	5/37 (13.5%)	$\chi^2 = 4.099$; $df = 1$; $p_{1\&2} = .053$
			$\chi^2 = 4.104$; $df = 1$; $p_{st} = 0.057$	
Staphylococcus aureus$^+$	29/65 (44.6%)	*sea*$^+$	13/29 (44.8%)	$\chi^2 = 3.985$; $df = 1$; $p_s = .046$
Escherichia coli$^+$ *and Staphylococcus aureus*$^+$	21/65 (32.3%)			
Statistical analysis	$\chi^2 = 3.097$; $df = 1$; $p_B = .065$			

p_1, p_2, and $p_{1\&2}$ show the statistical result testing hypothetical correlation between the presence of *Escherichia coli* and the presence of stx_1, stx_2, and $stx_1 + stx_2$, respectively.
p_{st} shows the statistical result testing hypothetical difference among the presence of stx_1, stx_2, and $stx_1 + stx_2$ in *E. coli* isolates recovered from falafel.
p_s shows the statistical result testing hypothetical correlation between the presence of *Staphylococcus aureus* and the presence of *sea* in *S. aureus* isolates recovered from falafel.
p_8 shows the statistical result testing hypothetical difference between the prevalence of *E. coli*, *S. aureus,* and both bacteria isolated from falafel.
stx_1, encoding gene for shiga toxin type 1 protein; stx_2, encoding gene for shiga toxin type 2 protein.

present in 43.2% (16/37), 27% (10/37), and 13.5% (5/37) of the falafel containing *E. coli*. Our findings show a correlation between the presence of either stx_1 or stx_2 genes and *E. coli* isolates of falafel ($p_1 = .000$; $p_2 = .002$). In addition, albeit stx_1 was more prevalent; the difference between the prevalence of stx_1, stx_2, and $stx_1 + stx_2$ was not statistically significant ($p_{st} = .057$).

Discussion

Different kinds of food can serve as suitable media for the growth of bacteria (Ananchaipattana et al., 2012). Kahledian et al. (2020) reported that out of the 121 samboosa and falafel samples in Hamedan, West Iran, 57 samples (47.5%) were contaminated with *S. aureus*. Compared to the present work in which 44.6% of the samples were contaminated with *S. aureus*, falafel is likely to stage a higher prevalence of *S. aureus* due to the higher number of positive samples of falafel in the present research. What is obvious, however, is that the similarity between the composition of samboosa and falafel correspond with the appropriateness of substances necessary for the growth of *S. aureus* (Ismail & Kucukoner, 2017; Raviv, 2003). The contamination of samboosa with *S. aureus* reportedly by Fazlara et al. (2005) stood at 13.7%, which is quite less than the corresponding result reported herein (44.6%). The difference could be explained by the possible variations in the composition of falafel in comparison with that of Samboosa. In other words, falafel may have more nutritious substances for the growth of *S. aureus* (Kahledian et al., 2020). Discovering the details of such differences would require deeper evaluations. Nonetheless, one definite reason behind this difference is the various conditions in which food hygiene is procured or otherwise neglected. We cannot find any legal regulation in Iran, regarding *S. aureus* and its enterotoxin in falafel. However, based on our literature review, the Food and Environmental Hygiene Department, China, established that the acceptable level of *S. aureus* in ready-to-eat food should be below 10^3 colony-forming units per gram (cfu/g) of food. If the amount of bacteria is greater than 10^4 cfu/g, the food is unsatisfactory and potentially hazardous for health and/or unfit for human consumption (Center for Food Safety (CFS), 2014b). Ingestion of nanogram to a microgram of staphylococcal enterotoxin contaminated food can cause serious illness ranging from minor skin infection to life-threatening diseases (Center for Food Safety (CFS), 2014b; Tsehayneh et al., 2021). The abovementioned limits could be a suggestion to test for next-step research, in particular for the present study area (i.e., Sistan) and study population (i.e., falafel). Many reports have emphasized the significant role of food handlers in distributing contamination. Poor personal hygiene is a leading cause by which bacterial diseases can spread (Dai et al., 2023; Nasrolahei et al., 2017; Onlen et al., 2017; Tsehayneh et al., 2021; Walker-York-Moore et al., 2017). A set of findings in Sudan revealed that nasal *S. aureus* is the most common pathogen being detected on nasal swabs and stool specimens collected from food handlers (Saeed & Hamid, 2010). The high prevalence of *S. aureus*, especially in the current research (44.6%), is based on the fact that this bacterial species resides naturally on the skin or may origin from nasal region to colonize on the skin of food handlers. Accordingly, the bacteria could be carried by healthy individuals too and be the cause of preliminary problems by going through the anterior nostrils of their noses (Quinn et al., 2002). A straight way of making the food contaminated with *S. aureus* happens when food handlers or vendors sneeze or cough on the edible material during food preparation. Matters become worse when the hands are not washed properly after touching the nose or when handling unclean money, either in the form of coins or notes, when customers pay for the price of food and the food handler has to handle the food together with the money for it (Nasrolahei et al., 2017). Regarding the Center for Food Safety (CFS) (2014a), *E. coli* amount greater than 10^2 cfu/g is harmful for human health. Although the colony count is necessary for more precise decision-making, the present research revealed that ready-to-fry falafels can be dangerously contaminated with *E. coli,* which renders the food unsuitable for human consumption (Center for Food Safety (CFS) 2014a). Previous data suggested that the fingernail samples of food handlers were colonized with *S. aureus*, followed by *E. coli* (Nasrolahei et al., 2017). The presence of a high rate of contamination by both *S. aureus* and *E. coli* (32.3%) in the present study can be seen in close connection with the previous report on food handlers' fingernails. Moreover, based on our results, the prevalence of contamination by *E. coli* (56.9%) was observed to be more than that of *S. aureus* (44.6%). It should be noted that the higher prevalence of *E. coli* than *S. aureus*, but not statistically significant, as obtained by the present study, might have been influenced by variations in the recipe of ready-to-fry falafel, the season of sampling, the ingredients of fast food (Ismail & Kucukoner, 2017; Nasrolahei et al., 2017), the state of sanitation inside the falafel stands, and the likelihood of contamination during the course of food preparation (Fazlara et al., 2005; Kahledian et al., 2020; Nasrolahei et al., 2017).

Our findings demonstrated that 44.8% of the isolates were confirmed to have borne the *sea* gene. As it occurred, the high presence of stx_1 in the isolates of *E. coli* (43.2%), compared to the presence of stx_2 in the same isolates (27%), would deepen public health concerns over food poisoning. Based on the literature review, the present work has the first record of stx_1 and stx_2 in falafel. Nonetheless, these findings cannot be compared comprehensively with previous reports on other foods due to the particularized differences between the various kinds of experimental research. However, the prevalence of stx_1 is reportedly higher than that of stx_2 in *E.coli* (Behzadian Nezhad et al., 2011; Dastmalchi & Ayremlou, 2012; Tahamtan et al., 2010), which comes in line with our data herein. Tahamtan et al. (2010) collected 420 samples consisting of recto-anal mucosal swabs from cattle to check for the presence of the stx_1 and stx_2 gene using multiplex-PCR every 1 week over a 1-year period (2007–2008). Out of total 146 strains of *E. coli*, 66, 129, and 51 strains contained the stx_1, stx_2 and $stx_1 + stx_2$ genes. Behzadian Nezhad et al. (2011) evaluated the prevalence of stx_1 and stx_2 virulence genes in non-O157 *E. coli* isolated from cattle stool samples. Out of total 39 non-O157 *E. coli*, 10 samples were found positive for stx_1 or stx_2 genes. Dastmalchi and Ayremlou (2012) screened a total of 124 *E. coli* isolates from clinically healthy and diarrheal calves belonging to six different farms located in West Azerbaijan province, Iran. Their PCR results showed that 6 (23.1%) isolates carried stx_1 gene, 7 (26.92%) possessed stx_2 gene, while 13 isolates (50%) gave positive amplicon both for stx_1 and stx_2 genes. Despite the fact that stx_2 is a more important virulence factor than stx_1, which is associated with human diseases and/or animal studies (Onlen et al., 2017), notably, some studies, except Onlen et al., (2017), have reported that the frequency of stx_1 is more than stx_2 in food-origin *E. coli*, which seems to be consistent with our results (Binandeh et al., 2020; Pavithra & Ghosh, 2013). Pavithra & Ghosh (2013) examined a total of 215 samples collected from fast foods, swabs from meat shops and fish markets, and nine (25%) *E. coli* possessed stx_{11}. Binandeh et al. (2020) identified that in non-O157:H7 *E. coli* isolated

from meat samples (beef and mutton), five (27.8%) isolates possessed stx_1 in Hamedan between 2015 and 2016. Onlen et al., (2017) examined a total of 327 salad samples for the frequency of stx_1 and stx_1 of foodborne *E. coli* O157:H7 in Hatay province, Turkey. Incidence of stx_1, stx_2, and both stx_1 and stx_2 in *E.coli* O157:H7 isolates were 6.3%, 15.6%, and 3.1%, respectively. Regarding *sea* investigation, the production of SE is seen to occur profoundly in foods that are high in protein and starch (Demirci et al., 2017). Our results are indicative of the increased likelihood that food poisoning appears commonly among the consumers of ready-to-eat falafel. Notably, only PCR of a toxin-producing gene does not clarify the toxin production; in vitro toxin analysis by enzyme-linked immunosorbent assay is necessary (Ge et al., 2002). The texture of ready-to-fry falafel may indicate the prevalence of the *sea* gene among the isolates. In this research, there was a higher prevalence of *sea* in the isolates of *S. aureus* in ready-to-fry falafels of the studied area compared to similar cases reported by Kahledian et al. (2020) where the prevalence of *sea* was 15% in raw falafel samples collected from licensed food vendors in Hamedan, Iran, and Xing et al. (2014) where the prevalence of *sea* was 7.8% in ready-to-eat foods collected from Shaanxi, China, indicating the horizontal gene transfer among isolates. Furthermore, this could be vindicated by the fact that bacterial colony numbers are reduced after frying the cooking foods. The potential health implications of consuming ready-to-fry falafel contaminated with toxin-producing bacteria (i.e., *S. aureus*) is highlighted when the capacity of *S. aureus* to grow at relatively low water activity ($a_w = 0.86$) is considered. As a result, since ready-to-eat falafel is a salted fast food and in turn provides a low water medium compared to ready-to-fry falafel, staphylococcal intoxication of the falafel with *S. aureus* originated from ready-to-fry falafel may be hypothetically increased (Argudín et al., 2010; Walker-York-Moore et al., 2017). Moreover, heat-stable enterotoxin (i.e., SEA) may resist after frying, emphasizing the potential health implications of consuming ready-to-fry falafel (Walker-York-Moore et al., 2017).

Variables that cause differences in the degree of bacterial infestation include the educational level of individuals, their different working classes, continued training and guidance on well-being, the vendors' knowledge on food safety, the availability of water to wash cooking utensils and hands, the accessibility to proper toilet facilities, and adequate levels of hygiene in the workplace (Nasrolahei et al., 2017). Individuals who come from lower socio-economic classes (and whose income is relatively low) are the people who become vendors at falafel stands in the studied area. When taken together with the absence of safe water supplies, poor environmental sanitation, and bad personal hygiene, an overall unawareness of health issues exacerbates the occurrence of pathogens in food. The prolonged, compact connection between vendors and consumers contributes to the high prevalence of the bacteria tested herein.

This is the case in Sistan where it is difficult to trace the source of infection of people affected and even more difficult to trace the source of contamination of the foods involved, partly due to limited capacity at district and provincial levels and the vast geographical areas that national food safety institutions have to cover. In such cases, studies such as the present one will provide the much-needed qualitative and quantitative data to aid strain monitoring and guide preventative measures for future possible outbreaks. Our findings provide necessary information to better understand and monitor *S. aureus* associated with food poisoning in Sistan.

Conclusion

Ready-to-fry falafel of study area can host the dangerous levels of contamination with *E. coli* and *S. aureus*, the two species of putative foodborne bacteria, containing *sea*, stx_1, and stx_2, the genes responsible for toxin-origin food poisoning. Our results call for strong requirements for serious training and education on hygiene for vendors. Several initiatives would need to enable action on behalf of microbiologists and food specialists, i.e., their monitoring of hygiene, coupled with the reinforcement of necessary requirements to that end. This work provides additional proof against the consumption of unreliable fast foods, especially falafel, owing to the irrecoverable impacts that these could have on public health in the studied region, particularly with regard to the increasing burden of microbial contamination and food poisoning in developing nations such as Iran.

Availability of Data and Materials: The data presented in this study are openly available in Mendeley at DOI: 10.17632/232c7nbgtx.1.

Ethics Committee Approval: This study does not involve any human or animal testing.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.R., M.A.E., S.S.; Design – M.R., S.S.; Supervision – M.R.; Resources – M.B., S.S.; Materials – M.B., S.S.; Data Collection and/or Processing – D.S., M.B.; Analysis and/or Interpretation – D.S., S.S.; Literature Search – M.B.; Writing Manuscript – M.R., S.S.; Critical Review – M.R., M.A.E., S.S.

Acknowledgements: Authors are grateful to Mr. Saeed Shahriari and Mis Sargolzaee for their technical assistance. This study was funded by University of Zabol (grant No. IR/UOZ/GR/1180). This study was carried out in partial fulfillment of the requirements for the MSc degree in food hygiene and quality control science student's thesis (MB).

Declaration of Interests: The authors declare that they have no conflict of interest.

Funding: This study was funded by University of Zabol (grant No. IR/UOZ/GR/1180).

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