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Laboratory Diagnosis of Staphylococcal Enterotoxins Causing Food Poisoning

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Abstract: Staphylococcal enterotoxins are members of a family of more than 20 different staphylococcal and streptococcal exotoxins that are functionally related and share sequence homology. It colonizes humans as well as domestic animals, and is a common opportunistic pathogen. It is estimated that *S. aureus* is persistent in 20% of the general population, while another 60% are intermittent carriers. Most frequently, the anterior nares is the site of colonization in humans, and this colonization increases the risk of infections when host defenses are compromised. This is supported by multiple observations. For instance, the frequency of infections is higher in carriers than in non-carriers. Non-carriers commonly acquire infections through contaminated food or when food handlers who are carriers contaminate food during preparation. Although there are more than 20 distinct staphylococcal enterotoxins, only a few of them have been studied in depth These bacterial proteins are known to be pyrogenic and are connected to significant human diseases that include food poisoning and toxic shock syndrome. These toxins are for the most part produced by *Staphylococcus aureus* (*S. aureus*) although other species have also been shown to be enterotoxigenic. *S. aureus* is an ubiquitous Gram-positive coccus of approximately 1 μm in diameter and forms clusters. There are several methods for detection of enterotoxigeneic bacteria. The phenotypical methods are not reliable in specificity, because staphylococcal enterotoxins serotypes are antigenically similar. On the other hand, commercial serological kits can not detect all the serotypes and are limited in serotypes (A-B). Therefore, molecular techniques such as multiplex PCR and real-time PCR are recommended for detection of *Staphylococcus aureus* enterotoxins genes.

Keywords: *Staphylococcus aureus*, Enterotoxigeneic Bacteria, Polymerase Chain Reaction (PCR)

1. Introduction

Staphylococcus aureus Food Poisoning (FP) is a common cause of food-borne disease worldwide [1]. Staphylococcal food poisoning (SFP) is caused by staphylococcal enterotoxins (SEs) preformed in food materials while SE genes are encoded on mobile genetic elements [2]. Symptoms of Staphylococcal food-borne disease (SFD) include nausea, vomiting, and abdominal cramps with or without diarrhea. Preventive measures include safe food handling and processing practice, maintaining cold chain, adequate cleaning and disinfection of equipment, prevention of cross-contamination in home and kitchen, and prevention of contamination from farm to fork. This paper provides a brief overview of SFD, contributing factors, risk that it imposes to the consumers, current research gaps, and preventive measures [3]. Classically, enterotoxins from *Staphylococcus aureus* strains can be classified into 18 serological types: A-U (except S, F and T) [4].

Most enterotoxin serotypes are heat stable and may resist inactivation by gastrointestinal proteases like pepsin. The B and C serotypes are cleaved by digestive enzymes in the cysteine loop site, but this cleavage is not effective against their toxicity and antigenic properties [5].

Staphylococcal enterotoxin (SE) A and SEB are two of the most important gastroenteritis causing agents. In some areas, more than 50% of FP is caused by SEA. SEA and SEB are the most FP agents (>60%) in USA and England [6]. The most common staphylococcal enterotoxins are SEA and SEB. SEA is the most common toxin in staphylococcus-related

food poisoning. SEB, while it is associated with food poisoning, has been studied for potential use as an inhaled bioweapon. Staphylococcal food-borne diseases acquired from eating enterotoxin-contaminated food are the second most commonly reported types of food-borne diseases. The high incidence of staphylococcal food poisoning is due to the insufficient pasteurization/decontamination of originally contaminated product source or its contamination during preparation and handling by individuals who are carriers of the organism. They are encoded by genes embedded in mobile genetic elements such as phags and pathogenicity islands [7].

Staphylococcus aureus nasal carriage is established constantly in 20%–40% of healthy human population and intermittently in 60% and only 10%–20% of people are non-carriers [8]. Staphylococcal enterotoxins are low molecular weight proteins (MW 26.900-29.600 KD). These are encoded by genes embedded in mobile genetic elements such as phages, (not in plasmids) and pathogenicity islands [9]. Therefore, molecular techniques such as PCR and real-time-PCR are recommended for detection of *Staphylococcus aureus* enterotoxin genes [10].

In this study, genotypic method is utilized to detect *Staphylococcal* enterotoxins A and B genes. Furthermore, we used these methods to examine the contamination rate of traditional dairy products by *Staphylococcus aureus.*

2. Materials and Methods

I. Sample collection.

One hundred samples of Milk and Milk products were collected randomly from different areas.

II. Processing of samples: [11].

III. Bacteriological identification:

Media used in cultivation:

1. Cooked meat with 9% NaCl (Oxoid, UK)
2. Baird Parker agar with Egg Yolk Emulsion (Oxoid, UK)
   a. Baird-Parker agar
   b. Egg Yolk Tellurite Emulsion: Sterile egg-yolk 200 ml; NaCl 4.25 g; potassium tellurite 2.1 g distilled water to give a final volume of 1000 ml.
3. Mannitol salt agar (OxoidUK)

IV. Identification of the suspected colonies:

1. A smear from the suspected colonies was stained by Gram stain and examined under microscope.
2. Catalase reagent [12].
3. Tube coagulase test

V. Mainteenance of the selected isolates

VI. Multiplex PCR for detection of sea & seb genes:

A) DNA extraction:

1. DNA extraction kit: i-genomic BYF DNA Extraction Mini Kit, (Cat. No. 17361) (iNtRON Biotechnology, Korea).
2. Additional required equipment and reagent:

B) DNA amplification:

Material for DNA amplification: PCR Premix: 2x PCR Master mix Solution (i-Taq™) tubes (Cat. No. 25027 "1ml", 25028 "5 ml") (iNtRON Biotechnology, Korea).

C) DNA detection by gel electrophoresis

VII. Statistical analysis of the results:

The results were calculated, tabulated and statistically analyzed. The collected data were entered, checked and analyzed using chi square (x^2) according to knapp and Odds ratio Using statistical computer program SPSS version II under windows 7 as follow:

$$x^2 = \sum \frac{(O-E)^2}{E}$$

3. Results

The present work was carried on 100 samples of milk and milk products collected randomly from different areas in Prot Said Governorate.

Cheese samples were 30; they were 10 samples of karish cheese, 5 samples of damitta cheese, 5 samples of rommy cheese, 5 samples of cheedar cheese and 5 samples of cooked cheese. Milk samples were 30; they were 20 samples of raw milk, 6 samples of pasteurized milk and 4 samples of powdered milk. Yoghurt samples were 20; they were 10 samples of canned yoghurt and 10 samples of hand-made yoghurt. Also 10 samples of cream and 10 samples of butter were examined

Table 1. *Positive samples for Staphylococcus aureus in milk and milk products.*

Sample type	No. of samples	Positive samples No. (%)	Negative samples No. (%)	Odds ratio 95% CI	X2	P. value
Milk	30	16 (53.3)	14 (46.6)	1.31 (0.42-4.09)	0.27	0.606
Cheese	30	15 (50.0)	15 (50.0)	1 (0.32-3.11)	0.0	1.0
Cream	10	4 (40.0)	6 (60.0)	0.44 (0.05-3.7)	0.8	0.371
Butter	10	4 (40.0)	6 (60.0)	0.44 (0.05-3.7)	0.8	0.371
Yogurt	20	7 (35.0)	13 (65.0)	0.29 (0.06-1.27)	3.6	0.06
Total	100	46 (46.0)	54 (54.0)			
X2		2.11				
P. value		0.716				

Figure 1 show the percentage of positive samples for *S. aureus* in different dairy products, it was (50%) for cheese, (53.3%) for milk, (30%) for cream, (40%) for butter and (45%) for yoghurt. The relation between positive samples for *S. aureus* in all

types of milk and milk products was statistically insignificant (P > 0.05).

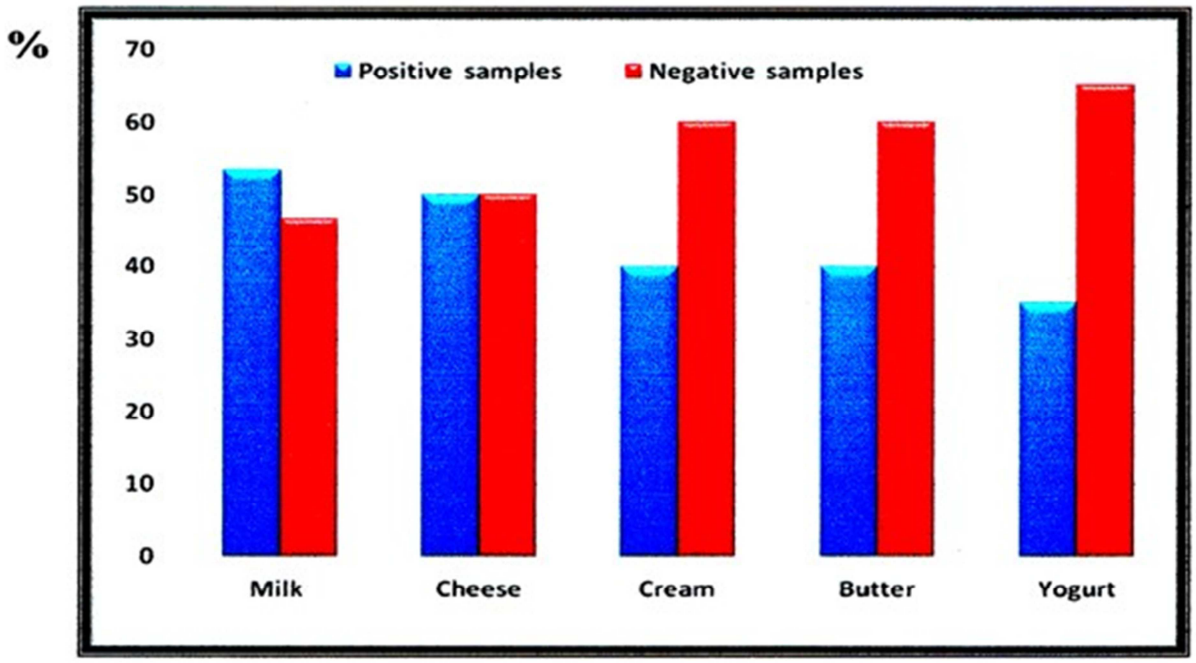

Figure 1. *Frequency of staphylococcus aureus in milk and milk products.*

Figure: 2 show that there is no statistically significant difference between positive samples for SE genes in different types of milk and milk products (P > 0.05). The percentage of SE genes in milk was (50%), also in cream it was (50%), while in yoghurt the percentage was (42.3%), (33.3%) in cheese and (25%) in butter.

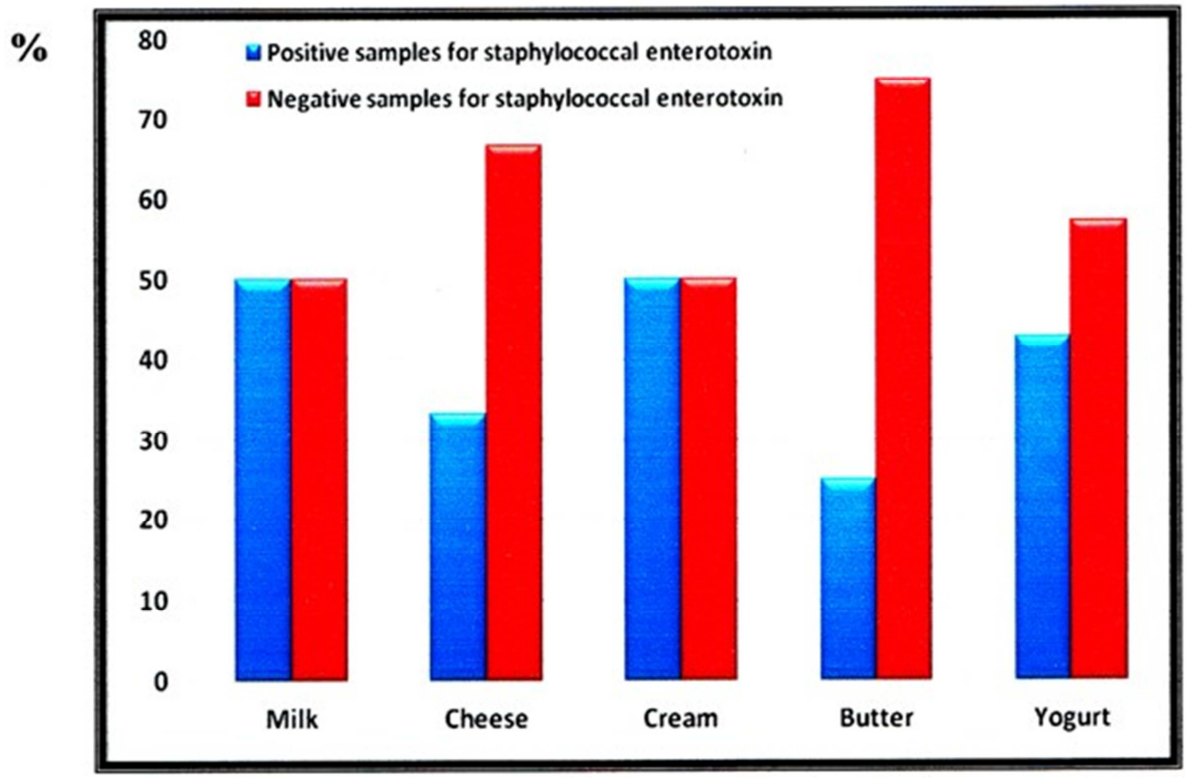

Figure 2. *Staphylococcal enterotoxin genes isolated by Multiplex PCR in milk and milk products.*

Figure 3 show number and percentages of Staphylococcal enterotoxins genes isolated by Multiplex PCR. The relation

between the detected SE genes (sea, seb, sea + seb) within all types of milk and milk products was statistically insignificant ($P > 0.05$)

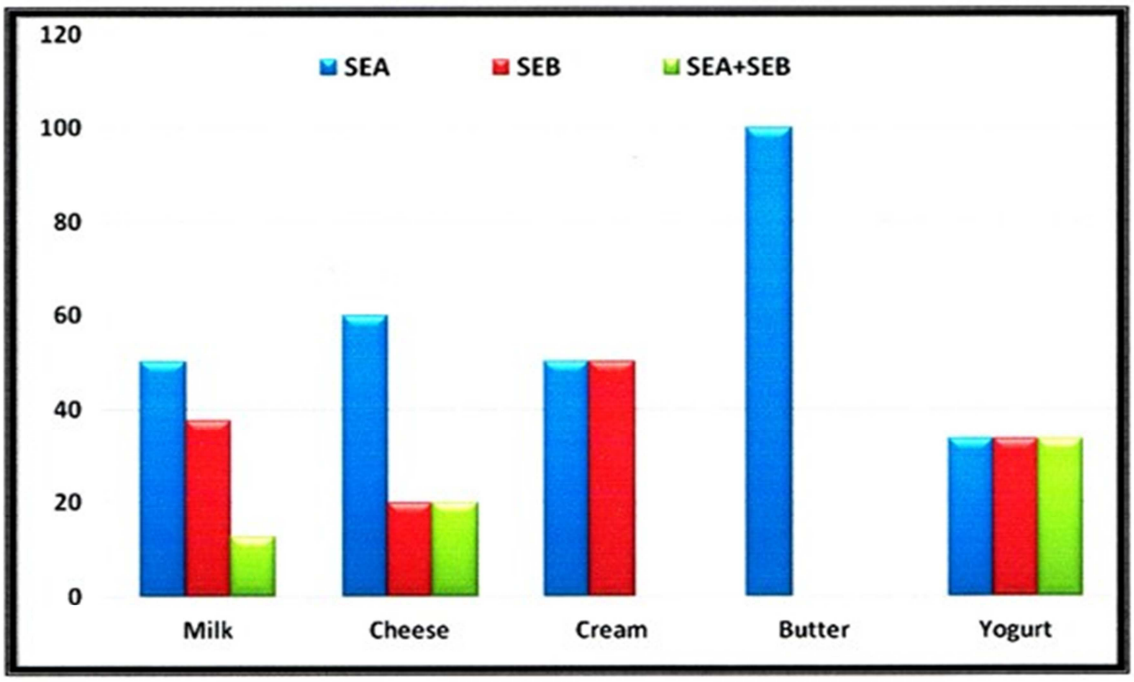

Figure 3. *Staphylococcal enterotoxin genes isolated by Multiplex PCR in Milk and Milk products.*

Figure 4 showing agarose gel electrophoresis for PCR of 270 bp sea gene and 165 bp seb gene. (Lane M) is the (100 bp ladder marker), (Lane 2) is for sea, (Lane 3) is for seb, (Lane 4) is for both genes (sea & seb)

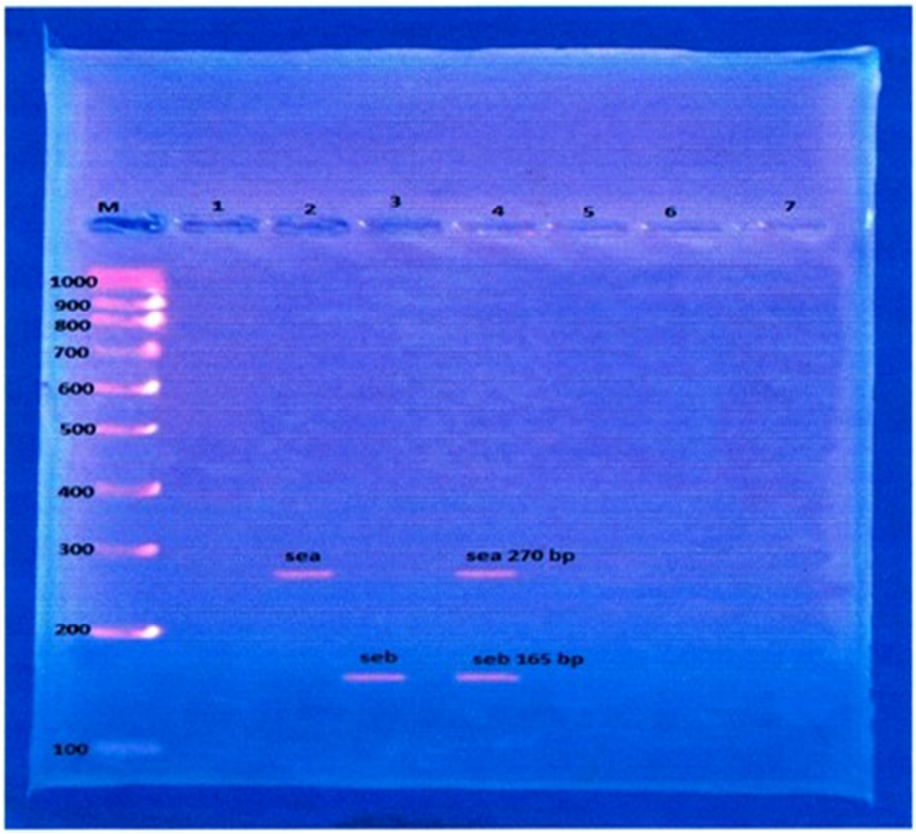

Figure 4. *Agarose gel electrophoresis for PCR of sea and seb genes.*

4. Discussion

Staphylococcus aureus is a facultative anaerobic Gram-positive coccus; it is non-motile and catalase and coagulase positive. Cells are spherical single or paired cocci, or form grape-like clusters (*staphylo* means grape in greek) [13].

Staphylococcal enterotoxins are members of a family of more than 20 different staphylococcal and streptococcal exotoxins that are functionally related and share sequence homology. *Staphylococcus aureus* is one of the most important pathogen in food poisoning, due to its wide spread and ability of many strains to synthesize one or more enterotoxin. It causes gastroenteritis symptoms like nausea, vomiting, abdominal cramps and diarrhea [14, 15].

Contamination of milk after pasteurization indicated transmission from food handlers or bad storage conditions. Pasteurized milk is deficient in microflora which compete the growth of *S. aureus,* so contamination of pasteurized milk is more dangerous and very hazardous to consumers [16]. Contamination of milk after pasteurization indicated transmission from food handlers or bad storage conditions. Pasteurized milk is deficient in microflora which compete the growth of *S. aureus,* so contamination of pasteurized milk is more dangerous and very hazardous to consumers [16].

SEA is most commonly reported in food stuffs. It is also considered as the main cause of Staphylococcal food poisoning, probably due to its extraordinary high resistance to proteolytic enzymes [17, 18]. While *S. aureus* organism is heat labile, its produced enterotoxin is heat stable. Hence the importance of Multiplex PCR technique in detecting genes encoding enterotoxigenic strains especially in food poisoning outbreak.

5. Conclusion

World health organization defines food-borne disease (FBD) as “disease of infectious or toxic nature caused by the consumption of food or water. *S. aureus* is a significant cause of food-borne disease. Identification of enterotoxigenic strains of *S. aureus* should be used as part of risk analysis of milk and milk products. Bacteriological detection of *S. aureus* is better by Baird-Parker agar medium. Multiplex PCR is a reliable and valuable method in detection of enterotoxigenic *S. aureus* strains.

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