MOLECULAR DETECTION OF *NUC* AND *SEA* GENES OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM COW AND SHEEP MEAT IN BASRAH CITY.

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ABSTRACT

A total of 100 samples collected randomly from different butcher shops, restaurants and fast food places at Basrah Province (60 raw meats and 40 cooked spiced meat burger). Molecular detection of the *nuc* gene showed that 13(76.47%) out of 17 isolates from the cow meat were positive for this gene. However, only10 (52.63%) out of the 19 isolates from the sheep meat were positive for the *nuc* gene. The present study found that 9/13 isolates of cow meat samples were positive for *sea* gene. Regarding to sheep meat samples, the current study found that out of 10 isolates of *S.aureus*, 7(70%)isolates were positive for *sea* gene, without significant differences, and 3/8 (37.5%) of restaurants isolates were positive for *sea* gene. In the current study, the molecular detection showed that 5/9(55.56%)of fast food isolates were positive for *sea* gene. In the current study demonstrate that the meat is adequate medium for growth of *S. aureus* and production of enterotoxins.

INTRODUCTION

S. aureus is a staining gram positive bacteria that is a considerable public health problem (1). *S. aureus* bacteria are characterised by non formation of spores and are facultative anaerobic bacteria that specialize in complicated nutritional demands for growth (2, 3). *S. aureus* bacteria is one of the main causes that transmit diseases through food and cause what is known as Staphylococcal food poisoning. Staphylococcal food poisoning is

an intoxication that produces from the consuming of foods comprising adequate amounts of one (or more) preformed enterotoxin (4, 5).

S. aureus are generally characterised by multiple virulence factors and because the concerted impact of several virulence determinants, which increase colonization of tissue, tissue injury, and consequently it causes illness (6).The capacity of *S. aureus* to induce infections are associated to expression of different virulence agents like formation of biofilm, surface proteins and exoenzymes, exotoxins. These bacteria attach to the tissues due to all these factors, causing pathogenesis and invade the immune system, causing toxic effect (7). The *nuc* gene is widely utilised as a specific target for the identification of *S. aureus* by Polymerase chain reaction (PCR), and this gene encodes thermonuclease (8, 9).

Staphylococcal enterotoxins (SEs) of *S. aureus* are represent as a virulence factors of a major category of pyrogenic toxin superantigens (PTSAgs) (10). Enterotoxins are excreted toxins of 20–30 kD that interfere with intestine role, they are the cause of vomiting and diarrhoea (11). Enterotoxin A is the widespread enterotoxin in intoxication of food, as it was found in 75% of cases of poisoning due to *S. aureus*, the quantity of enterotoxin A in demand to induce poisoning in human has been estimated less from one microgram (12). As for molecular biology assays, which include PCR technique, they are used to screen for enterotoxin-producing genes in the types of *S. aureus* isolated from contaminated foods. (13).The present study aimed to distinguish of *S. aureus* and reveal the prevalence of staphylococcal enterotoxin A(SEA) in samples of meat using PCR technique through presence of the *nuc* and *sea* genes.

MATERIAL AND METHODS

Samples collection

A total of 100 samples were collected randomly from different butcher shops, restaurants and fast food places in Basrah city (60 raw meats and 40 cooked spiced meat burger). Out of the 60 samples of raw meat, 30 samples were beef, and the rest were sheep meat. While the samples of cooked spiced meat burger included 20 samples of restaurants burger and the rest were fast food places burger (25 gm of each samples).

Isolation of *S. aureus*

The samples were inoculated after their collection on the medium of blood agar already prepared to this purpose, incubated at 37ºC for 24 hours. Isolates were examined based on their morphology, size, color, dyes, and hemolytic efficiency, there after they

were transmitted and streaked on mannitol salt agar MSA and incubated at 37°C for 24 hours.

Each colonies from primary cultures were purified by subculture on brain-heart infusion (BHI) agar and there after re- inoculated on (MSA) and incubated at 37°C for 24 hr (14, 15).

Identification of *S. aureus*

S.aureus was recognized based on the morphological characteristcs on culture media and biochemical assays (oxidase test, catalase test, coagulase test) according to Bergey's manual (16).

Genetic analysis

DNA Extraction

The chromosmal DNA of *S.aureus* isolates was extracted and purified using Genomic DNA Mini kit (Geneaid, Korea), according to instruction procedures. The Nanodrop was applied to determine the concentration of DNA. The Nanodrop was applied to measure the optical density (O.D) at wave lenth of 260 nm and 280 nm. An O.D of one corresponds to approximately 50 µg/ml for double stranded DNA (17).

Polymerase chain reaction PCR

PCR was used for amplifying the *nuc* and *sea* genes for all samples of genomic DNA extracted from *S.aureus* isolates. The oligonucleotide primers were dissolved in deionized water to give a ultimate concentration of (100 pmol/µl).The primers of *nuc* gene used were forward: 5'-GCTTGCTATGATTGTGGTAGCC-3' and reverse: 5'-TCTCTAGCAAGTCCCTTTTCCA -3' that were previously designed by (18). The reaction was performed in thermocycler under the following conditions: Initial denaturation step at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 40 sec, annealing at 58°C for 35 sec and extension at 72°C for 35 sec plus a final extension step at 72°C for 5 min. In related to *sea* gene, the primers used were forward: 5'-GGTTATCAATGTGCGGGTGG -3' and reverse: 5'-CGGCACTTTTTTCTCTTCGG-3' that were previously designed by (19). The reaction was performed in thermocycler under the following conditions: Initial denaturation step at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 35 sec, annealing at 58.3°C for 35 sec and extension at 72°C for 35 sec plus a final extension step at 72°C for 5 min.

For both of genes (*nuc* and *sea*), reaction of PCR was performed in a total volume of 20 µl (5 µl of DNA tamplet, 1 µl each of forward and reverse primers, 5 µl master mix and

8 µl distal water). Agarose gel electrophoresis of PCR products was used 2% agarose. An agarose gel was transmitted to UV light transilluminator and the result was photographed using a mobile phone camera.

Statistical analysis

The chi-square test was applied to analyse the results statistically. All statistical analyses were conducted using SPSS version 17 software. A P-value ≤ 0.05 was considered statistically significant.

RESULTS

DNA extraction

The bacterial genome was isolated from positive cultures of *S.aureus* then electrophoresis on 1% of agarose gel (Figure 1).

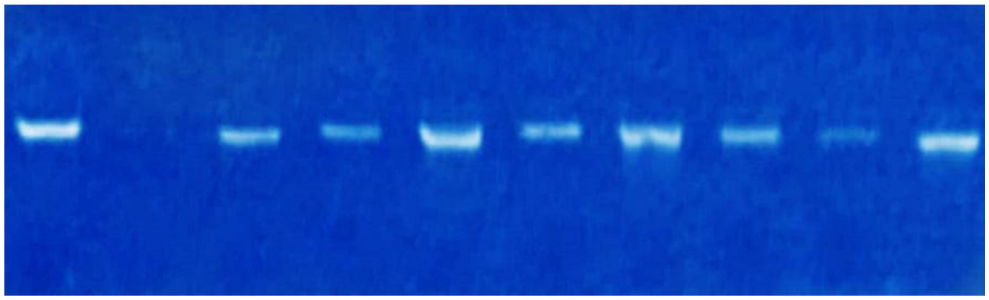

Figure 1: Genomic DNA isolated from *S.aureus* using 1% agarose gel

Detection of *nuc* gen

The current study demonstrate that the size of the gene amplified using PCR was 423 bp. PCR product was resolved on 2% agarose gel electrophoresis and detected by UV light transilluminator (Figure 2).

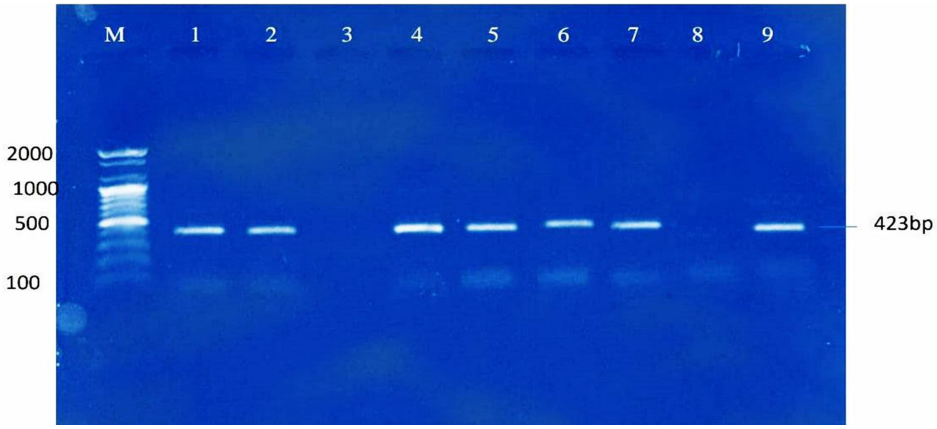

Figure 2 : Agarose gel electrophoresis of *nuc* gene amplification, where M:ladder,1-2, 4-7and 9 lanes are positive results, lane 3: negative result , lane 8 negative control

The findings of the molecular revelation of the *nuc* gene showed that 13 (76.47%) out of 17 isolates from the cow meat samples were positive for this gene. However, only 10 (52.63%)out of the 19 isolates from the sheep meat samples were positive for the *nuc* gene, the current study revealed that there was no significant differences (P-value = 0.137) (Table 1).

On the other hand, the current study found that 8 (66.67%) out of 12 isolates from restaurant samples were positive for the *nuc* gene. However, 9 (64.29%)out of 14 isolates from fast food samples were positive for this gene without significant differences at the level of probability P≤0.05 (P.value = 0.899) (Table 2).

Table (1): Results of *nuc* gene of *S. aureus* isolated from raw meat samples.

Type of samples	**No. *S. aureus* isolates**	***nuc* gene**		**P.value**
		Positive	Negative	
Cow meat	17	13 (76.47%)	4 (23.53%)	0.137
Sheep meat	19	10 (52.63%)	9 (47.36%)	
Total	36	23 (63.89%)	13 (36.11%)	
$\chi 2$ (df 1) = 2.21 P value ≤0.05 mean significant				

Table (2): Results of nuc gene of S. aureus isolated from cooked meat burger

Type of samples	**No. *S. aureus* isolates**	***nuc* gene**		**P.value**
		Positive	Negative	
Restaurants	12	8 (66.67%)	4 (33.33%)	0.899
Fast food places	14	9 (64.29%)	5 (35.71%)	
Total	26	17 (65.38%)	9 (34.62%)	
$\chi 2$ (df 1) = 0.016 P value≤0.05 mean significant				

Detection of *sea* gene.

Using PCR technique, 102 base pairs of the *sea* gene were amplified (Figure 3).

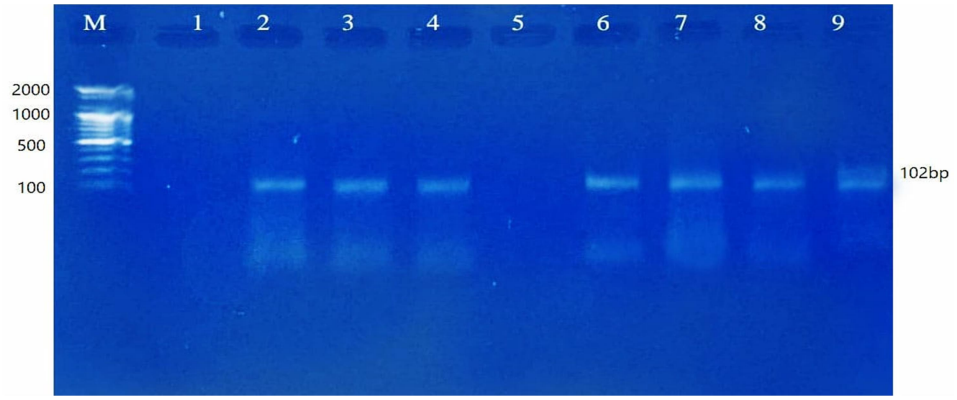

Fig (3): Agarose gel electrophoresis of *sea* gene amplification , where M: ladder, 1:negative result; 2-4,6-9: positive results; 5:negative control.

To detect staphylococcal enterotoxin determination, PCR technique was applied. Depending on PCR technique, the current study found that out of 9/13 isolates of cow meat samples were positive for *sea* gene. Regarding to sheep meat samples, the current study found that out of 10 isolates of *S.aureus*, 7 (70%) isolates were positive for *sea* gene, without significant differences (P>0.05) (Table 3).

Table (3): Results of enterotoxin (*sea*) gene of *S. aureus* isolated from raw meat samples.

Type of samples	**No. *S. aureus* isolates**	***SEA* gene**	
		Positive	Negative
Cow meat	13	9 (69.23%)	4 (30.77%)
Sheep meat	10	7(70%)	3(30%)
Total	23	16 (69.56%)	7 (30.43%)
$\chi 2$ (df 1)		0.002	
P.value		0.968	
P.value ≤0.05 mean significant			

In this study, 3/8 (37.5%) of restaurants isolates were positive for *sea* gene. The molecular detection of this study showed that 5/9 (55.56%) of fast food isolates were positive for *sea* gene, without significant differences (P.value>0.05) (Table 4).

Table (4): Results of enterotoxin (*sea*) gene of *S. aureus* isolated from cooked meat burger

Type of samples	**No. *S. aureus* isolates**	***sea* gene**	
		Positive	Negative
Restaurants	8	3 (37.5%)	5 (62.5%)
Fast food places	9	5 (55.56%)	4 (44.44%)
Total	17	8 (47.04%)	9 (52.94%)
$\chi 2$ (df 1)		0.55	
P.value		0.45	
P.value ≤0.05 mean significant			

DISCUSSION

The present results of the molecular detection of the *nuc* gene showed that 13 of the 17 isolates were taken from beef samples with a percentage of (76.47%). However, (52.63%) of the isolates that were taken from sheep meat showed a positive result for the test of the gene, and the study did not show any statistically significant differences when comparing results. Using polymerization reaction technique (20) identify a 100% *nuc* gene of *S. aureus* isolates diagnosed by conventional methods .Although *nuc* gene is specialized in *S. aureus* bacteria, this method can be used to diagnose *S. aureus* and dispense with other methods.

(21) showed amplification of the *nuc* gene in all *S. aureus* isolates that were diagnosed by conventional methods and not diagnosed in other types of bacteria. The present study is in agreement with findings of (22) in Iraq who demonstrate that the molecular detection of the selected isolates up to the species level by amplifying the *nuc* gene, which is specific to (*S.aureus*) for more accuracy using PCR technique and it was found that all isolates are positive for the existence of *nuc* gene after electrophoresis of the PCR products, the results revealed the presence of a DNA strand of the *nuc* gene with a molecular size (300 base pairs).

(23) showed in a study conducted in Saudi Arabia, that the *nuc* gene is amplified with 100% of *S.aureus* isolates.

The combination of molecular diagnosis by PCR technique and *nuc* gene amplification to identify the species of *S. aureus* in addition to the specific phenotypic characteristics of bacteria is possible, his study showed that all samples that included *S. aureus* bacteria were positive by direct testing in the *nuc* gene in the PCR product, and his results proved the potential for rapid diagnosis of staphylococcal infection by *nuc* PCR described in their study. We observed that the amount of PCR product was different with the number of *S. aureus* bacteria isolated from the sample (24).

A study of *staphylococcus* in cooked food, found that the prevalence of bacteria in chicken meat was 12.9%, while beef was 19.6%. These are meat products contaminated with *Staphylococcus*spp at a concentration greater than 102 per gram (25).

Depending on PCR technique, the current study found that out of 9/13 (69.23%) isolates of cow meat samples were positive for *sea* gene. Regarding to sheep meat samples, the current study found that out of 10 isolates of *S. aureus*, 7 (70%) isolates were positive for *sea* gene. In this study, 3/8 (37.5%) of restaurants isolates were positive for *sea* gene. The molecular detection of this study showed that 5/9 (55.56%) of fast food isolates were positive for *sea* gene.

(26) measured the percentage of *S. aureus* bacteria in frozen and fresh meats collected from retail stores in Karbala to detect the presence of enterococci and diagnose the gene by the method of polymerase chain reaction (PCR).

The results of a study showed that out of 100 samples of frozen and fresh beef, 57 (57%) were found to be contaminated with *S. aureus*. This current study does not agree with the findings of (20) on imported frozen and locally slaughtered meat in Egypt who explained negative isolates for *sea* and *sed* genes.

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الكشف الجزيئي لجينات nuc و sea لبكتريا المكورات العنقودية الذهبية المعزولة من لحوم الابقار و الاغنام في محافظة البصرة

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الخلاصة

هدفت الدراسة الحالية إلى التعرف على المكورات العنقودية الذهبية والكشف عن الذيفان المعوي نوع Aللمكورات العنقودية الذهبية من خلال تضخيم جين nuc وجين الذيفان المعوي sea باستخدام تقنية تفاعل البلمرة المتسلسل. تضمنت هذه الدراسة ١٠٠ عينة تم جمعها بشكل عشوائي من مختلف محلات الجزارة و المطاعم واماكن الوجبات السريعة في محافظة البصرة (٦٠ لحوم ابقار واغنام نيئة و ٤٠ برغر لحم مطبوخ). أظهرت نتائج الكشف الجزيئي لجين nuc أن ١٣ عزلة (٧٦.٤٧٪) من أصل ١٧ عزلة من عينات لحوم البقر كانت موجبة لهذا الجين ، بينما ١٠ فقط (٥٢.٦٣٪) من أصل ١٩ عزلة من عينات لحوم الأغنام كانت موجبة لجين nuc. وجدت الدراسة الحالية أن ١٣/٩ عزلة من عينات لحوم البقر كانت موجبة لجين الذيفان المعوي A . بالنسبة لعينات لحوم الأغنام ، وجدت الدراسة الحالية أنه من أصل ١٠ عزلات من بكتريا المكورة العنقودية ، كانت ٧ عزلات (٧٠٪) موجبة لجين الذيفان المعوي A ، دون فروق معنوية ، و ٨/٣ (٣٧.٥٪) من عزلات المطاعم كانت موجبة لهذا الجين. في هذه الدراسة ، أظهر الكشف الجزيئي أن ٩/٥ (٥٥.٥٦٪) من عزلات الوجبات السريعة كانت موجبة لجين الذيفان المعوي A ، وأظهرت الدراسة الحالية أن اللحوم هي وسط كاف لنمو بكتيريا *S. aureus* وإنتاج السموم المعوية.

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