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Prevalence of *Staphylococcus aureus* in Raw Milk and Some Dairy Products in Port Said Governorate

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Abstract: *Staphylococcus aureus* Food Poisoning is a common cause of food-borne disease worldwide. Of particular relevance is the ability of some *Staphylococcus aureus* strains to produce heat stable enterotoxins that cause *staphylococcal* food poisoning, which ranks as one of the most prevalent worldwide causes of gastroenteritis. Several studies have shown that 15% to 80% of the *Staphylococcus aureus* isolated from various sources (dairy products, ice heavy cream, meat products) is able to produce enterotoxin. Classically, enterotoxins from *Staphylococcus aureus* strains can be classified into 18 serological types: A-U (except S, F and T), most of these serotypes are heat stable. The percentage of samples of milk and milk products contaminated with *S. aurues* in the current study were 46% which is higher than that reported by who detected *S. aureus* in 17 % and 32 % respectively of the analyzed samples of milk and milk products. Staphylococcal enterotoxins are low molecular weight proteins (MW 26.900 – 29,600).They are encoded by genes embedded in mobile genetic elements such as phages and pathogenicity islands .There are several methods for detection of enterotoxigeneic bacteria. The phonotypical 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 polymerase chain reaction and real-time polymerase chain reaction are recommended for detection of Staphylococcus aureus enterotoxins genes.

Keywords: Staphylococcus Aureus, Gastroenteritis, Enterotoxins, Polymerase Chain Reaction

1. Introduction

Staphylococcus aureus Food Poisoning is a common cause of food-borne disease worldwide. Of particular relevance is the ability of some *Staphylococcus aureus* strains to produce heat stable enterotoxins that cause *staphylococcal* food poisoning, which ranks as one of the most prevalent worldwide causes of gastroenteritis [1].

The importance of the enterotoxins comes due to their heat stability and their resistance to inactivation by gastrointestinal proteases like pepsin. Although *Staphylococcus* can be killed at normal cooking temperature, the toxins remain active [2].

Also they are potent even in very small amount ranging from 20 ng to < 1μg can produce symptoms to human beings [3].

Classically, enterotoxins from *Staphylococcus aureus* strains can be classified into 18 serological types: A-U (except S, F and T), most of these serotypes are heat stable [4]. 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 [1].

Staphylococcal enterotoxins are low molecular weight proteins (MW 26.900-29.600 KD).There are several methods for detection of enterotoxigenic bacteria. The phenotypical methods (agglutination, SRID) are not reliable in specificity, because SE serotypes are antigenically similar [5].

2. Materials and Methods

One of the goals of this study is to determine the presence of enterotoxin genes among isolated strains of *S. aureus* from dairy products. To achieve this goal, Multiplex PCR technique was applied on *S. aureus* colonies from each positive sample isolated from culture on Baird- Parker agar. 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*.

2.1. Dairy Specimen Collection and Screening

A total 120 samples of Milk and Milk products were collected randomly from different areas in Prot Said governorate during the period form July 2017 to October 2017. All samples were kept at 4°C in insulated ice box and transferred to the dairy microbiology laboratory, Food control department, Zagazig University and analyzed within 4 h of collection.

Table 1. *Types and Number of Diary samples.*

Sample type	Number of samples
Raw Milk	25
Pasteurized Milk	6
Powdered Milk	4
Hand-made Yogurt	15
Canned Yogurt	15
Heavy cream	10
Unsalted butter	10
Karish Cheese	15
Damitta Cheese	5
Istanboly Cheese	5
Baramily Cheese	5
Rommy Cheese	5

Samples were collected divided into 5 groups, Group (I) Milk represented 35 of total Samples, while group (II) Cheese represented 35& group (III) Yogurt represented 30 &group (IV) Heavy cream represented 10 and group (V) Unsalted butter represented 10 illustrated by Table 1.

2.2. Processing of Samples: [6]

1 ml milk sample was added to 9 ml saline , or 10 ml gram of each dairy product sample was cutted by a steile knife and added to 90 ml saline, to be homogenized, the mixture was placed in disposable, sterile polyethylene bag (Stomacher bag) to be inserted in the Stomacher machine.

2.3. Bacteriological Identification

All collected samples were transferred to laboratory within 4 hours for cultivation and identification.

2.4. Identification of Suspected Colonies

A smear from the suspected colonies was stained by Gram stain and examined under microscope of the suspected colonies.

2.5. Biochemical Tests for Identification of Bacterial Isolate

1- Tube coagulase test, 2- Catalase test.

2.6. Multiplex PCR For Detection of Sea & Seb Genes

A) DNA extraction B) DNA amplification.

2.7. Statistical Analysis of Results

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

3. Results

120 samples of Milk and Milk products were collected randomly from different areas in Prot Said governorate.

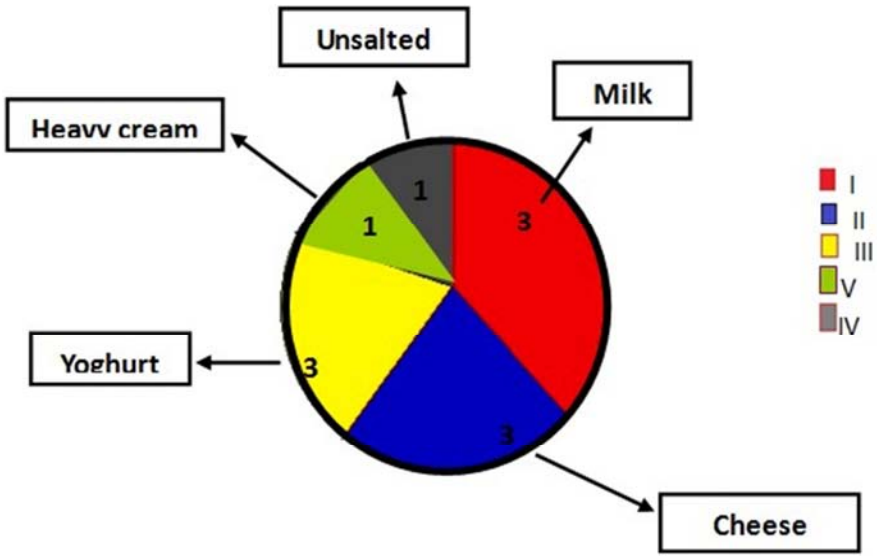

Figure 1. *Pie Chart Showing Frequency of Milk and Milk products different Groups.*

Table 2. *Comparison between media used in isolation of Staphylococcus aureus.*

Type of media	Positive samples for *S. aureus*	Percentage of positive samples	X2	Pvalue
Mannitol Salt agar	47	47%	1.85	0.23
Baird-parker agar	56	56 %		

Table 2 and figure 2 show the comparison between Mannitol Salt agar and Baird- Parker agar in isolation of *Staphylococcus aureus.* The percentage of positive samples by Mannitol Salt agar was (47%) while the percentage by Baird-Parker was (56%). This comparison was statistically insignificant (P > 0.05).

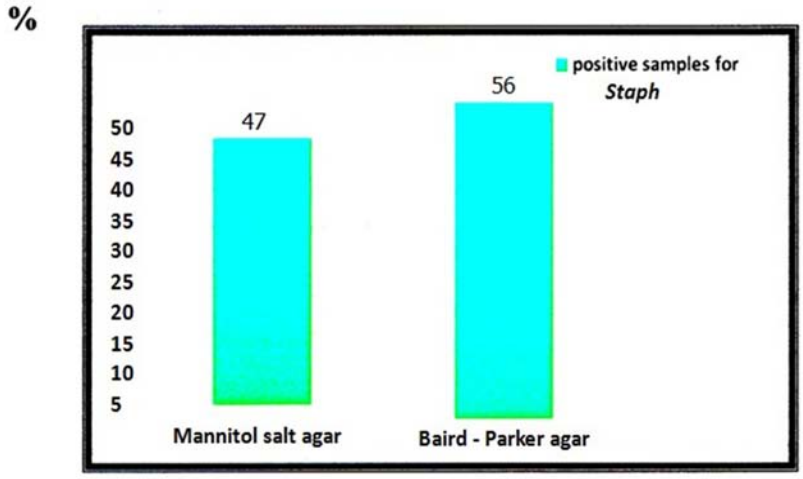

Figure 2. *Comparison between media used in isolation of Staphylococcus aureus.*

Table 3. *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
Cheese	35	15 (40.0)	20 (60.0)	1.31 (0.32-3.11)	0.15	0.21
Milk	35	22 (53.3)	13 (46.6)	1.38(0.42-4.09)	0.27	0.607
heavy cream	10	3 (30.0)	7 (70.0)	0.46 (0.05-3.7)	0.9	0.382
Unsalted butter	10	3 (30.0)	7 (70.0)	0.46 (0.05-3.7)	0.9	0.382
Yogurt	30	10 (35.0)	20 (65.0)	0.28 (0.06-1.27)	3.6	0.07
Total	120	53 (53.0)	67 (67.0)			
X2		3.11				
P. value		0.817				

Table 3 and figure 3 show the percentage of positive samples for *S. aureus* in different dairy products, it was (40%) for cheese, (53.3%) for milk,(30%) for heavy cream, (30%) for Unsalted butter and (35%) for yoghurt. The relation between positive samples for *S. aureus* in all types of milk and milk products was statistically inAsignificant (P > 0.05).

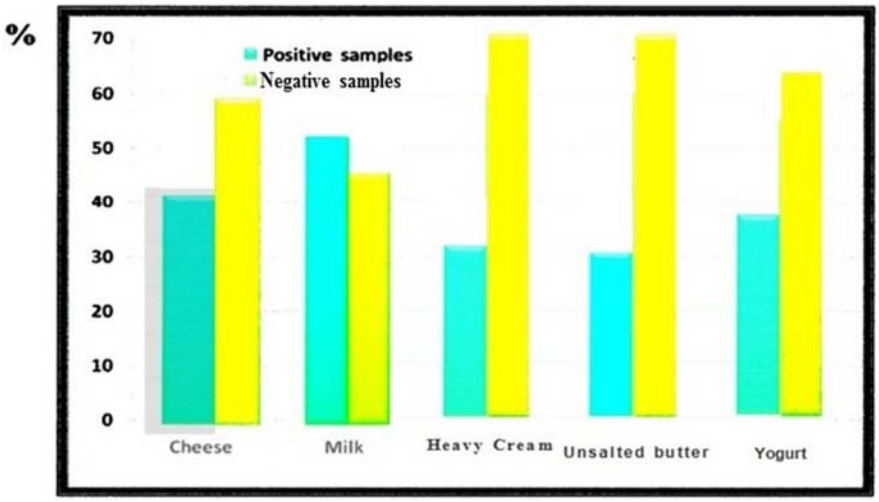

Figure 3. *Frequency of Staphylococcus aureus in milk and milk products.*

Table 4. *Positive samples for Staphylococcus aureus in different types of Cheese.*

Sample type	No of samples	Positive samples No (%)	Negative Samples No (%)	Odds ratio 95%CI	X2	P.value
Karish cheese	15	9 (75.0)	6 (25.0)	5.45 (0.58-63.02)	3.25	0.074
Damitta cheese	5	3 (60.0)	2 (40.0)	2.35 (0.1-68.48)	0.4	0.527
Rommy cheese	5	3 (60.0)	2 (40.0)	2.35 (0.1-68.48)	0.4	0.527
Istanboly cheese	5	1 (20.0)	4 (80.0)	0.07 (0.0-2.31)	3.7	0.06
Baramily cheese	5	1 (20.0)	4 (80.0)	0.07 (0.0-2.31)	3.7	0.06
Total	35	17 (40.0)	18 (60.0)			
X2		5.71				
P. value		0.235				

Table 4 and figure 4 show that there is no statistically significant difference between numbers of positive samples for *S. aurues* in different types of cheese (P > 0.05). The percentage was (75%) in karish cheese, (60%) in both damitta and rommy cheese, (20%) in both istamboly and baramily cheese.

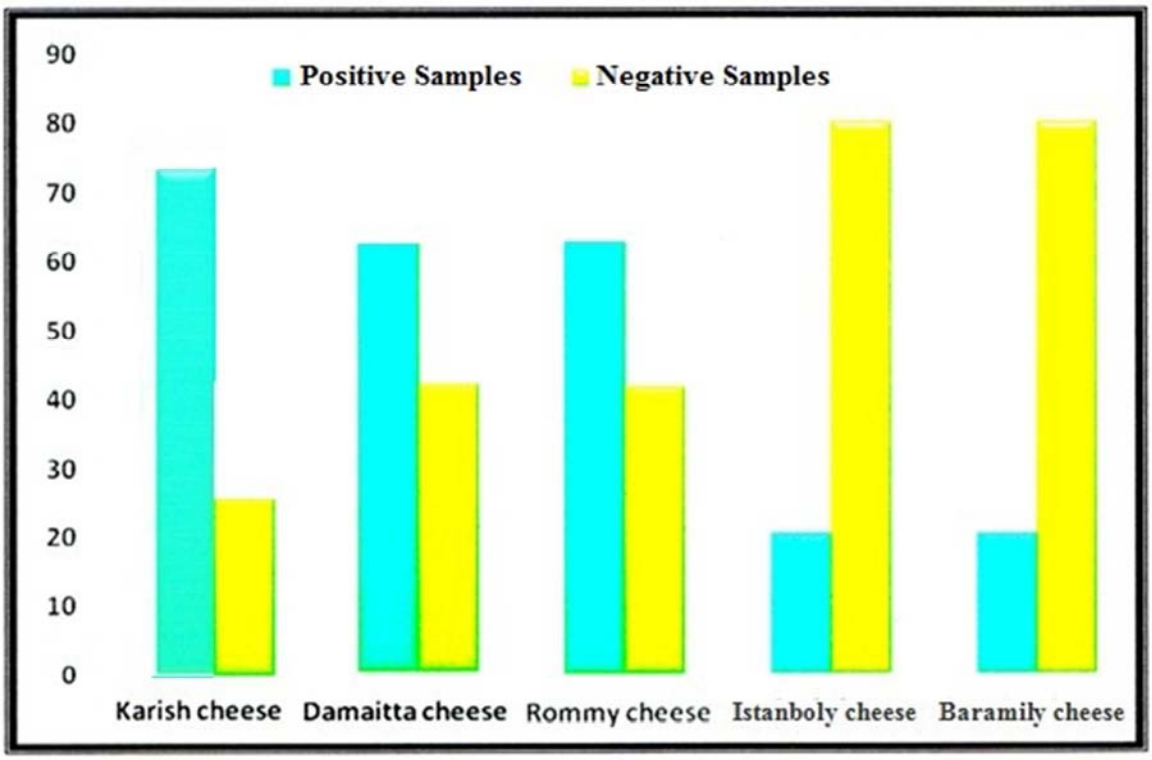

Figure 4. *Positive samples for Staphylococcus aureus in different types of Cheese.*

Table 5. *Staphylococcal enterotoxin genes isolated by PCR in milk and milk products.*

Sample type %	Positive samples for *S aureus*	Positive samples for enterotoxin by PCR No (%)	Negative samples for enterotoxin ByPCR No (%)	Odds ratio 95% CI	X2	P.value
Cheese	15	4 (33.3)	11 (66.7)	0.25 (004-1.43),	3.35	0.078
Milk	22	11 (50.0)	11 (50.0)	1 (0.2-5.01)	0.0	1.0
Heavy cream	3	1 (25.0)	2 (75.0)	0.21 (0.0-39.77)	2,0	0.432
Unsalted butter	3	1 (25.0)	2 (75.0)	0.11 (0.0-5.15)	2.1	0.457
Yogurt	10	4 (40.0)	6 (60.0)	0.56 (0.04-7.46)	0.29	0.593
Total	53	21 (41.3)	32 (58.7)			
X2		1.54				
P. value		0.843				

Table 5 and figure 5 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 heavy cream it was (25%), while in yoghurt the percentage was (40%), (33.3%) in cheese and (25%) in Unsalted butter.

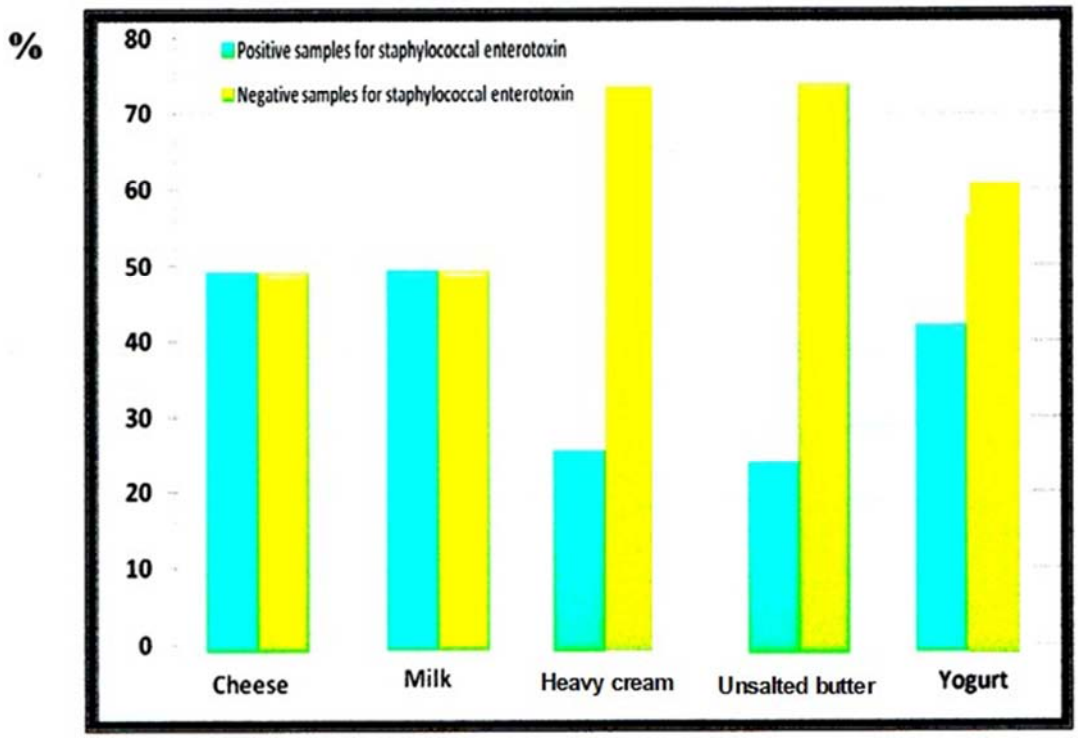

Figure 5. *Staphylococcal enterotoxin genes isolated by PCR in Milk and Milk products.*

Figure 6 showing agarose gel electrophoresis for PCR of 260 bp sea gene and 175 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).

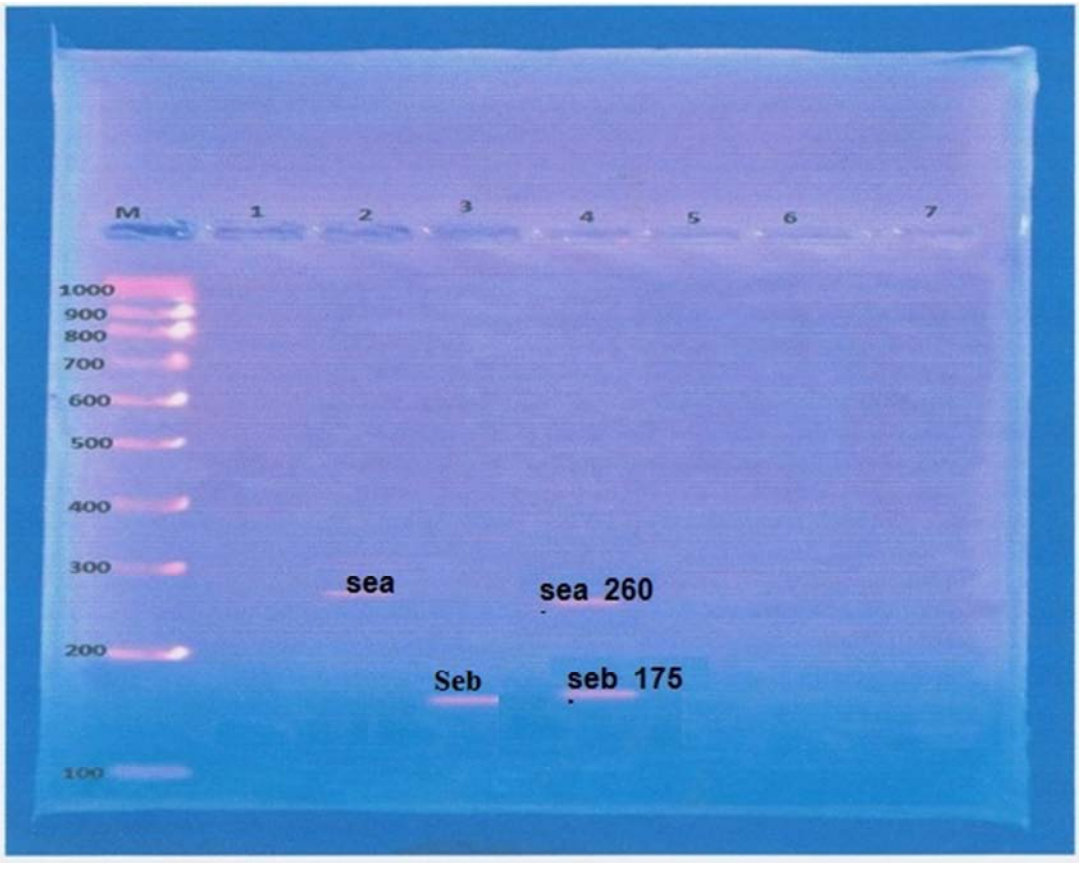

Figure 6. *Agarose gel electrophoresis for PCR of sea and seb genes.*

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) **[7].**

Staphylococcal enterotoxins are members of a family of more than 20 different staphylococcal and streptococcal exotoxins that are functionally related and share sequence homology [8].

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 [9].

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 [10, 11].

Milk products can cause sever health hazards to people as they are highly susceptible to variety of microorganisms because of their high nutritive value [12].

They are responsible of many outbreaks of food poisoning . *Staphylococcus aureus* strains were isolated by cultivation on two selective media, Mannitol Salt agar and Baird-Parker agar. Better results were obtained by Baird-Parker agar 56%.of samples were positive by Baird-Parker agar versus 47% by Mannitol Salt agar, This, was attributed to the enrichment of Baird-Parker medium by selective agents; glycine, lithium chloride and potassium tellurite [13].

The present study detected *S.aureus* in (30%) of the examined samples of both heavy cream and Unsalted butter, which came after milk (53.5%) and cheese (40%). This result differs from that reported by Imanifooladi *et al* [14].who found also the highest contamination rate in heavy cream (18%) and attributed that to excessive manipulation of heavy cream.

The presence of such organism compromises the safety of cheese and represets a hazard for the consumers, Karish cheese was the highest in *S. aureus* contamination (75%) among different types included in the present study.Rommy and damitta cheese also show a high rate contamination (60%). The least percentage was in istanboly and baramily cheese (20%) each [15].

The present study detected *S. aureus* in 30% of all examined samples. Canned yoghurt was *S. aureus* free after cultivation on Baird-Parker agar while (70%) of the examined hand-made samples were contaminated [16].

In the current study SE gene in 37 % of raw milk samples. Regarding genotypic findings by PCR, sea gene was isolated with a higher frequency than seb gene as it was detected in 52.6% of all isolated enterotoxin genes, followed by seb (31.6%) and lastly (sea+seb) (15.8%) [15].

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 [17].

5. Conclusion

In coclusion Sporadic presence of potentially staphylococcal enterotoxin producing strains in raw and raw milk products represents a health risk to consumers. *Staphylococcus aureus* is one of the most important pathogens in food poisoning. Many strains are capable of synthesizing one or more enterotoxin. Bacteriological detection of *S. aureus* is better by Baird-Parker agar medium. (80%) of raw milk was positive for *S. aureus* while pasteurized and powdered milk showed no growth. The Correlation between different types of milk was statistically significant. Raw milk and raw milk products were found to be highly contaminated. Strains produce enterotoxin (A) gene was found in .large extent than those produce enterotoxin (B) gene.

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