



ISOLATION AND IDENTIFICATION OF *STAPHYLOCOCCUS AUREUS* STRAINS FROM FRESH AND FROZEN MEAT IN KARBALA PROVINCE

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ABSTRACT

The present study aimed to evaluate the percentage of *S. aureus* in frozen and fresh meat collected from retail stores in Karbala province /Iraq and to detect the presence of *Staphylococcus enterotoxin A* gene by Polymerase Chain Reaction (PCR) method. For this purpose, 100 frozen and fresh meat samples (50 from each one), bacterial isolation was done by routine microbiology methods, identification by biochemical tests, enterotoxins of *S. aureus* isolates were confirmed through PCR assay for sea gene of enterotoxin A. The result showed that among 100 frozen and fresh bovine meat sample, 57 (57%) found to be identified with *S. aureus*. The highest rate of *S. aureus* isolates was observed in frozen meat as 64% followed by fresh meat as 50% with non- significant differences ($P > 0.05$) in the rate of *S. aureus* isolation among the different samples. PCR assay recorded that out of 57 isolates of *S. aureus* from bovine meats, 20(35%) isolates carried sea gene of SEA, 12 (37.5%) isolates from frozen and 8 (32%) isolates from fresh meat samples. It was concluded that bovine meats considered a main food borne disease in Karbala city.

KEYWORDS: Enterotoxin, meat, sea gene, PCR, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus is a main pathogen associated with nosocomial and community acquired infection and cause important public health problem as a result of wide range of infection from mild infection of soft tissue and skin to severe disease in humans and animals including life threatening pneumonia, sepsis, bacteremia, toxic shock syndrome, endocarditis and osteomyelitis^[1]. In addition it cause food poisoning ^[2] *S. aureus* normally colonized skin and nose passage of healthy people^[3], therefore these pathogen can easily contaminated the food through unhygienic handling and during stages of manufacturing^[4]. Food poisoning cause mostly by Staphylococcal enterotoxins (SEs) as a result of these types of toxin can resistant abnormal condition such as heat, proteolytic GIT enzymes like rennin, trypsin and pepsin, therefore they remain biological active during cooked process and through movement in GIT^[5]. However, low concentration of SEs in the food (20ng to 1ng /1g of food) cause clinical signs^[6], different foods particularly meats can easily contaminated by enterotoxigenic *S. aureus* ^[7] contaminated food by *S. aureus* occur by poor hygienic handlers and respiratory secretion^[8] or during stages of manufacturing^[4,9] found that retail meat and poultry meat were commonly contaminated with *S. aureus* particularly multiantibiotic resistance strains also *S. aureus* can contaminated meat products and different dairy products^[10]. Large percentage of *S. aureus* (15% to 80%) have ability to produced enterotoxins in different types of food such as meats ^[11,12], gastroenteritis and Saphylococcal food poisoning occur by enterotoxin A and enterotoxin B which encode by sea and subgenes respectively worldwide^[13]. There are few studies about the prevalence

of enterotoxigenic *S. aureus* in bovine meats in Iraq, therefore the aim of the present study was to determine the percentage of *S. aureus* in frozen and fresh bovine meats with determine the percentage of *S. aureus* isolates carried sea gene of enterotoxin A, by using Polymerase Chain Reaction (PCR) method.

MATERIALS & METHODS

All media were prepared according to the instruction of Manufactures Company.

Bacterial isolation and identification

One hundred fresh and frozen bovine meat samples (50 of each) were collected under aseptic condition from different supermarkets in kerbala province/Iraq. The period of collection beginning from October 2016 and until the end February 2017. The meat samples were homogenized with stomacher in 100ml of sterile normal saline then culture on selective media, mannitol salt agar for 24hr at 37°C then suspected Staph colonies were cultured on blood agar for 24 to 48hr at 37°C ^[14]. The morphology of suspected colonies was determined by shape, size, color, and microscopic examination include gram stain.

Identification of *S. aureus*

The suspected colonies were confirmed by biochemical reactions which composed Coagulase test, Catalase test, Oxidase, DNase test, and beta hemolysis test on blood agar ^[15].

Isolation of genomic DNA

An overnight grown of *S. aureus* was centrifuged at 4000 x g then; the pellets were transferred to 200µl of Gram positive buffer containing Lysozyme as (0.8 mg/200 µl per sample) to Gram positive buffer, DNA extracted with

PrestoTH Mini gDNA Bacterial Kit Protocol (Geneaid, Korea) according to the manufacture company.

Conformation of *S. aureus* by Polymerase chain reaction (PCR)

Oligonucleotide Primers

The oligonucleotide primers were obtained from [16], who consider being diagnosed enterotoxin gene A table 1, PCR was performed in a final reaction volume of 20µl table 2 in a thermal cycler (Techne TC-3000X Thermal cycler/

United Kingdom) with initial denaturation steps as 94°C for 5 min, Twenty-five amplification cycles (94°C for 30 s, 50°C for 30 s, and 72°C for 30 s) and a final extension step of 2 min at 72°C, PCR products were resolved on 1% agarose gel electrophoresis and visualized by UV trans illuminator apparatus.

DNA purity and concentration were determined by a Nano drop spectrophotometer at wave length of OD260/OD280 nm.

TABLE 1: Nucleotide sequences *staphylococcus aureus* primers

Gene name	Primer	Nucleotide Sequence	Size	No of nucleotides
EnteroAF	SA-U	TGTATGTATGGAGGTGTAAC	270 bp	20
EnteroAR	SA-A	ATTAACCGAAGGTTCTGT		18

TABLE 2: volume of PCR mixture of *Staphylococcus aureus*

No.	PCR mix	Volume (ul)
1	DNA samples	5 ul
2	Forward primer 10 pmol	1.5 ul
3	Reverse primer 10 pmol	1.5 ul
4	PCR water	12 ul
5	total	20 ul

The master mix reaction components were added to the standard PCR tube that containing the PCR Premix. PCR products along with 100 bp DNA ladder electrophoresed in 1% agarose gel containing ethidium bromide (5µl /100 ml) of ethidium bromide dye. Then the agarose was run at 100 V and 80AM for 1 hour [17].

RESULTS

The result revealed that at 24hr of incubation at 37 C on mannitol salt agar, the colonies of bacteria appear as

smooth round, yellowish zone due to mannitol fermenter (Fig:1) ,while under microscopic examination, the bacteria appear gram positive grape like cluster appearance (Fig: 2).

Biochemical examination

All staphylococcal isolates from different sources showed to coagulase positive, oxidase negative, catalase positive, and DNase positive, in addition beta hemolysis test on blood agar (Table: 3) hemolysis (, or hemolysis) on blood agar [18].

TABLE 3: The results showed the biochemical reaction of *S. aureus*

N	Biochemical test	Results
1	a. Slide Coagulase test	+
2	b. tube Coagulase test	+
3	Oxidase test	-
4	Catalase test	+
5	DNase	+

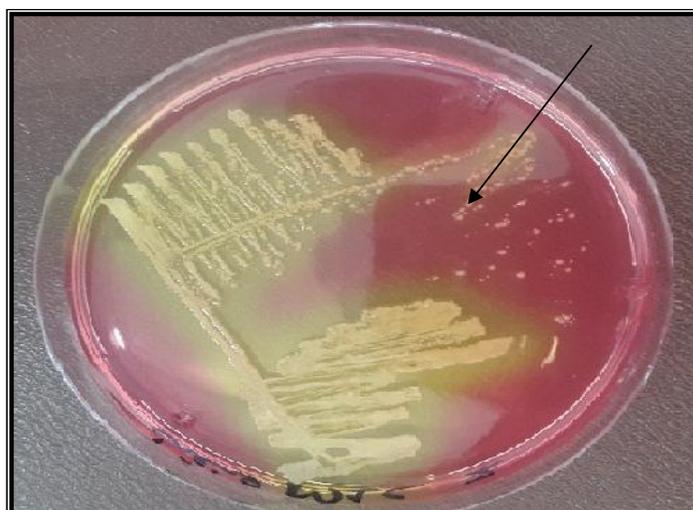


FIGURE 1: the arrow showed that colonies of *Staphylococcus aureus* on the Mannitol salts agar

According to the morphology of colonies, microscopic gram stain and biochemical tests, the bacterial isolates from bovine meats are considered *Staphylococcus aureus*. Our results showed out of 100 bovine meat samples there were 57(57%) meat samples showed *S. aureus* isolates,

otherwise, frozen meat samples showed that 32/50 (64%) of *S. aureus* positive while fresh meat samples revealed 25/50 (50%) of *S. aureus* isolates (Table:5).

TABLE 5: Showed number and percentage of bacterial isolates from bovine meat samples

Type of samples	No. of samples	No. <i>S. aureus</i>	(%)
Frozen meat	50	32	64%
Fresh meat	50	25	50%
Total	100	57	

$X^2 = 0.54$, Degree of freedom 1, Non-significant ($p > 0.05$)

PCR assay were used to detect *Sea gene* of *S. aureus* enterotoxin A the result showed that out of 25 isolates of *S. aureus* isolated from the frozen meat samples, 8 (32%)

isolates carried *sea gene* while 32 *S. aureus* of fresh meat isolates 12 (37.5%) isolates carried *sea gene* (Fig. 2; table: 6)

TABLE 6: Number and percentage of *Sea gene* of *S. aureus*

Type of samples	No. of samples	No. <i>S. aureus</i>	No. of negative <i>S. aureus</i>
Frozen meat	32	12 (37.5%)	20 (62.5%)
Fresh meat	25	8 (32%)	17 (68%)
Total	57	20 (35%)	37 (65%)

$X^2 = 0.09$, degree of freedom 1, non-significant ($p > 0.05$)



FIGURE 2: The arrow showed that PCR product as 270 bp represented *sea gene* on agarose gel electrophoresis, well 1: DNA ladder marker, well 3, 5 and 6 represented positive samples

DISCUSSION

The present result revealed that twenty (35%) out of 57 bovine meat samples both frozen and fresh meat samples contained *S. aureus*, these bacterial isolates from bovine meat collected from different local markets in Karbala, may indicated that bovine meat which considered one important source of *S. aureus* infection in the humans through consumption contaminated bovine meats, these result was in consistent with result of [19,20] and with [21, 22] also the present finding was agreement with [23] who recorded that among 370 samples of raw beef, lamb, goat and camel meat, 223 (60.3%) *S. aureus* were isolated in Iran, and (13.5%) of these isolates expressed enterotoxins and 14 of these isolates produced *sae gene*, particularly, the amount of *S. aureus* in the food depended on several factors including number of contaminated carriers, poor

hygiene handling of the food by workers, also transport system, these observation was agreement with [24] who showed that contaminated meats may occur through

varies stages of preparation food such as production, distribution and storage in the retailing in the supermarkets and during improper refrigeration temperature that allow to bacterial growth and produced enterotoxins, in addition to food handlers may be help in meat contamination by *S. aureus* [25]. The occurrence of bacterial isolated from frozen meats may indicated that commercial bovine meats were considered one important food vehicles for food borne *Staphylococcus aureus* infection, these idea was agreement with [26], who considered commercial poultry meats one essential vehicles for transmission food borne

pathogen and these pathogen may responsible for numerous foodborne disease in the world^[27]. The percentage of *S. aureus* was detected in fresh bovine meats samples (50%), in the present study these result may indicated the meat may contaminated from the animals through slaughter house or from worker who carried these bacteria as normal flora in nasal cavity and skin, these idea was in consist with^[28], who found that the meats can contaminated with *S. aureus* during manufacture and handling of the foods also^[29] found a high prevalence of *S. aureus* in raw meat (47%) isolated *S. aureus* from fresh bovine meats in the current study may indicated that the meat may contaminated with these pathogen during transmitted of the meat post slaughtering^[30] fresh meats can contaminated with *S. aureus* from contaminated water, air and dusts^[31,32], other results were similar to the current study on meats and meat products were performed by^[33,34] and^[35,36]. The percentage of *S. aureus* isolated from bovine meats in the current finding were higher than those reported by^[37] who recorded that the contaminated rates with *S. aureus* in meats were (16.4%), low incidence of *S. aureus* were isolated from beef meats by^[38,39] these variation between current finding and other results may due to differences in source of the meats, methods of prepared samples for culturing as well as process of bacterial isolation and identification in the laboratory procedures. According to present finding, it suggested that the food borne diseases are animal origin and these evidences were supported by observation of^[40]. The present finding investigated that among 57 *S. aureus* isolates, 57 isolates were coagulase positive, these result may indicate that all *S. aureus* isolated from bovine meat samples in Karbala are pathogenic strains not normal flora contaminated meat samples, these result was agreement with result of PCR assay that detected among 57 *S. aureus* isolated from bovine meat samples, 20(35%) strains expressed *S. aureus* enterotoxine A gene, these result may indicated these pathogen was considered a source of food poisoning in Karbala, these evidence was agreement with^[41] who investigated that enterotoxin A is considered a classical enterotoxin of *S. aureus* which considered important causes of Staphylococcal food poisoning, also it was reported that the most common outbreaks of food poisoning occur by enterotoxin A of *S. aureus* in united kingdom that form 77.8% of all food poisoning outbreaks^[42]. Detecting sea gene in *S. aureus* isolates (35%) from bovine meat was similar to the result of (43) who recorded that among the 170 isolates of *S. aureus*, 60.6% and 27.1% contained sea and seb genes, respectively. also it is not agreement with^[44] who found the high percentage of sea gene carrying *S. aureus* strain up to (77.8%) were recorded in USA and in other countries^[45,46,47]. The present investigation showed that (37.5 %) table (6) of *S. aureus* isolated from frozen meats were positive for sea gene of enterotoxin A, these result may indicated that frozen meats were considered one cause of food poisoning, symptoms of *S. aureus* food poisoning out breaks induced by enterotoxin A^[48] and as a result of unrefrigerated meat for several hr. particularly in Iraq, *S. aureus* can proliferation in the meat and produced enterotoxin A^[49,36] these toxin cannot destroyed by heat

treatment of the meats therefore consumption these contaminated meat lead to food poisoning^[36], on base of the result of current study, it was suggested that the commercial frozen meats may cause food poisoning in the Iraq people and these pathogen may cause death particularly in infants, old age and in the compromised immune response patients, these idea was agreement with^[50] who found that 1,000 deaths occur in each year due to *S. aureus* food poisoning, particularly frozen bovine meats were a common meat food using by Iraq people therefore these study may help the people to applied a good hygiene handling and using of the frozen meats, It concluded that the bovine meats both frozen and fresh type may considered important source of Staphylococcal poisoning food in Karbala city, Iraq.

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