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ORIGINAL

Enterotoxigenic profiles of *Staphylococcus aureus* strains isolated from sweet samples

Perfiles enterotoxigénicos de cepas de Staphylococcus aureus aisladas en muestras de dulces

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Abstract

Background: Enterotoxigenic *Staphylococcus aureus* is considered to be one of the major cause of foodborne diseases worldwide. The present investigation was done to study the enterotoxigenic genes distribution of *S. aureus* strains isolated from sweet samples.
Methods: Sixty *S. aureus* strains were isolated from sweet samples using culture. *S. aureus* strains were identified using the biochemical tests. Enterotoxigenic gene profile of the isolates was studied using the PCR.
Results: *Sec* (10.0%) harbored the highest distribution amongst examined isolates, while *seb* (1.0%) and *sed* (1.0%) harbored the lowest. Among all isolates of all examined sweet brands, brand C isolates harbored the highest distribution of *sea* (8.0%), *sec* (12.0%), and *sed* (4.0%) enterotoxigenic genes. Brand B isolates harbored the highest distribution of the *seb* (5.0%) enterotoxigenic gene.
Conclusion: Enterotoxigenic genes distribution was related to the types of samples. Simultaneous presence of several enterotoxigenic genes in some isolates showed an important public health issue.

Keywords: Prevalence, enterotoxigenic genes, *Staphylococcus aureus*, sweet.

Resumen

Antecedentes: El *Staphylococcus aureus* enterotoxigénico se considera una de las principales causas de enfermedades de transmisión alimentaria en todo el mundo. La presente investigación se llevó a cabo para estudiar la distribución de los genes enterotoxigénicos de las cepas de *S. aureus* aisladas de muestras de dulces.
Métodos: Se aislaron sesenta cepas de *S. aureus* de muestras de dulces mediante cultivo. Las cepas de *S. aureus* se identificaron mediante pruebas bioquímicas. Se estudió el perfil genético enterotoxigénico de los aislados mediante la PCR.
Resultados: *Sec* (10,0%) albergó la mayor distribución entre los aislados examinados, mientras que *seb* (1,0%) y *sed* (1,0%) albergaron la menor. Entre todos los aislados de todas las marcas de dulces examinadas, los aislados de la marca C albergaron la mayor distribución de genes enterotoxigénicos *seb* (8,0%), *sec* (12,0%) y *sed* (4,0%). Los aislados de la marca B albergaron la mayor distribución del gen enterotoxigénico *seb* (5,0%).
Conclusión: La distribución de los genes enterotoxigénicos estaba relacionada con los tipos de muestras. La presencia simultánea de varios genes enterotoxigénicos en algunos aislados puso de manifiesto un importante problema de salud pública.

Palabras clave: Prevalencia, genes enterotoxigénicos, *Staphylococcus aureus*, sweet.

Introduction

S. aureus is a Gram-positive and cocci-shaped bacterium responsible for nosocomial and community-acquired infections and foodborne diseases[1-3]. Foodborne diseases caused by *S. aureus* are characterized by nausea, vomiting, weakness, abdominal cramps, diarrhea, and toxic shock syndrome[4,5].

S. aureus bacteria secrete a group of extracellular enzymes, ease tissue extinction and dispersal, and membrane damaging toxins that cause catalytic effects on host cells and tissue damage[6]. They are low-molecular-mass and single-chain proteins with 23 different major types (SEA to SEV). *Sea, seb, sec*, and *sed* are the most commonly detected types in the cases of foodborne diseases and food poisonings[7].

Rendering the high importance of enterotoxigenic *S. aureus* and the absence of epidemiological surveys on its prevalence, the current research was done to study the distribution of enterotoxigenic genes of the *S. aureus* strains isolated from sweet samples.

Materials and methods

Isolates

Sixty *S. aureus* isolates of traditional sweet samples (three different brands) were included in this study. Isolates were cultured in Trypticase Soy Broth (TSB, Merck, Germany) supplemented with 10% NaCl and 1% sodium pyruvate (incubated at 37°C for 24 h). In addition, *S. aureus* isolates were identified using cultured into Baird-Parker agar supplemented with egg yolk tellurite emulsion (Merck, Germany) and Gram staining, hemolytic activity on sheep blood agar (Merck, Germany), catalase activity, coagulated test (rabbit plasma), oxidase test, glucose O/F test, resistance to bacitracin (0.04 U), mannitol fermentation on Mannitol salt agar (Merck, Germany), urease activity, nitrate reduction, phosphatase, deoxyribonuclease (DNase, Merck, Germany) test, Voges-Proskauer (Merck, Germany) test and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation tests[8].

DNA extraction

S. aureus isolates were sub-cultured on Tryptic Soy Broth media (TSB, Merck, Germany) and further incubated for 48 h at 37 oC[9]. Genomic DNA was extracted from bacterial colonies using the DNA extraction kit (Thermo Fisher Scientific, Germany), according to the manufacturer's instruction. After extraction, the DNA samples were quantified (NanoDrop, Thermo Scientific, Waltham, MA, USA), their purity checked (A260/A280), and their concentrations adjusted to 50 ng/µL. The integrity of the DNA was evaluated on a 2% agarose gel stained with ethidium bromide (0.5 µg/mL) (Thermo Fisher Scientific, Germany). The DNA concentration was also estimated by spectrophotometric absorbance at 257 nm (Hach, DR5000, USA). The DNA was stored at -20°C pending subsequent PCR analysis[10-17].

Enterotoxigenic genes identification

Table I represents the oligonucleotide primers and PCR conditions used to amplify enterotoxigenic genes[18]. A programmable DNA thermo-cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used in all PCR reactions. All runs included a negative DNA control consisting of sterile PCR grade water (Thermo Fisher Scientific, Germany) and positive DNA control consisting of positive DNA of each target gene. Fifteen microliters of amplified PCR products were subjected to electrophoresis in a 2% agarose gel in 1× TBE buffer at 90 V for 30-40 min, stained with SYBR Green (Thermo Fisher Scientific, Germany)[19-22].

Statistical analysis

Statistical analysis was done using the SPSS 25.0 statistical software (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact two-tailed test were used to assess any significant relationship between data collected in this survey. P-value <0.05 was considered as a statistical significant level[23-27].

Results

Enterotoxigenic gene distribution based on isolates

A total of 60 S. aureus isolates were assessed in the present study to found their enterotoxigenic gene profiles. **Table II**

Table I: PCR conditions used for enterotoxin genes detection[18].

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50µL)
Sea	F: TTGGAAACGGTTAAAACGAA R: GAACCTTCCCATCAAAAACA	120	1 cycle: 94°C ---------- 2 min. 30 cycles: 94°C ---------- 120 s 55°C ---------- 120 s 72°C ---------- 60 s 1 cycle: 72°C ---------- 8 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template (Thermo Fisher Scientific)
Seb	F: TCGCATCAAACTGACAAACG R: GCAGGTACTCTATAAGTGCC	478		
Sec	F: GACATAAAAGCTAGGAATTT R: AAATCGGATTAACATTATCC	257		
Sed	F: CTAGTTTGGTAATATCTCCT R: TAATGCTATATCTTATAGGG	317		

characterizes the enterotoxigenic gene profile of *S. aureus* strains isolated from sweet samples. *Sec* (10.0%) harbored the highest distribution amongst examined isolates, while *seb* (1.0%) and *sed* (1.0%) harbored the lowest.

Table II: Enterotoxin genes distribution amongst *S. aureus* strains.

Samples N (N *S. aureus*)	(%) isolates harbor each enterotoxigenic gene			
	sea	*seb*	*sec*	*sed*
Sweet samples (60)	3 (5.0)	1 (1.6)	6 (10.0)	1 (1.6)

Enterotoxigenic gene distribution based on brands of sweet samples

Table III assesses the *S. aureus* enterotoxigenic gene profiles according to the brand of sweet samples. Among all isolates of all examined sweet brands, brand C isolates harbored the highest distribution of *sea* (8.0%), *sec* (12.0%), and *sed* (4.0%) enterotoxigenic genes. Brand B isolates harbored the highest distribution of the seb (5.0%) enterotoxigenic gene.

Table III: Enterotoxigenic genes profile of *S. aureus* isolates according to the brands of sweet samples.

Samples N (N *S. aureus*)	N (%)isolates harbor each enterotoxigenic gene			
	sea	*seb*	*sec*	*sed*
Brand A (15)	-	-	1 (6.6)	-
Brand B (20)	1 (5.0)	1 (5.0)	2 (10.0)	-
Brand C (25)	2 (8.0)	-	3 (12.0)	1 (4.0)
Total (60)	3 (5.0)	1 (1.6)	6 (10.0)	1 (1.6)

Discussion

Several kinds of infectious diseases caused significant disorders globally[28-31]. In this survey, the role of enterotoxigenic *S. aureus* as an important cause of foodborne diseases was determined. *S. aureus* is one of the most frequent causes of food poisoning. The main food poisoning etiologic agents are staphylococcal enterotoxins. There are diverse SE kinds; types A (SEA) and B (SEB) are the most clinically significant enterotoxins. In this survey, sweet samples were the sources of enterotoxigenic *S. aureus*. *S. aureus* was also detected in sweet samples assessed in previous investigations conducted in Brazil (12.00%)[32], Pakistan (6.70%)[33], Spain (6.10%)[34] and Japan (19.40%)[35].

Some of probable reasons for the presence of enterotoxigenic genes amongst the *S. aureus* isolates were contamination of raw materials used to make sweets such as milk, eggs and even flour with the *S. aureus*, use of insufficient temperature to produce sweet samples, sweet maintenance at high temperatures and humidity, which has led to the enterotoxin production, and finally transmission of enterotoxin-producing strains from contaminated hands and nose of producing staffs during sweet production.

Heat stable SEs produced by enterotoxigenic *S. aureus* strains are considered as major cause of food poisoning. Findings showed that *sec* and *sea* were the most commonly detected enterotoxigenic genes. Song et al. (2015)[36] stated that 5.60% of *S. aureus* bacteria carried the sea, and 3.50% harbored the seb enterotoxins. Zhang et al[37] signifies that the *sea* gene was obtained in 24.10% and the *seb* gene in 4.20% of *S. aureus* bacteria. They also described that 20.50% of *S. aureus* isolates carried the *sed* gene, 6.80% carried the *sec* gene, and 0.60% carried the *see* gene. Kroning et al[32] described the high prevalence of *sea* (33.4%) and *seb* (16.6%) enterotoxigenic genes.

Conclusions

Sweet samples were considered as sources of enterotoxigenic *S. aureus*. Type A and C enterotoxigenic genes were identified as the most abundant. Simultaneous presence of enterotoxigenic gene in some S. aureus isolates must be considered as serious health hazard regarding the consumption of Iranian sweet samples. Proper time and temperature for cooking of sweet samples is important, but preventing cross-contamination is the most effective ways to prevent occurrence of *S. aureus* in sweet samples.

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