Original Article

Molecular Study of Enterotoxin Genes of *Staphylococcus aureus* Isolated Locally from Al-Diwaniyah City

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Abstract

Background: Following *Salmonella*, *Staphylococcus aureus* has been identified as the second most frequent origin of foodborne disease outbreaks. It was discovered that there are five main forms of enterotoxins, named the classic staphylococcal enterotoxin A, staphylococcal enterotoxin B, staphylococcal enterotoxin C, staphylococcal enterotoxin D, and staphylococcal enterotoxin E. **Objectives:** This study aims to isolate *S. aureus* and, then, study the molecular characterization of the enterotoxin genes that these bacteria possess by using the polymerase chain reaction (PCR) technique to determine the epidemiology of these genes. **Materials and Methods:** A total of 280 samples were collected from two different sources from December 1, 2019, to October 1, 2020, and distributed to 200 stool specimens from consulting Al-Diwaniyah clinics, Maternity and Children Teaching Hospital, and health centers, and the additional source of samples contained 80 samples of dairy items. **Results:** The findings revealed that there number of *S. aureus* was 15 isolates representing (20.83%) in samples of dairy ingredients, whereas it was discovered that (9.03%) of the all-inclusive was isolated from the stool of patients. *S. aureus* isolated from both origins had enterotoxin genes by utilizing the PCR technique, *S. aureus* isolates in both origins were characterized by a 100% presence of the *sea* gene, while the *seb* gene present with percentage (33%) and (40%), respectively, for milk and stool samples. In contrast, *S. aureus* isolates from both sources did not appear to contain genes of other enterotoxins, which include *sec*, *sed*, and *see*. **Conclusion:** It was found that the toxic metabolites that enable *S. aureus* bacteria to produce enterotoxins are genetically encoded and represented by the *sea* and *seb* genes, *sea* is the most prevalent compared with other genes followed by the *seb* gene.

Keywords: Dairy products, enterotoxins genes, PCR, *Staphylococcus aureus*, stool specimens

Introduction

Depending on its widespread giving out in nature and its toxicity to humans and other mammals *Staphylococcus aureus* has notable clinical value attracted the interest of researchers from all over the world.[1] Despite being a normal flora can develop into an opportunistic pathogen and is frequently the root of numerous clinical disorders. It was established that pathogenic strains enhanced their pathogenicity through the production of strong protein toxins.[2] It was found that it could cause illnesses, such as food poisoning.[3] Due to their ability to produce enterotoxins that are heat-stable when *S. aureus* is present in food protein short chains were formed. Following *Salmonella*, *S. aureus* has been identified as the second most frequent origin of foodborne disease outbreaks.[4] It was discovered that there are five main forms of enterotoxins, named the classic staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB), staphylococcal enterotoxin C (SEC), staphylococcal enterotoxin D (SED), and staphylococcal enterotoxin E (SEE).[5] Due to the significance of this bacteria from a medical point of view, the purpose of this study was to inquire into the prevalence of *S. aureus* isolate it from patients with intestinal infections and food poisoning in hospitals in the city of Al-Diwaniyah and from dairy products taken

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from the local market and determine the genetic basis for enterotoxins utilizing the polymerase chain reaction (PCR) technique for both sources to determine the epidemiology of these genes.

Materials and Methods

Two sources (patients and dairy products) provided 280 samples from December 1, 2019, to October 1, 2020, and divided into 200 feces specimens and 80 samples of dairy products obtained from vendors and markets in various parts of the city. Samples were cultivated using streaking technique culture media on blood agar and mannitol salt agar and, then, the plates were incubated at a temperature of 37°C for 24 h.[6] The isolates were identified according to the morphological traits of the bacterial colonies on the solid media and a series of biochemical assays, which included the catalase test, oxidase test, and coagulase test accordance.[6] The diagnostic process also used the Vitec system (Biomerieux, France) to affirm the diagnosis process.

Primers

Primers responsible for identifying enterotoxin genes were designed according to Jonhson[7] and Mehrotra *et al.*[8] [Table 1].

PreMix kit prepared

According to the manufacturer's instructions for Bioneer company (Korea), the AccuPower® PCR premix kit was used to prepare a PCR mixture.

Table 1: The study's primers used

Product (bp)	Sequence		Primers
120	TTGGAAACGGTTAAAACGAA	F	*sea*
	GAACCTTCCCATCAAAAACA	R	
478	TCGCATCAAACTGACAAACG	F	*seb*
	GCAGGTACTCTATAAGTGCC	R	
257	GACATAAAAGCTAGGAATTT	F	*sec*
	AAATCGGATTAACATTATCC	R	
317	CTAGTTTGGTAATATCTCCT	F	*sed*
	TAATGCTATATCTTATAGGG	R	
209	AGGTTTTTTCACAGGTCATCC	F	*see*
	CTTTTTTTTCTTCGGTCAATC	R	

Gel electrophoresis

The result of the PCR reaction for enterotoxin genes was read through gel electrophoresis, using 1.5% agarose gel for 1 h at a voltage difference of 100 V and a current of 80 A. The agarose gel was submerged in the solution Tris/Borate/EDTA buffer followed by the placement of the cover, its tight closure, and the turn-on of the device. Then the gel containing the PCR product was viewed in an ultraviolet light generator, and the apparent findings were then captured by a camera.

Statistical analysis

Results were analyzed statistically using the Chi-square statistical test (χ^2) according to Al-qisas.[9]

Ethical approval

According to document No. 11299 dated November 13, 2019, general ethical guidelines were followed when obtaining samples from patients to conduct the study by obtaining their consent and committing not to affect the patient's mental and physical health to achieve the study's goal.

Results

After isolates of *S. aureus* were diagnosed on culture media they showed as rounded, smooth, convex, slightly elevated opaque colonies, and were yellow to golden in color on the blood agar. It was also discovered that it was capable of fermenting mannitol because it formed golden colonies encircled by a broad yellow halo as shown in Figure 1. It was discovered that there were 15 samples of *S. aureus* isolates from dairy distributed among 7 (23.33%), 5 (22.72%), and 3 (15%) for milk, cheese, and cream, respectively, as the statistical analysis findings did not show any variation in the number of these bacteria in these different products, for stool samples *S. aureus* recorded (9.03%).

Detection of genes encoding enterotoxins

Enterotoxins were detected in *S. aureus* for both sources by using the PCR technique to investigate the enterotoxin

Figure 1: *S. aureus* colonies on (A) blood agar and (B) mannitol salt agar

genes possessed by the bacterial isolates under study. All bacterial isolates of *S. aureus* (30) isolates distributed among (15) isolates from dairy products and (15) from feces subjected to a PCR to investigate the *sea* gene, isolates contained the *sea* gene 100% of both sources, with a product size of 120 bp in marker ladder (1500–100 bp) [Figures 2 and 3].

As for the SEB, the *seb* gene encoding its production isolates from stool samples contained 40% of the *seb* gene [Figure 4]. The results showed that only five isolates of *S. aureus* isolated from dairy products contained a gene (*seb*) rate of 33% with a product size of 478 bp in the marker ladder (1500–100bp) [Figure 5]. Production of other enterotoxins that include SEC, SED, and SEE respectively after using the PCR technique, amplification results of these genes showed that all *S. aureus* isolates for both sources do not contain these genes, the statistical analysis results showed that there were highly significant differences in production enterotoxin genes at the level $P < 0.05$ in both groups of the studied samples.

Discussion

S. aureus isolated from dairy products at a high rate while it was found that it formed a lower isolation percentage in stool samples, which may be due to the difficulty of isolating it from stool samples because most cases of poisoning occur as a result of consuming the intestinal toxins of these bacteria.[10] These results were similar to what was obtained by Santana *et al.*,[11] it was found that the percentage of its isolation amounted to 18.80% from milk, whereas disagreeing with Al-Khafaji *et al.*[12] found the percentage of its isolation of dairy products reached 48%. In addition, disagree with Akingbade *et al.*[13] who found that the percentage of its isolation was 4.4% from fecal samples. The disparity in sample sizes could be the reason for the variation in this isolation rate in addition to the variations in the ambient circumstances from which the samples were obtained in terms of place and time.

Regarding the production of enterotoxins, it was also found that a gene *sea* appeared with a high rate of 100%. This high percentage of this enterotoxin explains that it is one of the common enterotoxins, which indicates its role in the pathogenicity of *S. aureus*. The reason for this may be attributed to enterotoxins characterized by high resistance to the action of intestinal enzymes.[14] This result was different from Bokaeian *et al.*[15] as they found that the percentage of this gene amounted to 2%. In addition, it was found that our results regarding *seb* converged with Nazari *et al.*[16] as they obtained an

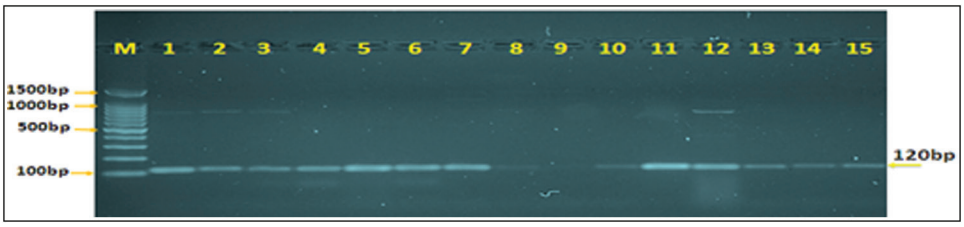

Figure 2: PCR assay of the *sea* in *S. aureus* from feces specimens, marker ladder (M) 1500–100 bp (1–15) are positive isolates to the test with a yield of 120 bp

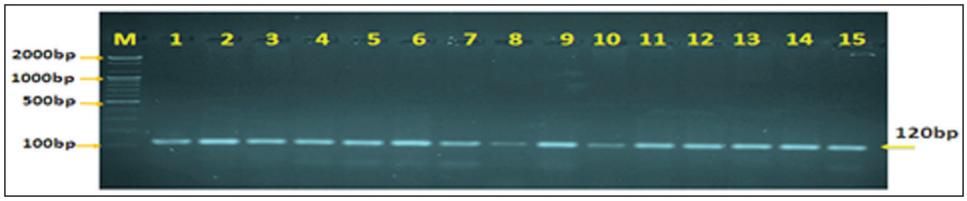

Figure 3: PCR assay of the *sea* in *S. aureus* from dairy products, marker ladder (M) 1500–100 bp (1–15) are positive isolates to the test with a yield of 120 bp

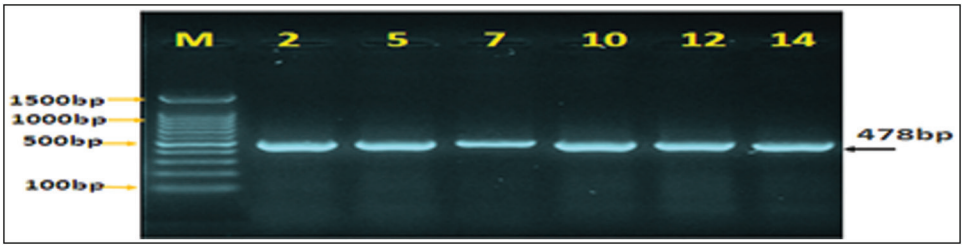

Figure 4: PCR assay of *seb* in *S. aureus* from feces specimens, marker ladder (M) 1500–100 bp, (2, 5, 7, 10, 12, and 14) are positive isolates to the test with a yield of 478 bp

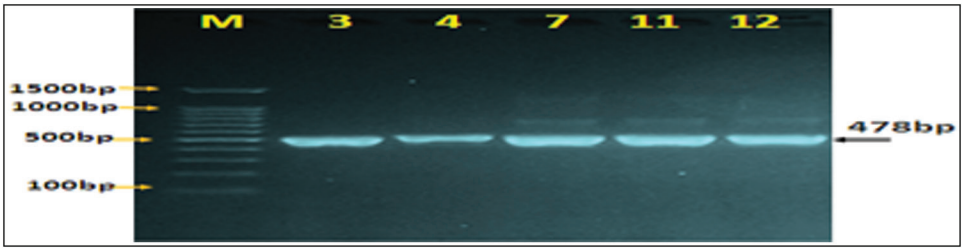

Figure 5: PCR assay of *seb* in *S. aureus* from dairy products, marker ladder (M) 1500–100 bp, (3, 4, 7, 11, and 12) are positive isolates to the test with a yield of 478 bp

isolation rate for this gene in 26.9% of milk samples. This variation is in proportion with *seb* production because number of parameters, such as *S. aureus* strain type as it was found that some strains contain pathological islands carrying the *seb* gene, and thus differ in the levels of SEB production.[17] As for the genes *sec*, *sed*, and *see*, *S. aureus* does not contain these genes, is due to the diversity of genetic elements some clinical isolates may carry it on the chromosome, whereas other strains may carry it on the plasmid.[18] In addition, several regulatory pathways regulate the enterotoxins, and these pathways are usually overlapping and influenced by environmental factors.[19]

Conclusion

S. aureus isolated from patients and dairy products had enterotoxin genes, it was found that *sea* and *seb* genes were encoded mainly the enterotoxin production in *S. aureus*, with the most prevalent of the *sea* followed by *seb* gene. Therefore, emphasis must be on increasing health awareness surrounded by the various groups of society and taking measures about hygiene and health care, food safety is the basis for preventing diseases caused by microbes transmitted through multiple foods.

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Conflicts of interest

There are no conflicts of interest.

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