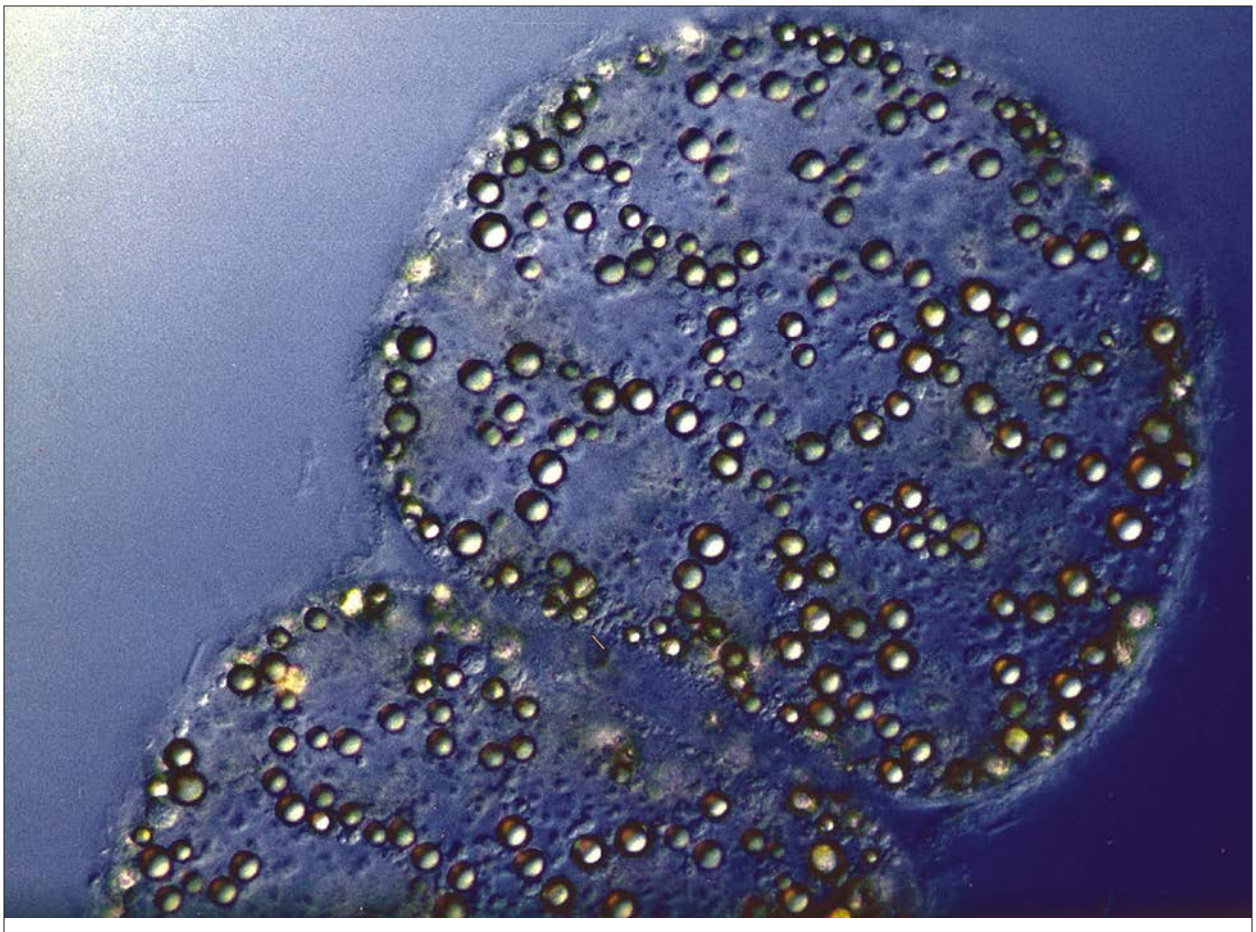

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Research Article
Characterization of Enterotoxins Produced by Food Isolates of *Staphylococcus aureus*

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Abstract

Background and Objective: Determination of the contamination of food, state evaluation health of animals intended for the food production both as subjects carriers and as infected. An investigation has been conducted to assess the *Staphylococcus aureus* contamination of certain categories of food of creature beginning. **Materials and Methods:** Out of the 350 items analyze, 14.0% were tainted with *S. aureus*, with pervasiveness of factors depending on the food group taken into account: 19.3% for meat preparations fresh, 13.3% new cheeses, 3.6% for the confectionery products and 7.7% for the gastronomic preparations. The strains of *S. aureus* isolates have been submitted for confirmation identification of the region16S rDNA and subsequently subjected to the PCR method and reverse latex agglutination (SET-RPLA) to detect those enterotoxigenic. **Results:** The outcomes were contrasted and the information got by exposing similar strains to the quest for qualities encoding enterotoxins (SE) *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei* and the Toxic Shock Syndrome Toxin-1 (TSST-1). To the RPLA test (8/49) 16.3 %of the strains were found to produce enterotoxins while at PCR (24/49) 48.97% carry one or more of the following genes for SE production found and thus potentially enterotoxigenic. **Conclusion:** This study revealed the prevalence of *S. aureus* in various food samples, The *S. aureus* isolates from foods harboured enterotoxin genes play critical roles in the pathogenicity and food poisoning cases, which pose a public health threat to the consumer.

Key words: Enterotoxin, food, PCR, reverse latex agglutination test, RPLA

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The wide diffusion of subjects with *Staphylococcus aureus* (more noteworthy than 30-50% of the populace), during handling the food or any of its ingredients may become contaminated, the permanence of the product at non-temperatures suitable and what represents the basic epidemiological characteristics that create the appropriate conditions for the emergence of an *S. aureus* infection is the ability of the microorganism to mutate under a wide range of conditions PH, free water, NaCl concentration, then many kinds of food products[1,2]. From analytics conducted on the occasion of outbreaks toxinfection, it was found that meat and products based on milk, the most matrices were found frequently involved[3,4]. It is basic, in the determination of the contamination of food, state evaluation health of animals intended for the food production both as subjects carriers and as infected[3].

Raw milk is known as a vehicle for the transmission of pathogenic microorganisms with a high number of positive samples for *S. aureus*. In literature, conflicting data are reported. Another study[5] report a percentage of raw milk contaminated with *S. aureus* by 27.5 -37% in dairies. Another[6] report low percentage levels of isolation. The tolerance criteria currently in force on the presence of *S. aureus* and related toxins have been identified in fresh meats and cheeses. Meat products (pork and salami), meat (chicken, beef and pork) and (salads), creamy pasta and dairy products are often associated with Staphylococcus poisoning[7].

Through the ability of some strains to synthesize one or more enterotoxins (SEs), the pathogenic activity of this microorganism is determined in smaller quantities, SED e SEB. Of the strains isolated from dairy products producing enterotoxin, 59.8% were found: SEA (26.8%), SED (25.7%), SEC (28.2%), SE (A, D) (28.7%)[8]. From low bacterial concentrations (102 g^{-1}) SE production can begin and after incubation times in humans for two hours at 37°C, for taking a very low dose of toxins (0.5 ng mL^{-1}), symptoms can occur[9]. Thermal resistance is one of the main characteristics of the SE . It can improve the chemical and physical properties of food (pH, NaCl concentration) and can determine the preservation of its biological activity even after heat treatment for industrial sterilization[3,9]. Production of toxic shock syndrome toxin-1 (TSST-1)[10-14], or more from additional exogenous proteins. The body itself produces hypersensitivity to endotoxins that release active substances into the vessels through vascular damage determined by both the direct effect of toxins on the endothelium[4,10].

The account of the contamination level assessment of some food classes of animal origin from *S. aureus* and the characterization of the enterotoxigenic strains were represented in this study. to determine the gene coding production of SE[15,16] and TSST-1[17-21] . The method of polymerase chain reaction (PCR) was adopted and compared with the results obtained by the RPLA application to detect toxins produced in the laboratory.

MATERIALS AND METHODS

Study area: samples were collected from several restaurants and randomly selected supermarkets in different regions of Baghdad, during the period between (January to August, 2020).

Sampling: Within a year, 350 food samples were selected for their ease to find and because it is widely used between which meats, fresh cheeses, products of pastry and gastronomic preparations. The products were picked up in the Baghdad market at retail outlets and transferred quickly under refrigeration conditions at the laboratory that performed the tests.

***Staphylococcus aureus* numbering:** Numbering of coagulase-positive Staphylococci has been performed according to the operating modes reported in the second part of the ISO 6888-1:1999. At the end of the incubation period at 37°C, colonies are grown on tripticase soy broth (TSA, Hi-media, India) and tested in Staphytect plus latex (Oxoid, Basingstoke to identify *S. aureus*) according to the another study[22] Biochemical identification is meticulously using the API staph system finalized later (BioMérieux, Marcy l'Étoile) according to the indications of the manufacturer.

Identification of strains with 16SrDNA: The strains of *S. aureus* isolated from food were confirmed in PCR with identification of the 16SrDNA region[19,23].

Identification and production of enterotoxins: *S. aureus* was identified as an enterotoxin producer and confirmed after strains testing *sea*, *seb*, *sec* and *sed* (*Sea-sed*) were

inoculated into brain infusion broth (oxoid) and grown at 37°C for 24 hrs, the broth was examined using a negative stacking assay. Reverse (SET-RPLA, Oxoid, England) as recommended by the manufacturer.

For the identification of genes (sea, seb, sec, sed, see and tsst) were performed single PCR as reported[24]. A multiplex PCR was moreover for *seg*, *seh*, *sei* and 16SrDNA domain, performed with the following cycles of amplification: denaturation at 94°C for 1 min, primers annealing at 55°C for 1 min and of primers extension at 72°C for 2 min. With the amplification of Thirty-five cycles using a Thermal Cycler GeneAmp PCR System 9700 (Applied Biosystems, California). PCR products were detected for each amplified sample and subjected to electrophoretic running in a 1.5% agarose (Hi-media, India) for 1 h at 100 volt. To evaluate the weight of the fragments achieved, a molecular weight standard marker Promega 50-2000 bp was used (Amplisize, Madison, USA). As verification of the conditions used for amplification and detection of PCR products, reference strains *S. aureus* were used as specific control for the toxins detected genes.

Statistical analysis: The percentage of encoding gene and distribution of toxigenic strains and the mean cell forming units were implemented by using EXCEL 2010 software.

RESULTS AND DISCUSSION

Contamination levels of *Staphylococcus aureus* for food analyzed: About 350 food samples tested, 49 (14%) were founded contaminated by *S. aureus.* Among the matrices tested, fresh meat preparations were considered for 19.3% of the positive samples and included fresh meat, which was 30% of the cases of contamination and in preparations of (sausages), contaminated in 16.7% as shown Table 1., the different outcomes may be due to differences in products technologies, such as in technologies of cheese production. The number of samples and like using milk was pasteurized or raw. Also might contribute un adequate hygiene standards during the manufacturing process in which cheese was produced and personnel included in production. Fig. 1 show the frequencies of *S. aureus* contamination in samples of fresh meat and fresh sausages. For fresh meat, the contamination levels are less than sausage.

The levels of contamination, comparison with the limits set by current legislation for this category of products and the concentrations of *S. aureus* necessary for the required production of enterotoxins are shown in Table 2 and Table 3. In the present study, *S. aureus* has been found in an average

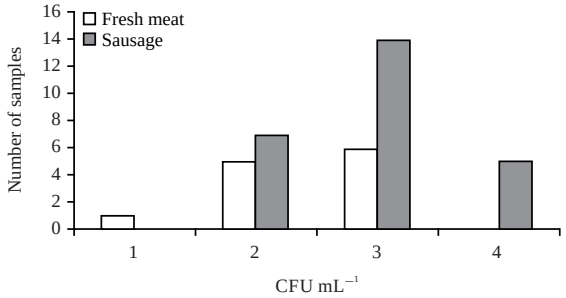

Fig. 1: Frequency of contamination levels of *Staphylococcus aureus* contamination in fresh meat samples

of 49 (14%) food samples. Of the fresh meat samples tested, only 11.1% was found to have complied with the proportions and below 100 CFU g^{-1}, since 18.5% had a load of more than 1000 CFU g^{-1}, a level also considered suitable for the production of SE toxins[9]. While for meat preparations (sausages) no samples were found with a load of more than higher than 1000 CFU g^{-1} (Table 3), Omoe *et al.*[25] have performed the isolation of 160 *S. aureus* from the samples of the food with animal origins in Italy. Similarly, the isolates of the *S. aureus* have been discovered enterotoxigenic and have shown resistance to a minimum of one tested antibiotic. Another research[26] isolates twelve of *S. aureus* from samples of Turkish cheese.

In fresh cheese samples, the percentage of contamination was 13.3%, the contamination was 33.3% for fresh sheep's cheese and 4.8% for fresh bovine's cheese. For fresh cheese, contamination levels ranged between 750 CFU g^{-1} and 2800 CFU g^{-1} shown in Table 4.

Identification of enterotoxigenic strains: At the RPLA set out, *S. aureus* isolates tested were found in SE producers[27]. The percentage of food contaminated with enterotoxigenic strains was 1 toxin A and 7 toxins C. 18 strains isolated from meat preparations were positive in PCR assay in the presence of one or more genes encoding enterotoxin. Table 4, that isolates of *S. aureus*, harboured one type of the SEs genes at least as reported by Sarah *et al.*[28], that was more frequently detected than the classical SE genes. Of the 4 strains isolated from cheese, only 1 strain is the positive result at PCR (sea). Out of 350 food samples, at retail sample outlets and belonging to the majority of prominent food categories, 49 (14%) were contaminated by *S. aureus*. The detection of *S. aureus* in animal-based foods was various and is probably because the foods in current study, raw milk was not included[2,18,29]. The most foods frequently found to be contaminated by *S. aureus* were preparations of meat and fresh cheese (19 .3 and 13 .3%,

Table 1: Distribution of fresh meat and cheese products and positive test results

Products	Number of samples examined	Number of samples positive for *Staphylococcus aureus*	Percentage of isolation
Fresh meat (minced meat, poultry meat)	40	12	30.0
Meat products (fresh sausages)	162	27	16.7
Total fresh meat products	202	39	19.3
Fresh sheep cheese	9	3	33.3
Fresh beef cheese	21	1	4.8
Total fresh cheeses	30	4	13.3
Pastry products (cream pastries)	55	2	3.6
Gastronomic products (timbale)	13	1	7.7
Seafood products (molluscs)	50	3	6.0
Total products	350	49	14.0

Table 2: Distribution of *Staphylococcus aureus* contamination levels in positive milk and cheese samples

Matrix	0-100	10-100	100-1000	>1000
Fresh sheep cheese	0	0	2 (50%)	1 (25%)
Fresh beef cheese	0	0	0	1 (25%)

Values are expressed in CFU g^{-1} for solid samples and CFU mL^{-1} for liquids

Table 3: Distribution of *Staphylococcus aureus* contamination levels in fresh meat samples and meat preparations

Matrix	10-100	100-1000	>1000
Fresh meats	3 (11.1%)	19 (70.4%)	5 (18.5%)
Fresh sausages	6 (50%)	6 (50%)	0

Values are expressed in (CFU g^{-1})

Table 4: Proportion of strains carrying genes encoding staphylococcal toxins

Products	Number of examined strains	Toxigenic strains	Percentage of positives out of total examined
Fresh meat products	39	18	46.2
Fresh cheese	4	1	33.3
Other products	6	5	83.3
Total	49	24	48.97

respectively). Because the number of sampling units per sample was not required by the regulations, therefor the results have been evaluated taking as reference the lower limit "m" (defined by the reference standard in force) for which the product limit is considered "satisfactory". As the upper value of the contamination ranges considered- 1000 CFU g^{-1} or mL (the solid or liquid nature of the analyzed product) that coincide with the number of *S. aureus* which reported by some authors production of SE[7,8,10].

Concerning fresh cheeses, no sample was found to be contaminated and the limits were discovered to be compromised with current legislation but 25% of the samples charge >1000 CFU g^{-1}, which is the limit for the *S. aureus* contamination level proposed in the literature[3,28] for the production of staphylococcal enterotoxin.

The number of enterotoxigenic strains isolated was 2.3%, which is superimposed on the national level (2.9%), enterotoxin C (7/8) and enterotoxin A (1/8) were the most common toxins as reported by Cécile *et al.*[30].

PCR results demonstrated that 24/49 (48. 9%) of the isolates were found to be potentially enterotoxigenic. These include 18 isolates were isolated from meat preparations and one isolate from cheese. The enterotoxins genes that encode the enterotoxins production (g, h, i and tsst) were not detectable by RPLA-test and these results match with Shijia *et al.*[29]. Fresh meat preparations isolates have been shown to carry genes encoding SE genes, In particular, 8 isolates partially possessed one gene encoding sea or sec genes, 11 isolates had two SE- encoding genes (*seg*+*sei*, *sec*+*sei*, *sec*+*seh*) and 3 isolates had 3 SE-encoding genes (*sea*, *seg* and *sei*, *sea*, *seg* and *sei*, *sec*, *seg* and *sei*, *sei*, *seg* and *tsst*). The isolated strain from cheese was positive for the sea gene by PCR. Shellfish isolates (5 strains) were positive for SE-encoding genes seg and sei. SE-encoding genes (*seg*+*sei*) attained the highest 50% strain shown in Fig. 2.

By comparing the results of the RPLA test for the detection of enterotoxins (A-D) with the presence of the comparable gene by PCR, it was observed that for 8 strains (16.3% of the total strains) there was a full match between the results. In fact, with the RPLA test one strain was the producer of toxin A and 7 strains of toxin C, in the same C toxin, in the same strains the PCR revealed the sea and sec genes,

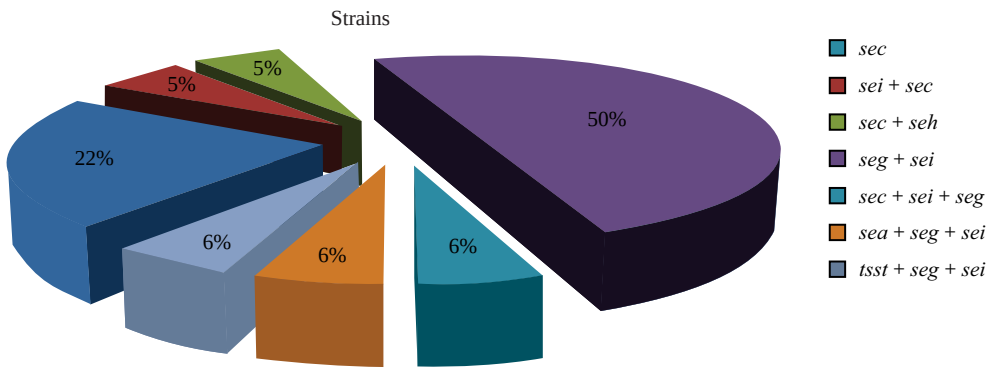

Fig. 2: Strains isolated from meat preparations
Showed positivity in the presence of one or more enterotoxin-encoding genes by PCR

respectively that 8 strains (20.4%) possessed the enterotoxin gene possess the gene for enterotoxins(A-D). This discordance could be related to the expression or lower production of enterotoxin below the detection limit of the RPLA test which is (1 ng mL^{-1}). This result was according to other studies[31-33].

Ultimately, for toxins detected with the RPLA set and PCR, good results were obtained by the two methods as further described by authors[22,29,34].

Although the legislator has identified *S. aureus* as an important risk factor as shown by numerous regulatory measures the numbering of the germ in various foodstuffs for human consumption. The current legislation on the hygienic quality of foodstuffs provides for the research for *S. aureus* toxins alone in the case of limits are exceeding and only as a small number of product types[35,36]. The number of *S. aureus* cells may, however, not be an indicator of the presence of enterotoxins in the product as not all strains enterotoxins producers and that the bacteria may no once be viable but may enterotoxin produced that remain in the food or the enterotoxin formed may be present in less amount below the detection limit of applicable methods[37].

CONCLUSION

The study reveals the widespread presence of *S. aureus* strains carrying genes encoding toxins other than those identifiable by traditional methods. For these toxins, whose the real significance of their presence in food and the actual impact of that they could be the cause of food-borne *S. aureus* toxins in humans, it is necessary to carry out further studies, combining classical and molecular methods. The use of innovative techniques for recognition of genes encoding enterotoxin production, in addition to classical detection methods, would allow identifying strains carrying genes that could produce, under the right conditions toxins other than those traditionally known toxins that could be capable of causing diseases to humans. The high proportion of cheese samples made from sheep's milk that are positive for Staphylococcal enterotoxin underlines the use of advanced animal management on the farm, especially the application of specific control programs to lower sub-clinical mastitis, that milk contaminate. An active reduction in contamination levels could be accomplished through improved hygiene procedures.

SIGNIFICANCE STATEMENT

This study could be preliminary to viewing the epidemiology of *S. aureus* isolates and recommended for more studies using molecular approaches for this purpose. A follow-up study using a large collection of *S. aureus* isolates from different regions of Iraq should be carried out to obtain a surveillance map that can be used for outbreaks. Such studies would involve molecular analysis of food poisoning, environmental and clinical isolates to evaluate sources of possible transmission.

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