Short Paper

Identification of the classical enterotoxin genes of *Staphylococcus aureus* in various foods by multiplex PCR assay

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(Received 12 Aug 2018; revised version 4 Feb 2019; accepted 18 Feb 2019)

Abstract

Background: An annual update of information about the prevalence of *Staphylococcus aureus* and staphylococcal enterotoxins (SEs) genes is required in every geographic area. **Aims:** This study was conducted to investigate the prevalence of the bacterium and type of associated enterotoxin genes in different food samples, using multiplex polymerase chain reaction (PCR) assay. **Methods:** In order to achieve these goals, 310 samples, divided into three groups (dairy products, meat, and traditional sweets groups), were collected. After determination of the prevalence of *S. aureus*, the existence of *16s rRNA*, *sea*, *seb*, *sec*, *sed*, and *see* genes were evaluated using multiplex PCR assay. **Results:** *Staphylococcus aureus* was isolated from 103 (33%) samples. Furthermore, the meat category had the most contamination rate of *S. aureus*. Additionally, the kebab samples (61.5%) were the most contaminated products, followed by hamburger (47.3%), and ice cream (33.8%). Of these 103 *S. aureus* isolates, 72 isolates (69.9%) harbored at least one type of the classical SEs genes. The prevalence of the type A enterotoxin gene was detected higher than the other SEs genes. **Conclusion:** The results indicated that inappropriate handling of the samples in the preparation and processing steps, especially for the meat products, can lead to the spread of more bacteria. The relatively high prevalence of some classical enterotoxin genes in the isolates revealed the potential power of this bacterium to produce enterotoxins, which can lead to food-poisoning.

Key words: Enterotoxin, Food, Polymerase chain reaction, *Staphylococcus aureus*

Introduction

Staphylococcus aureus is considered as a major public health concern, according to the definition of food-borne bacteria, published by World Health Organization (WHO) report (Le Loir *et al*., 2003; Angulo *et al*., 2008). In this regard, some, but not all strains of *S. aureus*, produce a wide variety of extracellular proteins and pyrogenic exotoxins, including staphylococcal enterotoxins (SEs), staphylococcal-like enterotoxins (SEls), toxic shock syndrome toxin 1 (TSST-1), and different types of hemolysins (Novick *et al*., 2001; Moraveji *et al*., 2014). These toxins cause various types of disease symptoms, including scalded skin syndrome, impetigo, toxic shock syndrome, and staphylococcal food poisoning (Novick *et al*., 2001; Le Loir *et al*., 2003).

Staphylococcal enterotoxins are resistant to heat, digestive enzymes, denaturing agents, and a wide range of pH. Hence, it does not degrade in the digestive tract, can pass through the stomach and attack the intestinal cells (Argudín *et al*., 2010). Among these enterotoxins, the five classical types (A, B, C, D, and E) are the most important and are responsible for 95% of staphylococcal food poisoning (Jay *et al*., 2005). Due to the growing of *S. aureus* in different extreme conditions, it can contaminate animal products during processing and preparation steps, and act as a source for SEs production and food poisoning. Therefore, there is always a potential risk of food poisoning associated with this bacterium, which needs annual checking (Chaibenjawong and Foster, 2011; Wang *et al*., 2017). For these reasons, rapid detection of SEs and efficient screening programs for the main source of food poisoning is critical. Therefore, the prevalence of *S. aureus* and the presence of classical SEs in different foods were investigated in this study, using a polymerase chain reaction (PCR) technique (Løvseth *et al*., 2004).

Materials and Methods

Sample collection

To assess the contamination level of *S. aureus* in foods, samples were aseptically collected between July 2014 and June 2015 in Gorgan, Iran. Foods were divided into three groups (dairy products, meat, and traditional sweets groups) and included raw milk, ice cream, cream, hamburger, kebab, sausage, and traditional creamy

sweets. Sample sizes were calculated based on the previous study and around 100 isolates were obtained from each food group (Peyravii *et al*., 2013). The samples were transported to the laboratory within 6 h in a cool box.

Isolation of *S. aureus*

The isolation of *S. aureus* was done according to the ISO 6888-1:1999/reaffirmed 2005 standard methodology (ISO 6888-1, 1999), using 25 g of each sample. The colonies were checked phenotypically and confirmed genotypically for identification of *S. aureus* (Løvseth *et al*., 2004; Pelisser *et al*., 2009).

DNA extraction and PCR analysis

DNA of each colony was extracted, using a DNA extraction kit (CinnaGen, Iran), according to the manufacturer's instruction. The purity and concentration of each DNA were evaluated by Spectrophotometry method (Nanodrop 1000; Thermo Scientific, Wilmington, DE, USA). *Staphylococcus aureus* and its five important toxins were identified, using two separate multiplex PCR reactions, according to Løvseth method (Løvseth *et al*., 2004). The PCR products were separated on agarose gels (1.5%) and electrophoresed at 100 V for 45 min. Each gel was stained with DNA safe-stain dye (CinnaGen, Iran), and visualized under a UV transilluminator (BTS-20, Japan). DNase free water, DNA of reference strains of *S. aureus* ATCC 29213 (SEA), and *S. aureus* NCTC 10655 (SEC) were used as negative and positive controls, respectively. A 1-Kb molecular weight marker (CinnaGen, Iran) was used to determine the size of the DNA bands.

Statistical analysis

The Pearson Chi-square and Fisher's exact two-tailed tests were used to analyze the differences between variables, using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA). The examined items included the association of different isolation rates of *S. aureus* and the type of foods, and the prevalence of the toxigenic genes in isolates with various origins. The statistical significance was determined as $P<0.05$.

Results

The frequency of *S. aureus* in the food materials

Among 312 food materials, 103 (33%) analyzed samples were contaminated with *S. aureus*. Although the meat category had the most contamination of *S. aureus*, no significant correlation was observed between three-food categories and the bacterium pollution rate (P=0.1). Additionally, the kebab samples (61.5%) were the most contaminated products, followed by hamburger (47.3%), and ice cream (33.8%). According to this, a weak significant correlation was detected between food types and the prevalence of *S. aureus* (P=0.047). Table 1 shows the details of the prevalence of *S. aureus* in the foods.

Detection of classical SEs genes

Of these 103 *S. aureus* isolates, 72 isolates (69.9%) harbored at least one type of the classical SEs genes. Among them, five isolates harbored two genes simultaneously, as follows; two isolates, obtained from ice cream, had *sea* + *sec*, and *sea* + *seb* genes, respectively. Furthermore, two milks' isolates had *sec* and *sed* genes. One isolate, which was obtained from hamburger, had *sea* and *sed* genes, simultaneously. None of the isolates had *see* gene. Table 1 also shows the genes distribution in detail. Based on the prevalence of the enterotoxin genes, the isolates with type A enterotoxin had a higher prevalence in the ice cream and traditional sweets samples, compared to the other food samples, but this difference was not statically significant (P=0.3).

Discussion

Annual review and analysis of food contamination with *S. aureus* and its enterotoxins in each geographic area in every country is crucial. In this study, the most contaminated foods were kebab and hamburger samples, in the category of meat product. It has been shown that the rate of contamination of *S. aureus* in food samples can be very variable. It has been reported that this rate

Table 1: Prevalence of *Staphylococcus aureus* in the various food samples and their enterotoxin genes

Category	Source/number of samples	No. of *Staphylococcus aureus* isolates (%)	Prevalence of enterotoxin genes (%*)				
			sea	*seb*	*sec*	*sed*	*see*
Meat	Hamburger/38	18 (47.3)	6 (33.3)	0 (0)	1 (5.6)	5 (27.8)	0 (0)
	Kebab/13	8 (61.5)	3 (37.5)	0 (0)	0 (0)	4 (50)	0 (0)
	Sausage/13	2 (15.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	All/64	28 (43.8)	9 (32.1)	0 (0)	1 (3.5)	9 (32.1)	0 (0)
Dairy products	Raw milk/90	28 (31.1)	6 (21.4)	0 (0)	3 (10.7)	11 (39.3)	0 (0)
	Ice cream/59	20 (33.8)	8 (40)	3 (15)	3 (15)	4 (20)	0 (0)
	Cream/13	2 (15.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	All/162	50 (30.9)	14 (28)	3 (6)	6 (12)	15 (30)	0 (0)
Traditional sweets	Traditional sweets/86	25 (29.1)	10 (40)	0 (0)	2 (8)	3 (12)	0 (0)
All samples	All samples/312	103 (33)	33 (32)	3 (2.9)	9 (8.7)	27 (26.2)	0 (0)

* Based on the isolates number in each source

can be very low, moderate, or even very high (Peyravii *et al*., 2013; Santos *et al*., 2014; Tang *et al*., 2015). Several factors, including different sampling, detection methods and their sensitivity, sample size, seasonal effects, the ecological and geographical origin of the isolates can be involved in this variation (Kamarehei *et al*., 2013). However, in most studies with any prevalence rate, the meat and dairy products are at the top of the contaminated foods, consistent with the results obtained from this study. In this regard, Wang *et al*. (2017) stated that one of the main reasons for the food-poisoning in the world is related to the meat products contaminated with *S. aureus*. One of the reasons for this contamination in the meat products can be more manipulation of the samples in the preparation and processing steps (Kraushaar *et al*., 2017; Wijesurendra *et al*., 2017). Manipulation of the food products, especially when other food materials such as onion and animal fats are added to them and mixed with hands, can lead to the transfer of the bacterium from other materials or workers' hands to food or at least it can increase the chance of the transfer. Both the kebab and hamburger are manipulated more than the rest of the analyzed samples, and the results of this study showed the high contamination rate in these products, which reinforces this theory. It seems that the use of automatic machines in food preparation and processing or disinfection of additive substances and hands of workers can help to reduce the chance of contamination. The prevalence of *S. aureus* in the dairy products obtained in this study was also consistent with Imani Fooladi *et al*.'s (2010) investigation published, which reported about 32% of the dairy products were contaminated. Non-standard milking, for example using dirty milking machine or hands, can also transfer the *S. aureus*, which is natural flora of cow's skin, to the dairy products.

Most of the isolates analyzed in this study possessed *sea*, followed by *sed* genes. Other investigators have also suggested the dominance of the *sea* (Rall *et al*., 2008; Argudín *et al*., 2012; Kamarehei *et al*., 2013; Seyoum *et al*., 2016). The data from the present research showed more than one enterotoxin gene can be present in one isolate, simultaneously. Although the prevalence of *S. aureus* with several genes is low, it should not be neglected. In order to reduce the risk of the *S. aureus* contamination in foods and improve health programs, more controlling strategies should be implemented in all stages of food preparation. In 2009, Pelisser *et al*. stated that approximately 10^6 CFU/g of *S. aureus* count is necessary for enterotoxins production. This point should be considered on issues related to food hygiene and several food preservation strategies should be implemented to reduce bacterial replication in food products.

The results of this study suggested that the contamination with *S. aureus* with the potential power of toxin production is still a potential health risk, especially in food with high manipulation at the time of preparation. The relatively high prevalence of the bacteria carrying enterotoxin A gene and their distribution in different food can be alarming, furthermore, the controlling programs are not still enough and more government oversight should be applied. Moreover, it was shown that more attention to food health issues should be the main concern of food factories and regulatory agencies. It also shows that molecular methods provide rapid, accurate, and economical means for *S. aureus* screening in food safety evaluations.

Acknowledgement

This work was supported by Food, Drug, and Natural Products Health Research Center, Golestan University of Medical Sciences, Gorgan, Iran.

Conflict of interest

There was no conflict of interest.

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