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Research article

Detection of *Staphylococcus aureus* enterotoxins genes in food collected from local markets at Baghdad city

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ABSTRACT

This study represents the record of assessment for the level of contamination by *Staphylococcus aureus* of certain categories of animal source food and characterization of enterotoxigenic strains. That was done dependent on Polymerase Chain Reaction (PCR) for identification genes coding for the production of toxin by *Staphylococcus* (SE). On basis of that information were compared the results obtained by PCR approximately the presence in the tested strains of genes encoding the enterotoxin. Number of coagulase-positive staphylococci was performed. At the end of incubation period of 24 h at 37 ºC, the typical colonies were sub-cultured on triptycase soy broth, biochemical identification was subsequently completed in micro-method with the API Staph system. *S. aureus* were isolated from foods and confirmed by PCR with the identification of genus, species and toxins gene. From 350 tested food samples, (48.9%) were contaminated with *S. aureus*. From all tested samples, the percentage of contamination of fresh meat was 30.2 % from total number of samples, also including fresh meat preparations like (sausages) the percentage of contamination was 18.7%. The fresh meat contamination levels were varied from 5 CFU/gm to 720 CFU/gm. It was found 19 toxigenic strains isolated from meat products. They have proven to be carriers of genes coding to SE, in other hand there were 5 toxigenic strains isolated from pastry, ground beef and shell fish they were tested as a positive for SE. Use of innovative techniques for identification of genes encoding the production of enterotoxins, in addition to classic method of detection, allow identify gene carriers strains they could produce, in suitable conditions, toxins also different from those traditionally.

Keywords: Bacteria, Enterotoxins, Food safety, PCR, *Staphylococcus aureus*, Toxigenic

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INTRODUCTION

Staphylococcus aureus is one of bacterial agents, frequently identified as a cause of food poisoning [1]. There were many strains of *S. aureus*, considered a producers of specific exotoxins include staphylococcal enterotoxins (SEs) [2]. Historically, at different countries, there were many outbreaks reported, for example, at USA. *S. aureus* caused about 241000 illnesses case per year [3]. In Europe, between 1993 and 1998, the outbreaks were caused by *S.*

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aureus and showed variations ranging from 0.9% in Netherlands 13.6 % in France, also in Italy 1.8 % of poisoning were reported in 1998 which were attributed to this microorganism. At USA it is estimated that every year there are 185,000 cases of Foodborne Outbreak of staphylococcal enterotoxin, 1750 persons were hospitalized and 2 deaths [4,5]. In Iraq, another studies, *Staphylococcus* spp. were isolated from milk and white cheese samples were collected from markets at Baghdad city, there were 200 Staphylococcal isolates were isolated from milk and white cheese samples were collected from markets Baghdad city, there were 200 Staphylococcal isolates, S. aureus are predominant species, which were (97) isolates represent (48%), followed by (82) isolates of *S. chromogenes* represent (41%) and (21) isolates are represent (11%) *S. epidermidis* isolates [6]. The wide diffusion of carriers subject to *S. aureus* are (greater than 30-50% of the population), the contamination of food or Its ingredient during handling permanence of the product at temperatures not suitable and the ability of the microorganism develop in a wide spectrum of conditions pH, concentration of free water Sodium chloride and then on a wide range of alimentary products, represent the Main epidemiological characteristics that create the right conditions for the emergence of borne by *S. aureus* [7]. Also the liquid foods, such as raw milk, which is known as a Carrier for many pathogenic agents, with highly number of samples positive for *S. aureus*. In literatures they are given conflicting data, [8] report a percentage of contaminated raw milk with *S. aureus* with value of 27.4 to 37% in dairies, [9] was reported a low percentage levels of isolation.

The tolerance criteria currently in force towards *S. aureus* and its toxins in fresh cheese and meat are set out in Regulation of Commission of the European Community (CEC) 1441/2007 [10]. Foods commonly associated staphylococcal intoxication such as meat types like (beef, pork and chicken) also meat products, salads, cream pastries and dairy products. The pathogenic action of this microorganism is determined by the ability of certain strains of synthesizing one or more enterotoxins (SEs). [11] The percentage of enterotoxigenic strains was estimated to represent about 25% of isolates. The production of SE can start with low bacterial concentrations (10^3 CFU /gm), after Incubation times of 2 hours at 37 °C. In humans the symptoms may occur after ingestion of very small amounts of toxin nearly about (0.5 ng/ml) [12].

of toxin nearly about (0.5 ng/ml) [12]. This feature can, It enhanced by chemical and physical conditions food (pH, concentration of chloride sodium) that can determine the maintenance biological activity even after thermal treatment of industrial sterilization [11,12].

This study represents the report assessment of the level for contamination by *S. aureus* of certain animal source foods categories and the characterization for enteroto-xigenic strains, with a method of PCR for identification of genes coding for the production of SE [13,14,15]. On basis of the information were compared with the results that obtained by PCR, approximately, the presence of genes encoding the enterotoxin were in the tested strains

MATERIALS and METHODS

Sample collection

Along study period for one year, 350 selected samples of foods were examined for their Ease of availability and because of wide using among other types of food which they were meats, fresh cheese, products pastry and gastronomical preparations. All products samples were collected from Baghdad markets and transferred rapidly under refrigeration to laboratory.

Number of *S. aureus* isolates

The numbering of Coagulase-positive staphylococci was performed according to the operating mode reported in the second part of the ISO6888: [16]. At the end of the incubation period at 37 °C, the typical colonies were subcultured on Triptycase soy broth and subjected to tests to latex Staphytect plus (Oxoid, Basingstoke) for identification of *S. aureus*, according as provided by the AOAC method 995.12 [17]. The biochemical identification it was subsequently completed in micromethod with API Staph system (bioMérieux Marcy l'Étoile) in accordance with the producer.

Primers and detection of specific coding genes

The strains of *S. aureus* were isolated from foods and confirmed by PCR with identification of genus, species and toxins gene. The sequences of oligonucleotide primers were reported in **Table 1.**

Table 1. sequences Oligonucleotide primers for the region

Gene	Primer	Oligonucleotide sequence(5'-3')	bp of gene	Product (bp)	References
	TstaG422	GGCCGTGTTGAACGTGGCAAATCA	422-226	370	[22]
	Tstage 765	TTACCATTTCAGTACCTTCTGGTAA	765-792		
	Sa442-1	AATCTTTGTCGGTACACGATATTCTTCACG	5-34	108	[23]
	Sa442-2	CGTAATGAGATTTCAGTAGATAATACAACA	83-112		
sec	GSECR-1	AGATGAAGTAGTTGATGTGTATGG	432–455	451	[24]
	GSECR-2	CACACTTTTAGAATCAACCG	863–882		
sed	GSEDR-1	CCAATAATAGGAGAAAATAAAAG	492–514	278	[13]
	GSEDR-2	ATTGGTATTTTTTTTCGTTC	750–769		
see	GSEER-1	AGGTTTTTTCACAGGTCATCC	237–257	209	[25]
	GSEER-2	CTTTTTTTTCTTCGGTCAATC	425–445		

Identification of *S. aureus* and enterotoxins gene

To identify *S. aureus* and *sea*, *seb*, *sec*, *sed* and *see* genes PCR method was used [18]. For *Staphylococcus* genus and species an initial denaturation at 94 °C for 5 min was followed by 35 cycles of amplification (denaturation at 94 °C for 2 min, annealing at 57 °C for 2 min, and extension at 72 °C for 1 min), ending with a final extension at 72°C for 7 min. For enterotoxins genes multiplex PCR was also carried out with following amplification cycles: denaturation for 1 min at 94 °C, annealing of the primers for 1 min at 55 °C and extension of the primers of 2min at 72 °C; 35 cycles were performed of amplification using thermal cycler gradient PCR 22331 System (eppendorf).
To detect PCR products, each sample, represented by 5 µl of amplified in 1µl of buffer (gel solution loading, concentration 1-6x, Sigma, St Louis, Missouri) was subjected to electrophoresis in agarose gel at 1.5% (Agarose, promega) at 100 volts for 1h. For estimating the number of base pairs of fragments standard weight molecular of 100-1500 bp (Amplisize TM Molecular Ruler, Bio-Rad, Hercules, USA) was used.

RESULTS and DESCUSION

Levels of contamination by *S. aureus* food examined

From 350 food samples 48.9% were contaminated with *S. aureus*. Fresh meat was contaminated with *S. aureus* (30.2%), this including fresh meat preparation (sausages 18.7%) (**Table 2**). For fresh meat, the contamination levels were varied from 5 CFU/gm to 720 CFU/gm.

Table 2. Distribution of meat products and fresh cheese with positive test results

Products	No. of tested samples	No. of positive samples of *S. aureus*	Percentage of isolates
fresh meat (ground meat, poultry)	40	12	30.2
meat products (sausages fresh)	162	27	18.7
fresh sheep cheese	9	3	33.3
fresh cow cheese	21	1	4.7
Pastry products (pastas cream)	55	2	3.6
Gastronomical products (timbale)	13	1	7.6
seafood (mussels)	50	3	6.0
Total products	350	49	14.0

While, for sausages, It it was observed that the level of contamination from 30 CFU/gm to 2900 CFU/gm. The contamination in fresh cheese samples is found to be 33.3% for sheep cheeses and 4.8% for bovine fresh cheese (**Table 2**). For fresh cheese, levels contamination ranged from 50 CFU/gm to 2800 CFU/gm. **Table 3** reported the percentage of carriers strains genes coding for staphylococcal toxins. The distribution of contamination levels, with reference to the limits, set by legislation force for this category of products and the concentrations of *S. aureus* necessary for the production of enterotoxins **(Table 3**, **4** and **Fig 1)**. Pattern electrophoretic PCR multiplex for display Staphylococcal genes identification (**Fig 3**). **Table 3** shows percentage of strains coding for Staphylococcal toxins of 4 isolates isolated from cheeses, only 1 strain positive for PCR (*sea*).

Table 3. Distribution of levels of contamination by *S. aureus* in positive samples of cheese (amounts are expressed in CFU/gm for solid samples and CFU/ml for liquids). a, limit "m" of acceptability for fresh cheeses according to CE/1441/2007; b, limit "M" of acceptability for fresh cheeses according to CE/1441/2007; c, level of contamination by *S. aureus* for the production of SE [13].

Matrix	0-10[a]	10-100[b]	100-1000	>1000[c]
fresh sheep cheese	0	0	2 x (50%)	1(25%)
fresh cow cheese	0	0	0	1(25%)

Table 4. Distribution of levels of contamination by *S. aureus* found in samples of fresh meat and meat preparations (amounts are expressed in CFU/gm). a, limit "m" of acceptability for fresh cheeses according to CE/1441/2007; b, limit "M" of acceptability for fresh cheeses according to CE/1441/2007; c, level of contamination by *S. aureus* for the production of SE [13]

Marix	0-100[a]	100-1000[b]	>1000[c]
Fresh meat	3(11.1%)	19(70.4%)	5(18.5%)
fresh sausages	6(50%)	6(50%)	0

Identification of enterotoxigenic strains

Forty nine isolates of *S. aureus*, 19 were producers SE. The percentage of food contaminated with enterotoxigenic isolates that isolated from meat gave positive to the presence of one or more genes encoding enterotoxin as shown in **table 5** and **fig 2**.

From the 350 food samples, were taken at retail outlets and belonging to the category of food most widespread, 49 (14%) were contaminated with *S. aureus*. Foods that with greater frequently they were contaminated with *S. aureus* were the meat preparations and the fresh cheese (19.3% and13.3%). The results were evaluated and taking as a reference, the lower limit "M" (defined by the reference standard force) for which product that respects this limit is considered "satisfactory", it was proposed 1000 CFU/gm or ml (depending on the liquid or solid nature of the analyzed product) which corresponds to the concentration of *S. aureus* in which some authors reported production of SE [19,20]. Specimen of fresh meat examined, only 11.1% was comply with the limits and therefore less than 100 CFU/gm, while the 18.5% experienced an upper charge to 1000 CFU

Table 5. Distribution of Enterotoxins genes SE among *S. aureus* isolates**,** X, Positive results

Strain No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
(Sec)	x	x	x	x	x	x	x	x	x	x					x	x			
(Sed)	x				x	x	x	x		x		x	x				x		
(See)	x	x							x	x	x	x		x	x	x	x	x	x

/gm, between another level considered suitable for the production of toxins SE [13]. However, as regards fresh cheese, 25% of samples result has more than 1000 CFU/gm, which is the limit level of contamination by S. aureus proposed in the literature for production of staphylococcal enterotoxin. PCR method showed 48.9% of isolates were enterotoxigenic, related to toxins C-E (sec, sed and see), genes also have been highlighted. Nineteen toxigenic strains were isolated from meat products, they have proven to be carriers of genes coding SE. as they proved by [21], 5 toxigenic strains were isolated from pastry, ground beef and shell fish were they tested as positive for SE. . In particular, (12) isolates have the gene encoding the sec, (9) isolates have two genes coding for SE (4 sec and sed, 3 sed and see, 2 sec and see), also (4) isolates of S. aureus encoding three genes (sec, sed and see) as shown in (**Table 5**).

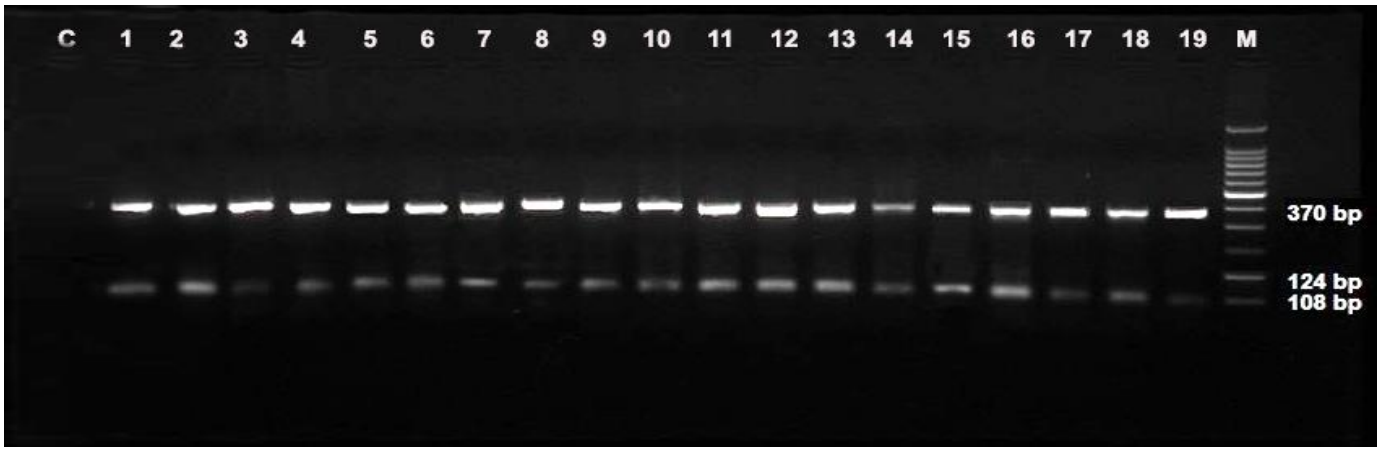

Fig 1. Amplification of *TstaG* and *Sa422*. M, 100-bp ladder; Lane 1-19, amplified *gene* of *TstaG* for *Staphylococcus spp with 370bp*; *Sa422* for *S. aureus* with 108 bp, negative control.

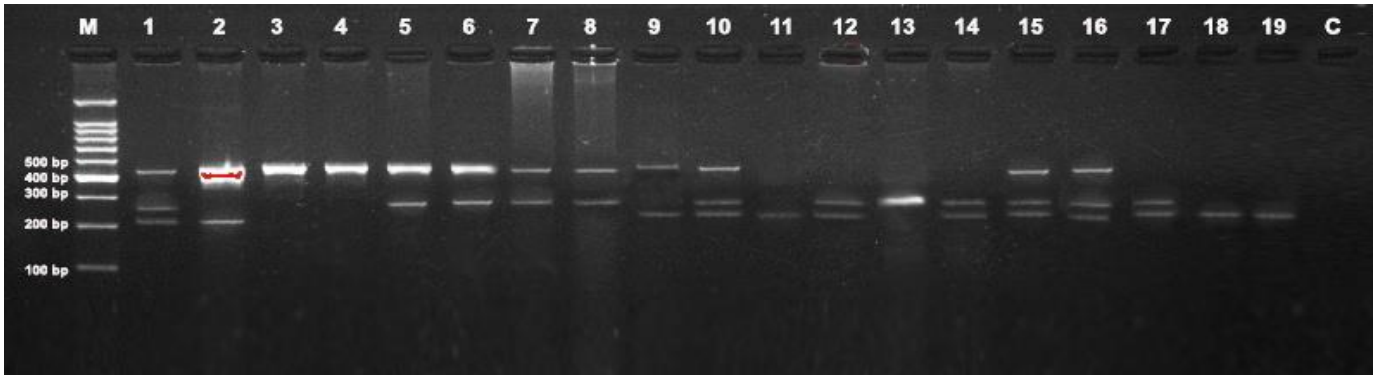

Fig 2. Agarose gel electrophoresis patterns showing multiplex PCR amplification products of *S. aureus* enterotoxins genes. Lanes M, DNA molecular size marker (100-bp ladder), lanes 1 to 19 PCR amplicons of *sec*, *sed* and *see* genes; C, negative control.

Number of S. aureus cells in different foods for human consumption prevailing standard for quality hygienic food health is planned search of staphylococcal toxins. The number of S. aureus cells can, however, not be an indicator of the presence of enterotoxins in the product and the microorganism may no longer be viable but having produced enterotoxin which persists in the food or the enterotoxin produced. It can be present in an amount less than the detection limit of the methods available in to detect the Staphylococcus spp.

This study shows a widespread presence of *S. aureus* which carriers of genes encoding toxins other than those identified with traditional methods. For the toxins, which is not known yet, the real meaning of their presence in food and actual impact that they may have as a cause of *S. aureus* food poisoning in humans, it's necessary to carry out further studies, supporting traditional methods and molecular methods. Use of innovative techniques for the identification of genes encoding the production of enterotoxins, in addition to classic method of detection,

which allow identify gene carriers strains, they could produce, in suitable conditions, toxins also different from those tradition-ally, that may be able to cause human disease . Effective reduction of the levels of contamination could be achieved through the improvement of hygiene procedures health. It can be concluded that S. aurues high prevalence among tested food samples highlighted the necessity of enforcement of hygienic implementations and practice. The presence of S. aureus in retailed meats and other foods. In this study, high numbers of S. aureus were observed in all common meat types sold in the market. Other types of super antigens (sec, sed and see) were detected in all meat samples. As future work, subsequent molecular and ecological characterization of isolated S. aureus should fellow.

Conflict of interest

The authors declare that they have no conflict of interests.

REFERNCES

1. **Le Loir Y, Baron F, Gautier M.** (2003) *Staphylococcus aureus* and food poisoning. *Genet Mol Res*, **2** (1): 63-76.
2. **Becker K, Roth R, Peters G.** (1998) Rapid and specific detection of toxigenic Staphylococcus aureus: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes and toxic shock syndrome toxin 1 gene. *J Clin Microbiol* **36**:2548-2553.
3. **Escallan RM, Hoekstra FJ, Angulo et al.** (2011) Foodborne illness acquired in the United States – major pathogens. *Emerg Infect Dis* **1**: 7-15.
4. **European Commission (EC).** (2003) Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on staphylococcal enterotoxin, especially in cheeses (adopted on 26-27 March 2003). EC, Directorato C - Scientific Opinion, Unit C2 - Management of the scientific committee, scientific cooperation and networks. Brussell. 73.
5. **Wieneke AA, Roberts D, Gilbert RJ.** (1993) Staphylococcal food poisoning in the United Kingdom, 1969-1990. *Epidemiol Infect* **110**: 519-531.
6. **Al-Khafaji MH, Flayyih MT, Sabah MA.** (2014) Methicillin resistance and enterotoxigenicity of Staphylococci isolated from Milk and white Cheese in Iraq. *Iraqi J Sci* **55**: 40-49.
7. **Firinu A, Virgilio DI, Mula GA, Poggio Zuccon F, Sias S.** (2003) *Staphylococcus aureus* in foods. Presence and characterization enterotoxic. *Food Industries*. **XLII**. 613-623.
8. **Oliver SP, Boor KJ, Murphy SC, Murinda SE.** (2009) Food safety hazards associated with consumption of raw milk. *Foodborne Pathog Dis* **6**: 793-806.
9. **D'Amico DJ, Groves E, Donnelly CW.** (2008) Low incidence of foodborne pathogens of concern in raw milk utilized for farmstead cheese production. *J Food Prot* **71**: 1580-1589.
10. **Commission of the European Community (CEC) N °** (2007) of Commission Regulation (EC). 1441/2007 of 5 December 2007 amending Regulation (EC) N °. 2073/2005 on microbiological criteria for foodstuffs. Off J, L 322, 12.7 / 2007.12-13.
11. **Bayles KW, Iandolo JJ.** (1989) Genetic and molecular analyses of the gene encoding staphylococcal enterotoxin D. *J Bacteriol* **171**: 4799–4806.
12. **Betley MJ, Mekalanos J.J.** (1988) Nucleotide sequence of the type A staphylococcal enterotoxin gene. *J Bacteriol* **170**: 34-41.
13. **Balaban N and Rasooly A.** (2000) Staphylococcal enterotoxins. *Int J Microbiol* **61**:1-10.
14. **Larsen, HD, Huda A, Eriksen, NHR, Jensen NE.** (2000) Differences between Danish bovine and human Staphylococcus aureus isolates in possession of superantigens. *Vet Microbiol* **76**: 153-162.
15. **Marrack P, Blackman M, Kushnir E, Kappler J.** (1990) The toxicity of staphylococcal enterotoxin B in mice is mediated by T cells. *J Exp Med* **171**:455–464.
16. **International standard. First edition** (2003) Microbiology of food and animal feeding stuff-horizontal method for the enumeration of coagulase – positive Staphylococci (*Staphylococcus aureus* and other spcies)-Part 1: technique using Baird-Parker Agar Medium. International Organization of Standardization. **1**-11.
17. **AOAC Afficial Method 995.12.** (1996) *Staphylococcus aureus* isolated from Foods Latex Agglutination Test Method. *J AOAC Int* **79**. 166.
18. **Morto-Bizot S, Talon S, Leroy S.** (2004) Development of a multiplex PCR for the identification of Staphylococcus genus and four staphylococcal species isolated from food. *J Appl Microbiol* **97**: 1087–1094.
19. **Lo Nostro A, Pesavento G. Tempestini E, Gariboldi A** (2003). Circulation of toxigenic strains of S. aureus in foods and surfaces in a catering business. National Conference Proceedings the Staphylococcus aureus and their toxins in the food chain: diseases, prevention, analytical research. Bologna. Oxoid, Bologna. 105-110.
20. **Firinu A, Virgilio DI, Mula GA. Poggio Zuccon F, Sias S.** (2003) *Staphylococcus aureus* in foods. Presence and characterization enterotoxic. *Food Industries* **XLII**, 613-623.
21. **Zschöck M, Botzelr D, Blöcher S, Sommerhäuser J, Hamann HP.** (2000) Detection of genes for enterotoxins (ent) and toxic shock syndrome toxin-1 (tst) in mammary isolates of *Staphylococcus aureus* by polymerase chain reaction. *Int Dairy J* **10**: 569-574.
22. **Martineau F, Picard, FJ, Ke D, Paradis S, Roy PH, Ouellete M, Bergeron MG.** (2001). Development of a PCR assay for identification of Staphylococci at genus and species level. J Clin Microbiol 39: 2541-2547.
23. **Martineau F, Picard, FJ, Ke D, Paradis S, Roy PH, Ouellete M, Bergeron MG.** (1998). Species-specific and ubiquitous-DNA- based assays for rapid identification of Staphylococcus aureus. J Clin Microbiol 36: 618-623.
24. **Bohach GA, Schlievert PM.** (1987). Nucleotide sequence of the staphylococcal enterotoxin C1 gene and relatedness to other pyrogenic toxins. Mol Gen Genet 209:15-20.
25. **Dinges MM, Orwin PM, Schlievert MPM**. (2000). Exotoxins of Staphylococcus aureus. Clin Microbiol Rev. 13(1): 16-34.

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