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Detection of Gene Encoding Enterotoxin A in *Staphylococcus aureus* Isolated from Cream Pastries

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HIGHLIGHTS

- Totally, 61 out of 160 (38.1%) samples were contaminated with *Staphylococcus aureus*.
- Among 61 *S. aureus* isolates, 16 (26.2%) harbored the gene encoding enterotoxin A.
- Prevalence of *S. aureus* in the hot/dry was significantly higher than the cold/wet seasons.
- The contamination rate was not significant between puff pastry and jelly roll samples.
- Appropriate measures should be applied to reduce the level of contamination in the confectionary products.

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Acronyms and abbreviations
SEA=Staphylococcal Enterotoxin A
PCR=Polymerase Chain Reaction

ABSTRACT

Background: *Staphylococcus aureus* has always been known as an important kind of bacteria causing food poisoning. This study was carried out to determine *S. aureus* contamination in cream pastries collected from popular confectioneries in Hamedan, Iran; also, presence of the gene encoding Staphylococcal Enterotoxin A (SEA) was studied in the isolates.

Methods: During April to October 2014 (as hot and dry seasons) and October to March 2015 (as cold and wet seasons), 80 puff pastry and 80 jelly roll samples were randomly purchased from confectionary markets of Hamedan, Iran. *S. aureus* colonies were isolated using culture media and identified by biochemical tests. Polymerase Chain Reaction was used for confirming identification of *S. aureus* using *femA* gene and detection of *sea* gene encoding the SEA. The data were analyzed by SPSS version 16.0.

Results: Out of 160 confectionary samples, 61 (38.1%) were contaminated with *S. aureus*. Among 61 *S. aureus* isolates, 16 (26.2%) contained the *sea* gene. Prevalence rates of *S. aureus* in the hot/dry and cold/wet seasons were 43.7% (35 out of 80) and 32.5% (26 out of 80), respectively showing significant difference ($p<0.05$). However, the contamination rate was not statistically significant between puff pastry and jelly roll samples ($p>0.05$).

Conclusion: Our results indicated a considerable level of contamination in puff pastry and jelly roll samples with enterotoxigenic *S. aureus*, which could be due to microbial contamination of raw materials such as cream and utensils used for pastry production and lack of personal hygiene of workers. Consequently, appropriate measures should be applied to reduce the level of contamination in the confectionary products in order to ensure public health safety.

Introduction

Among various types of food, cream pastries have high potentials for being contaminated by different pathogenic bacteria such as *Staphylococcus aureus* (Charlebois, 2002; Peles et al., 2007). A variety of

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Staphylococcus species may play role in food poisoning; however, *S. aureus* has always been known as an important kind of bacteria causing food poisoning. Since this bacterium exists in many raw ingredients, there is high risk of poisoning if the foods are not properly produced and maintained (Hetzel et al., 2004; Jomehpour et al., 2016). Some of the *Staphylococcus* strains create a kind of poison called enterotoxin, causing gastroenteritis in humans. Enterotoxin does not affect foods' taste, odor, and appearance; and it resists heat (Murray et al., 1994; Omoe et al., 2002; Orwin et al., 2003). The main *S. aureus* enterotoxins are included A, B, C, D, and E. Among them, enterotoxin A is the most potent for inducing the disease (Argudin et al., 2010; Balaban and Rasooly, 2000). Several factors such as inappropriate food handling, contaminated processing equipment, and using milk from staphylococcal mastitis cases are considered as possible sources of contamination with this bacterium. Moreover, individuals carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main sources of contamination. These people may transfer the bacterium via manual contact or respiratory secretions (Bhatia and Zahoor, 2007; Hennekinne et al., 2012; Kluytmans and Wertheim, 2005; Pinchuk et al., 2010). For instance, it has been reported that 32.4% of American people harbor *S. aureus* in their nasals (Kuehnert et al., 2006).

Confectionery products are supposed to be important sources of food poisoning in Iran. Pastry creams may promptly become contaminated with microorganisms like *S. aureus* and other microorganisms through their ingredients and preparation methods (Ray and Bhunia, 2014). It has been stated that molecular techniques have often acceptable accuracy as well as sensitivity for detection of some pathogenic hazards in food and foodstuff samples (Ahmadi et al., 2015; Eslami et al., 2017). Since there is little comprehensive data in this regard in Iran, this study was carried out to determine *S. aureus* contamination in cream pastries collected from popular confectioneries in Hamedan, Iran; also, presence of the gene encoding Staphylococcal Enterotoxin A (SEA) was studied in the isolates.

Materials and methods

Sampling

In this descriptive cross-sectional study, 40 popular confectionery markets of Hamedan, Iran were randomly chosen during April to October 2014 (as hot and dry seasons) and October to March 2015 (as cold and wet seasons). Totally, 160 samples (4 samples from each market), including puff pastry (n=80) and jelly roll (n=80) were randomly purchased, stored on ice packs (4 °C), and transferred to the laboratory under sterile conditions.

Isolation and identification of S. aureus

The samples were homogenized and then 10 g of each sample was diluted with 90 ml sterile peptone water (0.1%). Afterward, 0.1 ml of each dilution was streaked on Baird-Parker agar medium supplemented with egg yolk tellurite emulsion and incubated at 37 °C for 24 to 48 h. *S. aureus* isolates were identified by conventional tests, including Gram staining, production of coagulase, catalase, DNase, fermentation of mannitol, and other biochemical tests. All media were prepared from Merck, Darmstadt, Germany. The presumptively identified isolates as *S. aureus* were frozen at -20 °C in nutrient broth containing 30% glycerol until next experiments.

DNA extraction

DNA was extracted from each *S. aureus* isolate using a previously described protocol (Reischl et al., 2000). Briefly, the isolates cultured in nutrient broth in an overnight was centrifuged at 8000 rpm for 3 min. Afterward, 200 µl lysis buffer (1% Triton X-100, 0.5% Tween 20, 10 mM Tris-HCl, 1 mM EDTA, pH=8.0) was added to the pellets, boiled in water bath (100 °C) for 10 min, and followed by centrifugation at 10000 rpm for 2 min. The supernatant was transferred into the sterile microtube and stored at -20 °C for next steps.

Polymerase Chain Reaction (PCR)

Identification was done by PCR assay with the gene targets of *femA* as a *S. aureus* species-specific gene and *sea* as encoding the SEA (Mehrotra et al., 2000) using specific primer pairs which are presented in Table 1. Each PCR reaction was done in a volume of 25 µl containing of 100 ng genomic DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 U *Taq* DNA polymerase, and 10 pmol each primer. *S. aureus* ATCC 25923 was used as the positive control for both *femA* and *sea* genes (Pourmand et al., 2009). The negative control was the reaction without DNA as a template. The PCR amplification was performed under the following conditions: initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 2 min, annealing at 57 °C for 2 min, and extension at 72 °C for 1 min. The final extension was applied at 72 °C for 10 min (Mehrotra et al., 2000). In order to analyzing the PCR products, the 2.5% agarose gel electrophoresis was carried out.

Statistical analysis

The data were analyzed by SPSS version 16.0. The differences were considered significant when $p<0.05$.

Results

Out of 160 confectionary samples, 61 ones (38.1%) were contaminated with *S. aureus*. The species identification was confirmed by the amplicon fragment of 132 bp in length (Figure 1). Among 61 *S. aureus* isolates, 16 (26.2%) contained the *sea* gene encoding SEA.

As indicated in Table 2, the prevalence rates of *S. aureus* in the hot/dry and cold/wet seasons were 43.7% (35 out of 80) and 32.5% (26 out of 80), respectively showing significant difference (p<0.05). However, the contamination rate was not statistically significant between puff pastry and jelly roll samples (p>0.05).

Table 1: Sequences of the primers used in the PCR assay

Primer	Sequence 5'- 3'	Product size (bp)	Reference
femA-forward	AAAAAAGCACATAACAAGCG	132	Mehrotra et al., 2000
femA-reverse	GATAAAGAAGAAACCAGCAG		
sea-forward	GGTTATCAATGTGCGGGTGG	102	
sea-reverse	CGGCACTTTTTTCTCTTCGG		

Table 2: *S. aureus* prevalence in confectionary samples during April to September (as hot/dry seasons) and October to March (as cold/wet seasons)

Month	Sample size	*S. aureus* No. (%)	*sea* gene No.
April	12	2 (16.6)	0
May	14	5 (35.7)	1
June	14	6 (42.8)	2
July	20	12 (60)	4
August	0	0 (0)	0
September	20	10 (50)	4
Total	**80**	**35 (43.7)**	**11**
October	14	9 (64.2)	3
November	14	5 (35.7)	1
December	12	4 (33.3)	0
January	16	4 (25)	1
February	14	2 (14.2)	0
March	10	2 (20)	0
Total	**80**	**26 (32.5)**	**5**

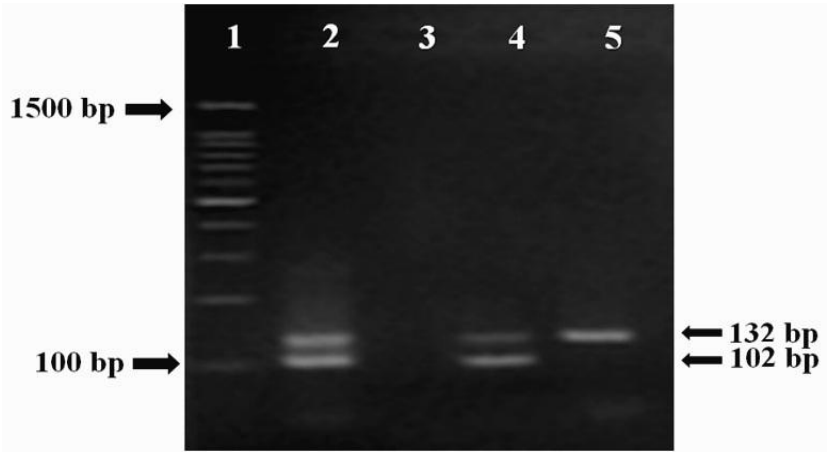

Figure 1: Agarose gel electrophoresis of the PCR products. Lane 1: 100 bp DNA ladder, lane 2: a standard *S. aureus* strain (ATCC 25923) as positive control for *sea* gene, lane 3: negative control contained no template DNA, lane 4: *S. aureus* isolate with *sea* gene, lane 5: *S. aureus* isolate without *sea* gene

Discussion

In this study, we found relatively high prevalence rate of *S. aureus* in puff pastry and jelly roll samples purchased from confectionery stores in Hamedan, Iran which the *sea* gene encoding the SEA was identified in 26.2% of the *S. aureus* isolates. Such high contamination rate highlights that cream pastries are suitable environments for growth and proliferation of microorganisms causing food poisoning in consumers.

Similar to our findings, Jamshidi et al. (2017) found high contamination of Enterobacteriaceae in supplied cream pastries in Arak province of Iran. In the present study, contamination rate in puff pastry and jelly roll samples was relatively similar to each other. Nikniaz et al. (2011) showed that 51 out of 160 (31.2%) tested pastry cream samples produced in Tabriz, Iran were contaminated with *S. aureus*. In another study, various food samples, such as cake, pastry cream, cream, milk, etc. in Tehran, Iran were examined for contamination with *S. aureus*; out of 50 samples, 30% were contaminated with *S. aureus* and 32.1% of the isolates had SEA (Norouzi et al., 2012). Shabani et al. (2014) indicated that 90% of puff pastry and 30.6% of jelly roll samples purchased from Gorgan, Iran were contaminated with *S. aureus*. In another survey conducted on the cream products of Greece, contamination with *S. aureus* was detected in 12.5% samples (Kotzekidou, 2013). Also, Normanno et al. (2005) detected *S. aureus* in 3.5% of pastry cream samples in Italy, whereas none of the isolates had gene encoding SEA. Comparison of the results of this study and similar ones in Iran and the other countries indicates the risk of microbial contamination in such products. The poor personal hygiene and contaminated ingredients may be the most effective factors in increasing the microbial contamination of cream pastries (Smith et al., 2004).

We found that *S. aureus* contamination in samples collected in hot/dry seasons was significantly higher than those taken in cold/wet seasons which is similar with the findings of Hosseini Jazani and Babazadeh (2013) who studied *S. aureus* contamination in collected cream pastries from Urmia, Iran. In the hot/dry seasons, the high temperature can provide a favorable condition for accelerating the growth of *S. aureus* in condition of lack of sanitation, improper handling, and imperfect cold chain.

Conclusion

This investigation indicated a considerable level of contamination in puff pastry and jelly roll samples with enterotoxigenic *S. aureus*, which could be due to microbial contamination of raw materials such as cream and utensils used for pastry production and lack of personal hygiene of workers. Consequently, appropriate measures should be applied to reduce the level of contamination in the confectionary products to ensure public health safety.

Conflicts of interest

The authors declare that there is no conflict of interest.

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