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Molecular Characterization of Methicillin-Resistant Enterotoxin-Producing *Staphylococcus aureus* Isolated From Samosa and Falafel in Iran

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Abstract

Objective: This study aimed to determine the contamination rate of *Staphylococcus aureus* in Samosa and falafel as most popular snacks, detect the classic enterotoxins, *mecA*, and *tst* genes and investigate antimicrobial resistance in the isolates.

Materials and Methods: The samples were examined using bacterial culture and the suspected isolates were characterized by biochemical tests. The identity of *S. aureus* isolates and the presence of enterotoxin-encoding genes were assessed using a multiplex polymerase chain reaction (PCR) assay and antibiotic resistance of the isolates was determined.

Results: The results revealed that 56 (46.67%) samples were contaminated with *S. aureus*, among which 45 isolates (80.35%) were characterized as enterotoxigenic *S. aureus*. The highest prevalence rate belonged to *sea* encoding gene as 20 isolates (35.71%) were positive for this gene followed by *sed* gene which was detected in 14 *S. aureus* isolates (25%). Most isolates (75%) were resistant to cefoxitin. Moreover, the results of PCR assays indicated that 10 (17.58%) and 7 (12.5%) isolates were positive for *mecA* and *tst* genes, respectively.

Conclusion: The results of the present study demonstrated that staphylococcal contamination of Samosa and falafel should be considered as a potential health risk for consumers.

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Background

Samosa and falafel are the among most popular snacks widely consumed in many Asian and Middle East countries including Iran.[1] However, as these foods are generally produced and served by hand, there is a high risk of contamination with food-borne pathogens. Among the pathogens, *Staphylococcus aureus* is considered an important bacterium as it is a common organism of the natural microflora of human skin, nasal passage, hair, and mucous membranes. The lack of personal hygiene in food handlers and equipment can increase the risk of transmission of this pathogen to foods.[2]

Staphylococcus aureus is a bacterium with various virulence factors including hemolysins, nuclease, coagulase, leukocidins, enterotoxins, toxic shock syndrome toxin-1 (TSST-1), and exfoliative toxin which may also show high frequency of antibiotic resistance.[3,4] For this reason, it has a high potential to cause diseases in humans. This bacterium is able to produce different enterotoxins designated as classic staphylococcal enterotoxins A to E. Staphylococcal enterotoxins as the main causatives agents of food poisoning are single chain, low molecular mass, highly thermo-stable and resistant proteins to most proteolytic enzymes and different environmental conditions.[5-10]

The emergence of antibiotic-resistant pathogenic bacteria is a major public health concern worldwide. In the past decade, *S. aureus* strains isolated from foods have shown high resistance to various antibiotics. The strains of this bacterium which are resistant to the beta-lactam antibiotics are known as methicillin-resistant *S. aureus* (MRSA).[11,12] Methicillin resistance in *S. aureus* is mediated through an altered protein called penicillin binding protein (PBP2a). PBP2a is encoded by *mecA* gene which is present in chromosomal mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*).[13] The MRSA strains isolated from different sources are resistant to a wide range of antibiotics such as tetracycline, erythromycin, aminoglycosides, and fluoroquinolones.[14] In recent studies, vancomycin and oxacillin are regarded as the drug of choice for the treatment of infections caused by MRSA strains.[15,16]

The isolation of MRSA strains has been reported from various foods including poultry, pork, beef, milk, and

vegetables, suggesting that foods may serve as sources for staphylococcal food poisoning.[17] Consequently, the present study aimed to investigate the contamination rate of raw samosa and falafel samples, as two of the most commonly used snacks, with enterotoxin-producing *S. aureus* strains for the first time in Iran with emphasis on the detection of MRSA strains.

Materials and Methods

Sample Collection

This descriptive cross-sectional study was carried out from 2015 to 2016 in Hamedan province, western Iran. Totally, 120 samples including 60 samosa and 60 falafel samples were collected in sterile zip-lock bags from licensed food vendors in Hamedan and transferred on ice to the Laboratory of Food Safety, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran.

Bacterial Culture

Culturing on Baird-Parker Agar Medium

Isolation of *S. aureus* was conducted according to ISO standard 6888-1.[18] Briefly, 25 g of each homogenized sample of samosa and falafel was diluted in 225 mL peptone water (0.1%) in a sterile zip-lock bag. Then, 0.1 mL of each dilution was spread in duplicate on Baird-Parker agar medium (Merck, Germany) containing 0.01% potassium tellurite and egg yolk emulsion and incubated for 24 to 48 hours at 37°C. Thereafter, black colonies with bright halos were considered as suspected *S. aureus* isolates.

Biochemical Tests

Suspected *S. aureus* strains were further examined using conventional microbiological assays including Gram staining, coagulase, catalase, DNase, and oxidase tests, and culturing on mannitol salt agar medium. Finally, bacterial strains confirmed as *S. aureus* using phenotypic methods were stored at -20°C until molecular analysis.

Determination of Antibiotic Resistance of Isolated Strains

Antibiotic susceptibility profiles of the isolates were determined according to the instruction of Clinical and Laboratory Standards Institute (CLSI), using disk diffusion method[19] with commercial antibiotic disks (Padtan Teb, Iran). The resistance of the isolates was assessed against 11 antibiotic disks including tetracycline (30 µg), gentamycin (10 µg), oxacillin (1 µg), vancomycin (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), penicillin (10 µg), lincomycin (2 µg), streptomycin (10 µg), and cefoxitin (5 µg).

DNA Extraction and Multiplex PCR

The extraction of genomic DNA from isolates was performed according to a previously described protocol.[20] Briefly, the isolates were cultured in nutrient broth for 18 hours followed by centrifugation at 8000 rpm for 3 minutes. Then, 200 µL of lysis buffer containing 1% Triton X-100, 0.5% Tween 20, 10 mM Tris-HCl, and 1 mM EDTA, with pH=8.0, was added to the pellets and the suspensions were incubated in a water bath (100°C) for 10 minutes. After centrifugation at 10 000 rpm for 2 minutes, the supernatants were transferred into the sterile microtubes and stored at –20°C until further analyses.

To confirm the identity of S. aureus strains, a multiplex Polymerase chain reaction (PCR) assay was conducted to simultaneously detect *femA* (*S. aureus* species-specific gene), *mecA*, and *tst* genes as well as encoding genes of classic enterotoxins as described previously.[21] Primer sequences and expected size of the multiplex PCR products are given in Table 1.

The multiplex PCR reactions contained 5 µL of extracted DNAs, 10 µL of a commercial PCR master mix (Ampliqon, Denmark), 0.75 µL of each of the primers (50 pmol), and H2O in a volume of 25 µL. The PCR amplification was performed under the following conditions: initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 2 minutes, annealing at 57°C for 2 minutes, extension at 72°C for 1 minute (35 cycles) and a final extension at 72°C for 7 minutes. The amplified products were detected by electrophoresis on 2.5% agarose gels stained with ethidium bromide (0.5 µg/mL). The standard strains of *S. aureus* used as positive control for the investigated genes in the PCR reactions were as follows: *S. aureus* ATCC 33591 (*femA*), ATCC 29213 (*sea*), ATCC 14458 (*seb*), ATCC 19095 (*sec*), ATCC 23235 (*sed*), ATCC 27664 (*see*), ATCC 43300 (*mecA*), and ATCC 13566 (*tst*). In addition, a sample containing no DNA was used as negative control in all PCR runs.

Table 1. Primer Sequences Used for PCR Reactions

Primer	Oligonucleotide sequence (5′-3′)	Size of Product (bp)
*fem*A-F	AAAAAAGCACATAACAAGCG	132
*fem*A-R	GATAAAGAAGAAACCAGCAG	
sea-F	GGTTATCAATGTGCGGGTGG	102
sea-R	CGGCACTTTTTTCTCTTCGG	
seb-F	GTATGGTGGGTGTAACTGAGC	164
seb-R	CCAAATAGTGACGAGTTAGG	
sec-F	AGATGAAGTAGTTGATGTGTATGG	451
sec-R	CACACTTTTAGAATCAACCG	
sed-F	CCAATAATAGGAGAAAATAAAAG	278
sed-R	ATTGGTATTTTTTTTCGTTC	
see-F	AGGTTTTTTCACAGGTCATCC	209
see-R	CTTTTTTTTCTTCGTCAATC	
*mec*A-F	ACTGCTATCCACCCTCAAAC	163
*mec*A-R	CTGGTGAAGTTGTAATCTGG	
tst-F	ACCCCTGTTCCCTTATCATC	326
tst-R	TTTTCAGTATTTGTAACGCC	

Results

Out of 120 raw samosa and falafel samples, 57 were found to be contaminated with *S. aureus* by bacteriological tests; however, using multiplex PCR, 56 of these isolates (46.7) possessed *femA* gene confirming their identity as *S. aureus* (Figure 1).

Lane M: a 100-bp DNA marker; all lanes contain a 132-bp amplicon of *fem*A which is *S. aureus* species-specific gene; lanes 1 and 2: a 326-bp amplicon of *tst* gene for the positive control and an isolate, respectively; lanes 3 and 4: a 102-bp amplicon of sea for the positive control and an isolate; lanes 5 and 6: a 164-bp amplicon of *seb* for the positive control and an isolate; lanes 7 and 8: a 451-bp amplicon of *sec* for the positive control and an isolate; lanes 9 and 10: a 278-bp amplicon of *sed* for the positive control and an isolate; lanes 11 and 12: a 209-bp amplicon of *see* for the positive control and an isolate, respectively.

The results of antibiotic susceptibility test revealed that 75% of isolates were resistant to cefoxitin while all of the isolates were susceptible to vancomycin, gentamycin, ciprofloxacin, and lincomycin (Table 2).

Among 56 *S. aureus* isolates, 45 strains (80.35%) had one or more enterotoxin genes. The frequency of each enterotoxin encoding gene is presented in Table 3. Overall, the gene encoding staphylococcal enterotoxin A (*sea*) showed the highest prevalence rate since it was present in 20 *S. aureus* isolates (35.71%) followed by *sed* gene which was detected in 14 *S. aureus* isolates (25%). Comparison of S. aureus isolated from two products revealed that the prevalence of the genes encoding enterotoxins A, C, D, and E in *S. aureus* isolates from samosa samples were more than that of falafel samples; however, the number of *S. aureus* isolates which possessed gene encoding enterotoxin B was equal in both groups.

Additionally, 10 (17.58%) and 7 (12.5%) isolates possessed *mecA* and *tst* genes, respectively. However, only 7 *mecA*-positive isolates (12.5%) were resistant to oxacillin in disk diffusion method. The prevalence of the investigated genes in *S. aureus* isolated from raw samosa and falafel samples are compared in Table 3.

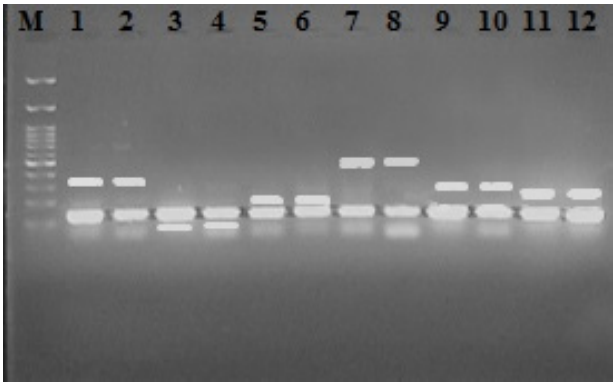

Figure 1. Gel Electrophoresis of Multiplex PCR Products.

Discussion

Staphylococcus aureus is one of the most common and important food-borne pathogens causing food poisoning worldwide. As some individuals carry this bacterium in their nose, its transmission through improper manipulation could increase food contamination.[10]

In this study, bacteriological and molecular techniques were used to determine the contamination rate of *S. aureus* in samosa and falafel in Hamedan, Iran. About 46.67% of the collected raw samosa and falafel samples were contaminated with *S. aureus*. Several studies have been performed to determine the level of *S. aureus* contamination in hand-processed foods.[22] Researchers have reported relatively high levels of contamination of ready-to-eat and street foods with *S. aureus*.[23,24] However, in some studies, low contamination rates of street foods have been reported.[25] Differences between our results and those obtained in other studies may be due to the studied food types (samosa and falafel) since poor personal hygiene habits in hand-processed foods may be associated with *S. aureus* contamination. As *S. aureus* is one of the most common and important food-borne pathogens causing food poisoning worldwide and apparently healthy individuals might carry this bacterium in their nose, its transmission through improper manipulation could increase food contamination.[10]

Table 2. Antibiotic Susceptibility of the Isolated *Staphylococcus aureus* Using Disk Diffusion Method

Antibiotics	Number (%) of *S. aureus* isolates (n=56)		
	Resistant	Intermediate	Sensitive
Tetracycline	5 (8.92)	4 (7.14)	47 (83.92)
Lincomycin	0 (0)	0 (0)	56 (100)
Ciprofloxacin	0 (0)	0 (0)	56 (100)
Oxacillin	7 (12.5)	1 (1.78)	48 (85.71)
Streptomycin	3 (5.35)	0 (0)	53 (94.64)
Penicillin	5 (8.92)	7 (12.5)	44 (78.57)
Cefoxitin	42 (75)	4 (7.14)	10 (17.85)
Vancomycin	0 (0)	0 (0)	56 (100)
Gentamycin	0 (0)	0 (0)	56 (100)

Table 3. Frequencies of Enterotoxin Encoding Genes Among *Staphylococcus aureus* Isolates

Gene profile	No. (%) of *S. aureus* Isolates		
	Samosa	Falafel	Total
femA	29 (100)	27 (100)	56 (100)
mecA	5 (17.24)	5 (18.51)	10 (17.58)
tst	4 (13.79)	3 (11.11)	7 (12.5)
sea	11 (37.93)	9 (33.33)	20 (35.71)
seb	3 (10.34)	3 (11.11)	6 (10.71)
sec	4 (13.79)	2 (9.52)	6 (10.71)
sed	8 (27.58)	6 (22.22)	14 (25)
see	7 (24.13)	5 (18.51)	12 (21.42)
sea+seb	1 (3.44)	1 (3.70)	2 (3.50)
sea+sed	2 (6.89)	-	2 (3.50)
sea+see	1 (3.44)	-	3 (5.26)
seb+sed	1 (3.44)	-	1 (1.75)
seb+see	1 (3.44)	-	1 (1.75)
sec+sed	1 (3.44)	1 (3.70)	2 (3.50)
sed+see	-	1 (3.70)	1 (1.75)

Staphylococcal food-poisoning has mostly been associated with SEA and SED.[26-28] Nevertheless, no study has been performed to evaluate the contamination of samosa and falafel with enterotoxin-producing *S. aureus* strains. The results of multiplex-PCR assay revealed that *sea* gene was the most detected gene, whereas *seb* and *sec* genes had the lowest prevalence rates among the identified genes in our study. In some studies on *sea*, it was reported as the most prevalent enterotoxin encoding gene[9,29]; however, in some other studies on *sec* and *sed* genes, prominent enterotoxin genes were found.[23,30] Differences in the distribution of enterotoxin encoding genes could be due to the ecological origins and geographical varieties of *S. aureus* isolated from foods, livestock, and humans.[31] In our study, *tst* gene was also detected in 12.5% of the samples, suggesting that contamination could occur through food handling by carriers of TSST-positive *S. aureus*. Some researchers who tried to find *tst* gene in bacteria isolated from various foods have achieved results similar to ours.[32-34] It should be considered that staphylococcal enterotoxins are generally heat stable and their presence in foods, even at very low levels, may result in food poisoning in consumers.[35] Therefore, many steps should be envisaged to prevent contamination of such food products with *S. aureus* including on-the-job training of foodstuffs, preparation of fresh raw materials on a daily basis, disinfection of vegetables, and wearing gloves and even masks, given that almost 25%-30% of people may be asymptomatic carriers of this bacterium.[36]

PCR results for *mec*A gene in *S. aureus* strains isolated from falafel and samosa samples showed that 17% of them were methicillin-resistant, which is absolutely important from the public health point of view and should be recognized as a serious health problem. In other studies carried out on hand-processed foods, the contamination rate of isolates containing *mecA* gene was higher than that of our study.[37,38] It should be noted that street food vendors are the main source of food contamination.[39]

In antibiotic susceptibility test, 75% of the isolates were resistant to cefoxitin by disk diffusion method. However, all isolates were sensitive to lincomycin, ciprofloxacin, vancomycin, and gentamycin. In most studies, *S. aureus* isolates were resistant to beta-lactam antibiotics group.[23,39] Arbitrary consumption of antibiotics, especially in street food workers, can lead to an increase in antibiotic-resistant strains.

Conclusion

In our study, samosa and falafel samples showed a high rate of staphylococcal contamination, and the majority of isolated *S. aureus* isolates possessed enterotoxin encoding genes. Besides, resistance against various antibiotics, especially methicillin, was observed among the isolates, indicating that generally such snacks may be contaminated with enterotoxin-producing MRSA strains. Hence, the result of the present study demonstrated that samosa and falafel may have a potential health risk for consumers. It is suggested that more appropriate hygiene practices should be taken in the production of such foods in order to reduce the possibility of contamination and infection and food handlers be educated on them.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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Ethical Approval

Not applicable.

Authors' Contributions

Saeid Khaledian: Investigation, Performed laboratory operations and Writing - Original Draft;
Mohamadreza Pajohi-Alamoti: Conceptualization, Methodology, Writing- Reviewing and Editing;
Pezhman Mahmoodi: Methodology, Reviewing and Editing.

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