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# Molecular Detection of the clumping factor (fibrinogen receptor) in the Enterotoxigenic *S. aureus* isolated from Raw Milk and Traditional Cheese

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**Abstract :** The objective of this study was to determine the occurrence of the enterotoxigenic *S. aureus* within 205 raw milk and traditional cheese samples examined. The *S. aureus* strains isolated from raw milk and traditional cheese samples examined were 50 (58.8%, 50/85) and 55 (45.8%, 55/120) respectively however both camel milk and cream cheese samples had not any *S. aureus* detected. The isolated *S. aureus* were screened for the presence of the clumping factor encoding gene (*clfA* gene) by PCR; all of the isolated 105 (100%, 105/105) *S. aureus* strains carried the *clfA* gene. The isolated *S. aureus* were molecular screened for the presence of the staphylococcal enterotoxin encoding genes (SEs); *Sea*, *Seb*, *Sec*, *Sed*, and *See* by multiplex PCR; 6 (5.7%, 6 /105), 4 (3.8%, 4 /105), and 3 (2.9%, 3 /105) carried the, *Seb*, *See, Sed* genes respectively. The detection of the 13 (12.4%, 13/105) enterotoxigenic *S. aureus* strain in the present investigation is considered a potential public health hazard which should be taken into consideration to find the possible strategies for prevention. The most frequent enterotoxin encoding genes detected were *seb* 6 (5.7%, 6/105), *see* 4 (3.8%, 4/105), and *sed* 3 (2.9%, 3/105) respectively. There was not found any *S. aureus* carried neither for *Sea* nor *Sec* genes.
**Keywords :** Enterotoxigenic, *S. aureus,* clumping factor, *clfA*, *Sea, Seb, Sec, Sed, See*, Milk, Cheese.

## Introduction

*Staphylococcus aureus* is considered one of the most common causes of worldwide food borne poisoning. Milk and dairy products are an important vehicle that cause staphylococcal food poisoning outbreaks [1,2] because they are ideal growth media for *S. aureus* multiplication due to their carbohydrates, proteins, fats, vitamins, and mineral components[3,4].

*S. aureus* can produce several virulence factors; surface-exposed virulence factors (fibrinogen, protein A, fibronectin binding proteins) and secreted virulence factors (enterotoxins, extracellular toxins, haemolysins and coagulase) which allows *S. aureus* to colonize, invade, and multiply in the host tissues, causing severe infections and which representing risks for both humans and animals[5-10]. The wild-type clumping factor locus ClfA is a surface-associated fibrinogen-binding surface protein of *S. aureus*, which cause clumping of *S. aureus* in the suspension[11.12]. This clumping factor is very important for the virulence of *S. aureus*, and almost all *S. aureus* strains have the *clfA* gene[12,13]. Serologically, there are five main staphylococcal enterotoxins SEs (SEA-SEE) which are commonly responsible for staphylococcal food poisonings[8]. Staphylococcal enterotoxins SEs are group of heat-stable single-chain globular proteins belonging to a large family of pyrogenic toxin

superantigens (PTSAgs) encoded by phage (SEA), chromosome (SEB and SEC), or plasmid genes (SED), these SEs are water-soluble and pepsin-resistant[8,14-18]. Although the heat treatment of food contaminated with the enterotoxigenic *S. aureus*, it can kill *S. aureus* bacterium, however, it has not any eliminating effect on the *S. aureus* performed enterotoxin[4,15,19]. Not only *S. aureus* SEs are resistant to heat but also to inactivation by gastrointestinal protease enzymes like pepsin[15] and moreover, they can easily infect the gastrointestinal tract, causing nausea, vomiting, abdominal pain and diarrhea which occurred within two to six hour incubation period[15,20,18,21]. Interestingly, *S. aureus* strains harbor different virulence genes encoding staphylococcal enterotoxins (SEs) expression which can help us to identify the enterotoxigenic *S. aureus* strain.

The aim of the present study was thus to determine the occurrence of *S. aureus* in raw milk and traditional cheese. The isolated *S. aureus* strains were then subjected to phenotypic characterization, molecular detection of the clumping factor encoding gene (*clfA* gene) by PCR and molecular detection of the *S. aureus* enterotoxin encoding genes (SEs); *Sea*, *Seb*, *Sec*, *Sed*, and *See* by multiplex PCR.

## Materials and Methods

### Samples Collection

A total of 205 samples were purchased from different sources within Great Cairo territory; 85 raw milk (40 cattle milk were purchased from supermarkets and shops, 30 camel milk were purchased from Ministry of Agriculture sale outlets, and 15 sheep milk were collected from apparently health sheep from sheep farms) and 120 traditional cheese samples (45 white cheese, 20 cheddar cheese, 10 cream cheese, and 45 other cheese {Old Egyptian cheese (Mesh), Dan blue (sheep milk), Italian Parmisian, Kashkaval (cow milk), Labina, Old waxy Turkish, New Turkish, Flamenco, Dutch yellow} were purchased from supermarkets and shops. The samples were collected and transferred in insulated ice box to the department of Microbiology and Immunology, Veterinary Division, National Research Centre, Cairo, Giza, Egypt, where they were investigated immediately for the presence of *S. aureus* bacterium.

### Isolation and Identification of *S. aureus* bacterium

In the case of cheese samples, twenty five grams of each cheese sample were homogenized with 225 ml of 0.2% sodium citrate in a stomacher for 2 min, then 1 ml of that homogenized mixture was inoculated into 5 ml Brain-Heart Infusion broth (BHI, Oxoid). While in the case of milk samples, 1 ml of each sample was added to 5 ml BHI broth. All cheese and milk inoculated broths were then incubated at 37ºC for 24 hours, each broth was then streaked into three agar medium; Mannitol-Salt agar (MSA, Oxoid), Baird-Parker agar (BP, Oxoid) supplemented with Egg Yolk-Tellurite Emulsion (SR0054, Oxoid), and Blood agar (Oxoid) supplemented with 5% sheep red blood cells. All plates were then incubated again aerobically at 37°C for 24-48 hours. *S. aureus* suspected colonies were confirmed by gram staining, catalase, Voges Proskauer, DNase, coagulase and finally, API-*Staph* identification Kit (bio-Merieux) according to the manufacturer's instructions. The confirmed *S. aureus* isolates were preserved for subsequent research work in 15% glycerol at -80°C.

### Genomic DNA extraction

The genomic bacterial DNA extraction was achieved using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) according to the manufacturer's recommendations with some modifications. Briefly, *S. aureus* isolates were cultivated on nutrient blood agar base and the colonies were collected in micro-centrifuge tubes with the aid of 1ml saline. 200 µl of that suspension was then incubated with both 10 µl of proteinase K and 200 µl lysis buffer for 10 min at 56°C. After incubation, 200 µl of 100% ethanol was added to that lysate. The samples were then washed and centrifuged following the manufacturer's recommendations. Nucleic acids were eluted with 100 µl of elution buffer provided in the kit. All the extracted DNA were stored at −20°C.

### Molecular Detection of the clumping factor encoding gene by PCR

The isolated 105 *S. aureus* isolates were molecular investigated for the clumping factor through PCR detection of the *clfA* gene. The amplification was performed in a total reaction volume of 25 µl containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

The *clfA* primer sequence is presented in Table 1 (Metabion, Germany). After mixing the mixture in the PCR tube, the mixture was overlaid with 40µl mineral oil to avoid evaporation then the tubes were placed in a programmable thermal cycler (PTC100Mil Research, USA) and the amplification was performed under the following cycling condition; initial denaturation at 94°C for 5 min., followed by 35 amplification cycles consisting of secondary denaturation at 94°C for 30 Sec., annealing at 55˚C for 45 Sec., and the extension was at 72°C for 45 Sec., while the final extension was at 72°C for 10 min.. *S. aureus* strain KH1 was used as a positive control[24] and *S. epidermidis* was used as a negative control.

**Table 1. Primer sequences, amplicon sizes and annealing temperature**

| Target gene | Primer oligonucleotide sequences | Annealing Temp. | Amplified segment (bp) | Reference |
|---|---|---|---|---|
| ***clfA*** | GCAAAATCCAGCACAACAGGAAACGA | 55˚C | 638 | [22] |
| | CTTGATCTCCAGCCATAATTGGTGG | | | |
| ***Sea*** | GGTTATCAATGTGCGGGTGG | 50˚C | 102 | [23] |
| | CGGCACTTTTTTCTCTTCGG | | | |
| ***Seb*** | GTATGGTGGTGTAACTGAGC | | 164 | |
| | CCAAATAGTGACGAGTTAGG | | | |
| ***Sec*** | AGATGAAGTAGTTGATGTGTATGG | | 451 | |
| | CACACTTTTAGAATCAACCG | | | |
| ***Sed*** | CCAATAATAGGAGAAAATAAAAG | | 278 | |
| | ATTGGTATTTTTTTTCGTTC | | | |
| ***See*** | AGGTTTTTTCACAGGTCATCC | | 209 | |
| | CTTTTTTTTCTTCGGTCAATC | | | |

**Molecular Detection of the enterotoxin encoding genes by Multiplex PCR**

For the amplification of *S. aureus* enterotoxin encoding genes (*Sea, Seb, Sec, Sed,* and *See*), a multiplex PCR was applied. The amplification was performed in 50 µl total reaction volume; 25 µl of Emerald-Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 10 µl of DNA template, and the final volume was completed by 5 µl of sterile distilled water. The 5 oligonucleotide primer sequences, amplicon sizes and references were listed in Table 1 and all of them were supplied by Metabion (Germany). The thermal cycling amplification was performed as the follows; initial denaturation at 94°C for 5 min., followed by 35 amplification cycles consisting of secondary denaturation at 94°C for 30 Sec., annealing at 50°C for 45 Sec., and the extension was at 72°C for 45 Sec., while the final extension was at 72°C for 10 min. The following strains were used as positive controls; ATCC 13565 (*Sea*), ATCC 14458 (*Seb*), ATCC 19095 (*Sec*), 90-S-1025 (*Sed*), ATCC 27664 (*See*)[25] and *S. epidermidis* was used as a negative control.

**Agarose gel electrophoresis**

The PCR products were separated by electrophoresis in 1X TBE buffer using 1.5% agarose gel (Applichem, Germany, GmbH) stained with ethidium bromide. The electrophoresis of 5 V/cm gradients was done at room temperature. 20 µl of the uniplex PCR products and 40 µl of the multiplex PCR products were loaded in each gel slot. 100 bp DNA ladder and a 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) were used to determine the PCR product's fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data were analyzed through computer software.

## Results

**Isolation and Identification of *S. aureus***

From the results presented in Table 2, it is clearly shown that, out of the examined 205 raw milk and traditional cheese samples, 105 (51.2%, 105/205) samples were positive for *S. aureus* contamination. The total *S. aureus* isolated from the examined 85 raw milk and 120 traditional cheese samples were 50 (58.8%, 50/85) and 55 (45.8%, 55/120) respectively.

*S. aureus* contamination rates within each examined group sample type were as follows; 40 (100%, 40/40), 10 (66.7%, 10/15), 25 (55.6%, 25/ 45), 10 (50%, 10/ 20), 20 (44.4%, 20/ 45) within raw cattle milk, raw sheep milk, other cheeses, cheddar cheeses, and white cheese samples respectively.

It is evident that, the 50 *S. aureus* strains that isolated from raw milk samples were coagulase positive (CP) (100%) while the coagulase negative (CN) and coagulase positive (CP) *S. aureus* strains isolated from the examined cheese samples were 50 (41.7%) and 5 (4.2%) respectively. Furthermore, it is evident that, both of the examined camel milk and cream cheese samples have not any detected *S. aureus* bacterium.

**Table 2: *S. aureus* isolation and identification**

| Total Examined Samples | | Results | | | | | |
|---|---|---|---|---|---|---|---|
| Types of Samples | No. | Coagulase –ve *S. aureus* | | Coagulase +ve *S. aureus* | | Total isolated *S. aureus* | |
| | | No. | % | No. | % | No. | % |
| Camel Milk | 30 | - | - | - | - | - | - |
| Sheep Milk | 15 | - | - | 10 | 66.7 | 10 | 66.7 |
| Cattle Milk | 40 | - | - | 40 | 100 | 40 | 100 |
| **Total Examined Raw Milk** | **85** | **-** | **-** | **50** | **58.8** | **50** | **58.8** |
| White Cheese | 45 | 15 | 33.3 | 5 | 11.1 | 20 | 44.4 |
| Cheddar Cheese | 20 | 10 | 50 | - | - | 10 | 50 |
| Cream Cheese | 10 | - | - | - | - | - | - |
| *Other Cheese | 45 | 25 | 55.6 | - | - | 25 | 55.6 |
| **Total Examined Cheese** | **120** | **50** | **41.7** | **5** | **4.2** | **55** | **45.8** |
| **Total Examined Sample** | **205** | **50** | **24.4** | **55** | **26.8** | **105** | **51.2** |

*(Other Cheese): Old Egyptian cheese (Mesh), Dan blue (sheep milk), Italian Parmisian, Kashkaval (cow milk), Labina, Old waxy Turkish, New Turkish, Flamenco, and Dutch yellow, (-) = negative.

## Phenotypic features

The Phenotypic features of the isolated *S. aureus* strains were presented in Table 3; 55 (52.4%), 34 (32.4%), and 16 (15.2%) of the isolated *S. aureus* strains produced creamy, golden yellow, and white pigmented colonies respectively. Moreover, 53 (50.5%) and 52 (50%) of the isolated *S. aureus* strains showed β-hemolysis and α-hemolysis respectively. Furthermore, 55 (52.4%) of the isolated *S. aureus* strains had the tellurite reduction capability while 50 (47.6%) had not that capability.

**Table 3. The phenotypic features of the isolated *S. aureus* strains**

| Types of the Examined Samples | No. of *S. aureus* detected | Colony pigment | | | | | | Hemolytic activity | | | | Tellurite reduction | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | White | | Creamy | | Golden yellow | | β-hemolysis | | α-hemolysis | | Positive | | Negative | |
| | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| Sheep Milk | 10 | 2 | 20 | 1 | 10 | 7 | 70 | 10 | 100 | - | - | 10 | 100 | - | - |
| Cattle Milk | 40 | 14 | 35 | 5 | 12.5 | 21 | 52.5 | 37 | 92.5 | 3 | 7.5 | 40 | 100 | - | - |
| **Total Examined Milk** | **50** | **16** | **32** | **6** | **12** | **28** | **56** | **47** | **94** | **3** | **6** | **50** | **100** | **-** | **-** |
| White Cheese | 20 | - | - | 14 | 70 | 6 | 30 | 6 | 30 | 14 | 70 | 5 | 25 | 15 | 75 |
| Cheddar Cheese | 10 | - | - | 10 | 100 | - | - | - | - | 10 | 100 | - | - | 10 | 100 |
| *Other Cheese | 25 | - | - | 25 | 100 | - | - | - | - | 25 | 100 | - | - | 25 | 100 |
| **Total Examined Cheese** | **55** | **-** | **-** | **49** | **89.1** | **6** | **11** | **6** | **11** | **49** | **89.1** | **5** | **9.1** | **50** | **91** |
| **Total Examined Sample** | **105** | **16** | **15.2** | **55** | **52.4** | **34** | **32.4** | **53** | **50.5** | **52** | **50** | **55** | **52.4** | **50** | **47.6** |

(-)= negative

### Genotypic features

From the results presented in Table 4, it is clear that, all of the isolated 105 (100%, 105/105) *S. aureus* strains carried the clumping factor encoding gene (*clfA*) with the amplified products were at 638 bp as shown in Figure 1. On the other hand, 6 (5.7%, 6 /105), 4 (3.8%, 4 /105), and 3 (2.9%, 3 /105) of the isolated *S. aureus* strains carried the enterotoxin encoding genes; *Seb*, *See,* and *Sed* respectively, and the amplified products were at 164, 209, 278 bp, respectively as shown in Table 4 and Figure 2. Furthermore, there was not any *S. aureus* carried neither *Sea* nor *Sec* genes.

**Table 4. The genotypic features of the isolated *S. aureus***

| Types of the Examined Samples | No. of *S. aureus* detected | Genes Detected by PCR assay | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *clfA* | | *Sea* | | *Seb* | | *Sec* | | *Sed* | | *See* | |
| | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| Sheep Milk | 10 | 10 | 100 | - | - | - | - | - | - | - | - | - | - |
| Cattle Milk | 40 | 40 | 100 | - | - | 6 | 15 | - | - | - | - | 4 | 10 |
| **Total Examined Milk** | **50** | **50** | **100** | **-** | **-** | **6** | **12** | **-** | **-** | **-** | **-** | **4** | **8** |
| White Cheese | 20 | 20 | 100 | - | - | - | - | - | - | 3 | 15 | - | - |
| Cheddar Cheese | 10 | 10 | 100 | - | - | - | - | - | - | - | - | - | - |
| *Other Cheese | 25 | 25 | 100 | - | - | - | - | - | - | - | - | - | - |
| **Total Examined Cheese** | **55** | **55** | **100** | **-** | **-** | **-** | **-** | **-** | **-** | **3** | **5.5** | **-** | **-** |
| **Total Examined Sample** | 105 | 105 | 100 | - | - | 6 | 5.7 | - | - | 3 | 2.9 | 4 | 3.8 |

(-)= negative

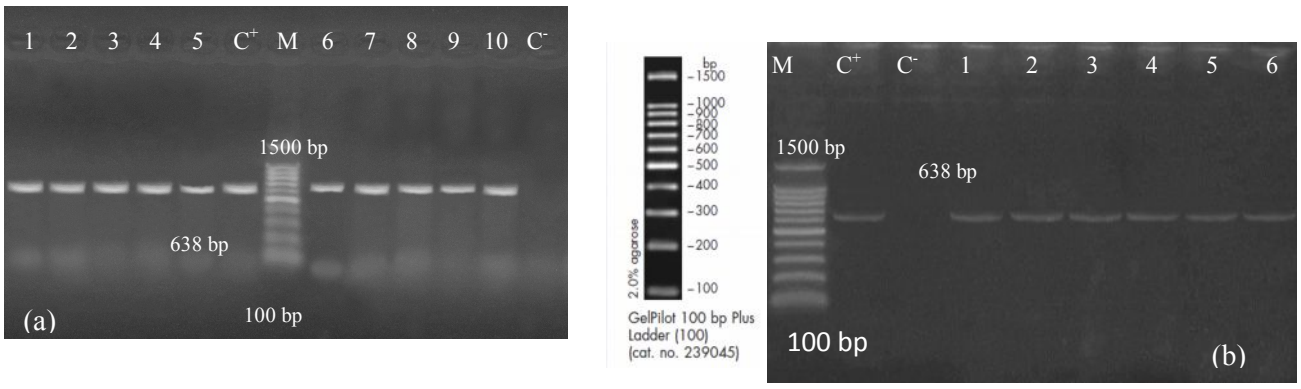


**Fig.1. PCR amplified products of the clumping factor encoding gene (*clfA*) at 638 bp among *S. aureus* isolates. (a) *S. aureus* isolates isolated from milk samples; lanes (1-10): *S. aureus* isolates, lane (M): QIAGEN GelPilot DNA 100 bp plus ladder, lane (C+): *S. aureus* strain KH1 as a positive control, lane (C-): *S. epidermidis* as a negative control. (b) *S. aureus* isolates isolated from cheese samples; lanes (1-6): *S. aureus* isolates, lane (M): QIAGEN GelPilot DNA 100 bp plus ladder, lane (C+): *S. aureus* strain KH1 as a positive control, lane (C-): *S. epidermidis* as a negative control.**

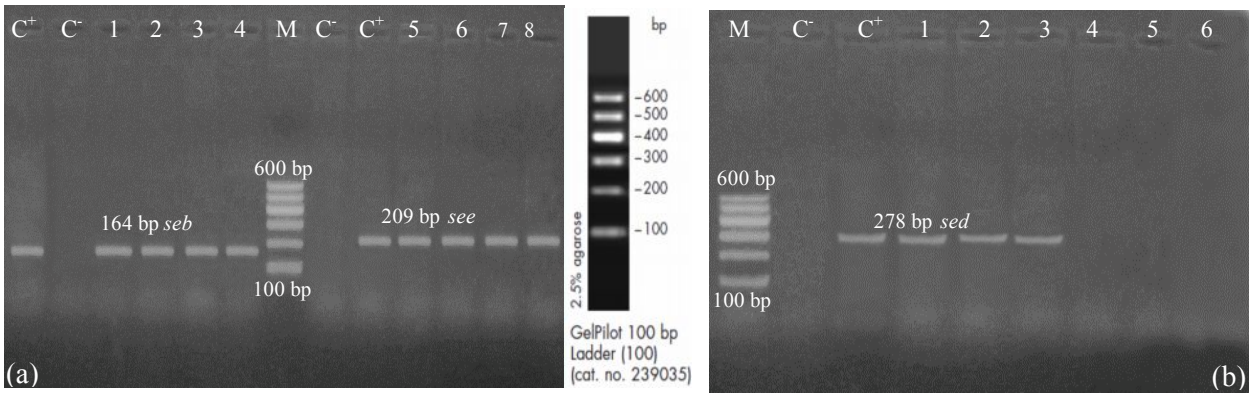


**Fig.2.** PCR amplified products of the enterotoxins encoding gene among *S. aureus* isolates. (a) ***S. aureus* isolates isolated from milk samples**; lane ($C^+$): *S. aureus* strain ATCC 14458 and ATCC 27664 as a positive control for *Seb* and *See* respectively, lane (C-): *S. epidermidis* as a negative control, lanes (1-4): enterotoxins gene *seb* amplification at 164 bp, lane (5-8): enterotoxins gene *see* amplification at 209 bp, lane (M): QIAGEN GelPilot DNA 100 bp ladder. (b) ***S. aureus* isolates isolated from cheese samples**; lane ($C^+$): *S. aureus* strain 90-S-1025 as a positive control for *Sed*, lane (C-): *S. epidermidis* as a negative control, lane (1-3): enterotoxins gene *sed* amplification at 278 bp, lane (M): QIAGEN GelPilot DNA 100 bp ladder.

## Discussion

From the results presented in Table 2, it is clear that there were high level of *S. aureus* contamination rate within raw milk which was 50 (58.8%, 50/85) and within traditional cheese which was 55 (45.8%, 55/120) with the total contamination rate of 105 (51.2%, 105/205) for both of them. Furthermore, *S. aureus* contamination rates were 40 (100%, 40/40), 10 (66.7%, 10/15), 25 (55.6%, 25/45), 10 (50%, 10/20), and 20 (44.4%, 20/45) within the analyzed raw cattle milk, raw sheep milk, other cheese, cheddar cheese, and white cheese samples respectively. Meanwhile, other research articles reported variable rates than the present study; for example, 49 (10.9%) traditional cheeses, 162 (15.7%) raw cow milk, and 86 (9.6%) raw sheep milk examined samples were contaminated with *S. aureus*[26]. Furthermore, 32% of the examined dairy products were positive for *S. aureus* contamination[27]. *S. aureus* was also detected in 5.8% of the examined milk and dairy products[28]. Moreover, 5% of the examined white cheese were contaminated with *S. aureus*[8]. Furthermore, 109 (17%) *S. aureus* contaminated samples of milk and dairy products were detected[18]; all of the above mentioned research article reported low *S. aureus* rates than the present article. However, out of the 90 cheese samples examined, 37 (41.1%) were contaminated with *S. aureus*[2] which is nearly similar to the present study.

*S. aureus* can get into raw milk either directly or indirectly; directly through mastitis or through the infected mammary glands while indirectly through animal skin, mucosal surfaces, unsanitary milking processes, unhealthy behavior of milking workers, contaminated milking utensils, milkers' hands, environment, contact, and non-contact surfaces in cheese factories[2,3,4,29]. The differences in the *S. aureus* contamination rate in milk and traditional cheeses within different research articles may be due to several factors such as; differences between countries, the ecological origin of the strains, hosts, level of hygiene, worker handlers, dairy farm sanitation systems, transportation systems, seasons, usage of preharvest surveillance to control *S. aureus* in the farm, storage conditions, and the cheese manufacturing techniques (like hygiene conditions during milking and in cheese plants, level of raw milk contamination, use of raw or pasteurized milk in cheese manufacturing, and the number of the colonized cheese handlers)[2,8,10,27,30]. Interestingly, it is evident in the present investigation that, both of the examined camel milk and cream cheese samples had not any *S. aureus*. Indeed, the camel milk negative result doesn't stun us because many previous research articles showed low level of *S. aureus* contamination within camel milk; for example, *S. aureus* herd

prevalence rate in Kenya within six herds (207 lactating camels examined) were varied between 0 and 13% [31]. Moreover, the *S. aureus* prevalence within the analyzed 348 lactating camel milk was 2.9% (39/1,362)[32]. However, 28 (8.75%) out of the 320 camel milk samples collected from 160 apparently healthy camels in Sudan were positive for *S. aureus*[33]. The absence of *S. aureus* in camel milk, which noticed in the present investigation may be due to high levels of hygiene as it produced under the supervision of the Ministry of Agriculture, Egypt. Moreover, camel milk was able to reduce the resistance of *S. aureus* in experimental challenge, and in addition, camel milk has a synergistic effect with ciprofloxacin against *S. aureus*[34]. Camel milk contains also lysozyme (LZ), lactoferrin (LF), lactoperoxidase (LP), immunoglobulin G, and secretory immunoglobulin A and the activities of these protective proteins was assayed against *S. aureus*[35].

Table 3 shows the phenotypic features of the isolated *S. aureus* in the form of colony pigment, hemolytic activity, and tellurite reduction. In the present investigation, it is clear that 55 (52.4%), 34 (32.4%), and 16 (15.2%) of the isolated *S. aureus* strains produced creamy, golden yellow, and white pigmented colonies respectively. Although, the golden yellow colonies were the predominant pigmented colonies in *S. aureus* isolates and although the wild type pigment producing strains withstood exposure to higher concentrations of $H_2O_2$ than the isogenic, pigment knockout mutants[36]. However, the lack of the common golden yellow pigment was further noticed in 126 *S. aureus* isolates[37]. Furthermore, 2 (10%) and 18 (90%) *S. aureus* strains isolated from bovine milk showed creamy colonies and golden yellow colonies respectively, while there was no isolate showed white colonies[38]. Interestingly, the white pigmented colonies of the isolated *S. aureus* strains observed in the present study may be due to lacking of the genes responsible for formation of staphyloxanthin, the carotenoid pigment that responsible for the characteristic golden yellow pigmented *S. aureus* which protect the bacterium from the oxidative stress[37]. Moreover, the white colony *S. aureus* phenotype strain actually lacked the *crtOPQMN* operon[39]. Furthermore, the absence of the characteristic golden yellow pigment attributed to mutations in the cold shock gene *cspA* which is may be due to environmental stress[40]. Furthermore, the dramatic effect on pigment production seems to correlate with altered expression of virulence determinants[41].

From the data presented in Table 3, it is clear that, 53 (50.5%) and 52 (50%) of the isolated *S. aureus* strains showed β-hemolysis and α-hemolysis respectively. Moreover, Wei *et al*. (2012) stated that 88.51% *S. aureus* strains had hemolytic characteristics. Furthermore, 18 (90%) of the *S. aureus* strains isolated from bovine milk showed also hemolytic activities[38]. In the present investigation, it is clear that 55 (52.4%) of the isolated *S. aureus* strains had the ability for tellurite reduction while 50 (47.6%) had not this ability. Meanwhile, 19 (95%) of the isolated *S. aureus* strains from bovine milk showing tellurite reduction activity[38].

From the results presented in Table 4, the detection of 13 (12.4%, 13/105) enterotoxigenic *S. aureus* strain in the current study is a potential public health hazard which should be taken into consideration to find possible strategies in order to control these hazards. Enterotoxin SEA-SEE are considered hazards in dairy products made of raw milk[42,43,44,45]. Similar to the present study, *S. aureus* was also involved in enterotoxin production in milk samples[44,46]. Furthermore, *S. aureus* strains that were isolated from Turkish cheeses were also found to be enterotoxigenic (mainly *SEC* and *SED*)[8]. On the other hand, out of 852 *S. aureus* strains that isolated from cheese samples made of raw milk in France, 7.3% were enterotoxigenic[47]. Moreover, 41 (19.2%) enterotoxigenic *staphylococci* were isolated from 214 traditional cheese samples in Ankara[48].

Toxin production is affected by the extensive growth, which is required to produce sufficient toxins to can cause illness and also affected by pH, $a_w$ (49, 50, 51, 52, 53, 8). The presence of the enterotoxigenic *S. aureus* especially in the fermented dairy product were mainly due to contamination of the bulk milk[9,20,54].

It is evident that, the most frequent enterotoxin encoding gene detected out of the 13 enterotoxigenic *S. aureus* strains in the present investigation was the *seb* gene 6 (46.2%, 6/13) followed by *see* gene 4 (30.8%, 4/13), which both were isolated from raw cattle milk followed by the *sed* gene that was detected in 3 (23.1%, 3/13) *S. aureus* strains that were isolated from white cheeses. Indeed, the SEs that were most commonly involved in the past in cases of SFP were the SEA and SED, followed by SEB[15]. Also, Berdgoll reported that, the most commonly SEs involved in the food poisoning were the SEA and SED[55]. There were lots of variations in the prevalence of an enterotoxin encoding gene within raw milk and traditional cheeses among many previous research articles. For example, Holecková isolated 47.4% enterotoxigenic *S. aureus* from cheese samples and the enterotoxin encoding gene was mainly the *seb* which is similar to the present study[56]. Furthermore, it was found that 33.6% of the isolated strains were enterotoxigenic SED *S. aureus* isolates, followed by SEA (18.4%), SEC (15.2%), and SEB (6.4%)[18]. Also, Milk and dairy products were often

contaminated with enterotoxigenic *S. aureus* strains and most of the isolated strains produced SED (33.6%), followed by SEA (18.4%), SEC (15.2%), and SEB (6.4%)[18]. Moreover, 7 (18.9%, 7/37) *S. aureus* strains that were isolated from cheeses, were found to be carrying to at least one of the following enterotoxin encoding genes SEs; sec (3/7; 42.8%) was the most frequent, followed by sea (1/7; 14.3%) and see (1/7; 14.3%)[2]. Furthermore, the most frequent enterotoxin encoding genes detected in *S. aureus* strains isolated from raw and pasteurized milk were the *sea* (41%), *sec* (20.5%), and *sed* (12.8%)[57].

## Conclusion

It is evident that, the detection of 13 (12.4%, 13/105) milk and traditional cheese samples contaminated with the enterotoxigenic *S. aureus* is regarded as a potential public health hazard facing their consumers. To control the SFP cases and in order to prevent the enterotoxigenic *S. aureus* of animal origin from passage through the food chain to the human, it is better to correct the unhygienic behavior during milk production and cheese manufacturing with strict enterotoxin preventive measures. In conclusion, Regular examination of the milk and traditional cheese for the presence of the enterotoxigenic *S. aureus* is required to provide an indication for the public health hazards associated with their consumption.

## References

1. Bianchi D.M., Gallina S., Bellio A., Chiesa F., Civera T., Decastelli L., Enterotoxin gene profiles of *Staphylococcus aureus* isolated from milk and dairy products in Italy. Letters in Applied Microbiology, 2014, 58(2): 190-196.
2. Basanisi M.G., Nobili G., La Bella G., Russo R., Spano G., Normanno G., La Salandra G., Molecular characterization of *Staphylococcus aureus* isolated from sheep and goat cheeses s in southern Italy. Small Ruminant Research, 2016, 135: 17-19.
3. Anderson M., Hinds P., Hurditt S., Miller P., McGrowder D., Alexander-Lindo R., The microbial content of unexpired pasteurized milk from selected supermarkets in a developing country. Asian Pacific Journal of Tropical Biomedicine, 2011, 1(3): 205-211.
4. Akindolire M.A., Babalola O.O., Ateba C.N., Detection of Antibiotic Resistant *Staphylococcus aureus* from Milk: A Public Health Implication. International Journal of Environmental Research and Public Health, 2015, 12(9): 10254-10275.
5. Heilmann C., Adhesion mechanisms of staphylococci. Adv. Exp. Med. Biol. 2011, 715: 105-123.
6. Le Maréchal C., Jardin J., Jan G., Even S., Pulido C., Guibert J.M., Hernandez D., François P., Schrenzel J., Demon D., Meyer E., Berkova N., Thiéry R., Vautor E., Le Loir Y., *Staphylococcus aureus* seroproteomes discriminate ruminant isolates causing mild or severe mastitis. Vet. Res., 2011a, 42(1): 35-55.
7. Le Maréchal C., Seyffert N., Jardin J., Hernandez D., Jan G., Rault L., Azevedo V., François P., Schrenzel J., van de Guchte M., Even S., Berkova N., Thiéry R., Fitzgerald J.R., Vautor E., Le Loir Y., Molecular basis of virulence in *Staphylococcus aureus* mastitis. PLoS One 2011b, 6(11): e27354.
8. Yeşim Can H., Haluk Çelik T., Detection of enterotoxigenic and antimicrobial resistant *S. aureus* in Turkish cheeses. Food Control 2012, 24(1-2): 100-103.
9. Peton V., Le Loir Y., *Staphylococcus aureus* in veterinary medicine. Infection, Genetics and Evolution 2014, 21:602-615.
10. Zhang L., Li Y., Bao H., Wei R., Zhou Y., Zhang H., Wang R., Population structure and antimicrobial profile of *Staphylococcus aureus* strains associated with bovine mastitis in China. Microbial Pathogenesis, 2016, 97; 103-109.
11. Peacock SJ, Moore CE, Justice A, Kantzanou M, Story L, Mackie K, O'Neill G, Day NP., Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*. Infect Immun 2002, 70(9):4987–4996.
12. Delfani S., Mobarez A.M., Fooladi A.A.I., Amani J., Emaneini M., Protection of mice against *Staphylococcus aureus* infection by a recombinant protein ClfA–IsdB–Hlg as a vaccine candidate. Med Microbiol Immunol, 2016, 205:47–55.
13. Josefsson E, Higgins J, Foster TJ, Tarkowski A., Fibrinogen binding sites P336 and Y338 of clumping factor A are crucial for *Staphylococcus aureus* virulence. PLoS One, 2008, 3(5):e2206.

14. Johnson W.M., Tyler S.D., Ewan E.P., Ashton F.E., Pollard D.R., Roze K.R., Detections of genes for enterotoxins, exfoliative toxins and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. J. Clin. Microbiol. 1991, 29(3): 426-430.
15. Balaban N., Rasooly A., Staphylococcal enterotoxins. Int. J. Food Microbiol. 2000, 61(1): 1-10.
16. Dinges M.M., Orwin P.M., Schlievert P.M., Exotoxins of *Staphylococcus aureus*. Clin. Microbiol. Rev., 2000, 13(1): 16-34.
17. Proft T., Fraser J.D., Bacterial superantigens. Clin. Exp. Immunol. 2003, 133(3): 299-306.
18. Normanno G., La Salandra G., Dambrosio A., Quaglia N.C., Corrente M., Parisi A., Santagada G., Firinu A., Crisetti E., Celano G.V., Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. International Journal of Food Microbiology, 2007, 115(3): 290-296.
19. Barker G.C., Gomez-Tome N., A risk assessment model for enterotoxigenic *Staphylococcus aureus* in pasteurized milk; a potential route to source level inference. Risk Anal., 2013, 33(2): 249-269.
20. Le Loir Y, Baron F, Gautier M. (2003): *Staphylococcus aureus* and food poisoning. Genet Mol Res. 2(1): 63-76.
21. Arfatahery N., Mirshafiey A., Abedimohtasab T.P., Zeinolabedinizamani M., Study of the Prevalence of *Staphylococcus aureus* in marine and farmed shrimps in Iran aiming the future development of a prophylactic vaccine. Procedia in Vaccinology, 2015, 9:44-49.
22. Mason W.J., Blevins J.S., Beenken K., Wibowo N., Ojha N., Smeltzer M.S., Multiplex PCR Protocol for the Diagnosis of Staphylococcal Infection. Journal of clinical microbiology, 2001, 39(9): 3332-3338.
23. Mehrotra M., Wang G., Johnson W.M., Multiplex PCR for Detection of Genes for *Staphylococcus aureus* Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance. Journal of clinical Microbiology, 2000, 38(3): 1032–1035.
24. Nemati M., Hermans K., Devriese L.A., Maes D., Haesebrouck F., Screening of genes encoding adhesion factors and biofilm formation in *Staphylococcus aureus* isolates from poultry. Avian Pathology, 2009, 38(6): 513-517.
25. Saadati M., Barati B., Doroudian M., Shirzad H., Hashemi M., Hosseini S.M., Chaleshtari A.R.S., Bahmani M., Hosseinzadeh S., Imani S., Detection of *Sea, Seb, Sec, Seq* genes in *Staphylococcus aureus* isolated from nasal carriers in Tehran province, Iran; by multiplex PCR. Journal of Paramedical Sciences (JPS), 2011, 2(2): 34-40.
26. Jamali H., Paydar M., Radmehr B., Ismail S., Dadrasnia A., Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk and dairy products. Food Control, 2015, 54; 383-388.
27. Fooladi I.A.A., Tavakoli H.R., Naderi A., Detection of enterotoxigenic *Staphylococcus aureus* isolates in domestic dairy products. Iran. J. Microbiol., 2010, 2(3): 137-142.
28. Rahimi E., Enterotoxigenicity of *Staphylococcus aureus* isolated from traditional and commercial dairy products marketed in Iran. Braz. J. Microbiol. 2013, 44(2): 393-399.
29. Ateba C.N., Mbewe M., Moneoang M.S., Bezuidenhout C.C., Antibiotic resistant *Staphylococcus aureus* isolated from milk in the Mafikeng Area, North-West province, South Africa. S. Afr. J. Sci., 2010, 106 (11/12), 1-6.
30. Ertas N., Gonulalan Z., Yildirim Y., Kum E., Detection of *Staphylococcus aureus* enterotoxins in sheep cheese and dairy desserts by multiplex PCR technique. Int. J. Food Microbiol., 2010, 142(1-2), 74-77.
31. Younan M, Ali Z, Bornstein S, Müller W., Application of the California mastitis test in intramammary *Streptococcus agalactiae* and *Staphylococcus aureus* infections of camels (Camelus dromedarius) in Kenya. Prev Vet Med., 2001, 51 (3-4): 307-316.
32. Regassa A, Golicha G, Tesfaye D, Abunna F, Megersa B., Prevalence, risk factors, and major bacterial causes of camel mastitis in Borana Zone, Oromia Regional State, Ethiopia. Trop Anim Health Prod., 2013, 45(7):1589-95.
33. Shuiep ES, Kanbar T, Eissa N, Alber J, Lämmler C, Zschöck M, El Zubeir IEM, Weiss R.. Phenotypic and genotypic characterization of *Staphylococcus aureus* isolated from raw camel milk samples. Res Vet Sci., 2009, 86 (2): 211-215.
34. Yassin M.H., Soliman M.M., Mostafa S.A., Ali H.A., Antimicrobial Effects of Camel Milk against Some Bacterial Pathogens. Journal of Food and Nutrition Research, 2015, 3(3): 162-168.
35. el Agamy EI, Ruppanner R, Ismail A, Champagne CP, Assaf R., Antibacterial and antiviral activity of camel milk protective proteins. J Dairy Res., 1992, 59(2):169-75.

36. Goerke C, Gressinger M, Endler K, Breitkopf C, Wardecki K, Stern M, Wolz C, Kahl BC., High phenotypic diversity in infecting but not in colonizing *Staphylococcus aureus* populations. Environ Microbiol., 2007, 9(12):3134-42.
37. Holt DC, Holden M.T.G., Tong S.Y.C., Castillo-Ramirez S., Clarke L., Quail M.A., Currie B.J., Parkhill J., Bentley S.D., Feil E.J., Giffard P.M., A very early-branching *Staphylococcus aureus* lineage lacking the carotenoid pigment staphyloxanthin. Genome Biol Evol., 2011, 3:881-895.
38. El-Jakee J., Samir A., Al-Saify S., Shimaa T. Omara and Hedaia R., "Screening of Toxigenic *S. aureus* isolated from Bovine and Human origin", International Journal of Current Research, 2015, 7 (9): 20647-20651.
39. Antonic V., Stojadinovic A., Zhang B., Izadjoo M.J., Alavi M., *Pseudomonas aeruginosa* induces pigment production and enhances virulence in a white phenotypic variant of *Staphylococcus aureus*. Infect Drug Resist., 2013, 6: 175-186.
40. Duval B.D., Mathew A., Satola S.W., Shafer W.M., Altered growth, pigmentation, and antimicrobial susceptibility properties of *Staphylococcus aureus* due to loss of the major cold shock gene cspB. Antimicrob Agents Chemother., 2010, 54(6): 2283-2290.
41. Lan L., Cheng A., Dunman P. M., Missiakas D., He C., Golden Pigment Production and Virulence Gene Expression Are Affected by Metabolisms in *Staphylococcus aureus*. Journal of bacteriology, 2010, 192 (12): 3068- 3077.
42. Joffe R., Baranoviès E., Bovine mastitis as the primary contamination source of milk and milk products with *S. aureus* enterotoxins. *Veterinaria Ir Zootechnika* T., 2006, 36 (58): 21-26.
43. Jørgensen HJ, Mørk T, Høgasen HR, Rørvik LM., Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. J Appl Microbiol, 2005, 99 (1):158-166.
44. Moon JS, Lee AR, Kang HM, Lee ES, Joo YS, Park YH, Kim MN, Koo HC., Antibiogram and coagulase Diversity in staphylococcal enterotoxin-producing *Staphylococcus aureus* from Bovine Mastitis. *J Dairy Sci.,* 2007, 90(4):1716-1724.
45. Neder VE, Canavesio VR, L.F. Calvinho., Presence of enterotoxigenic *Staphylococcus aureus* in bulk tank milk from Argentine dairy farms. Rev Argent Microbiol*.,* 2011, 43(2): 104-6.
46. Adesiyun AA., Characteristics of *Staphylococcus aureus* strains isolated from bovine mastitic milk: bacteriophage and antimicrobial agent susceptibility and enterotoxigenicity. *Zentralbl Veterinarmed B*., 1995, 42(3):129-39.
47. Lamprell H., Villard L., Chamba J.F., Beuvier E., Borges E., Maurin F., Mazerolles G., Noel Y., Kodjo A., Identification and biotyping of coagulase positive staphylococci (CPS) in ripened French raw milk cheeses and their in vitro ability to produce enterotoxins. Revue de Medicine Veterinaire, 2004, 155(2), 92-96.
48. Kuplulu O., Sarimehmetoglu B., Celik T.H., Determination of the enterotoxigenicity of coagulase positive staphylococci isolated from cheese by ELISA. Milchwissenschaft, 2004, 59(1): 17-19.
49. Jay J.M., Loessner M.J., Golden D.A., Staphylococcal Gastroenteritis. In J. M. Jay, M. J. Loessner, & D. A. Golden (Eds.), Modern food Microbiology 2005 (pp. 545-566). USA: Springer Science.
50. Munson S.H., Tremaine M.T., Betley M.J., Welch R.A., Identification and characterization of staphylococcal enterotoxin types G and I from *Staphylococcus aureus.* Infection and Immunity, 1998, 66 (7), 3337-3348.
51. Omoe K., Ishikawa M., Shimoda Y., Hu D., Ueda S., Shinagawa K., Detection of seg, seh, and sei genes in *Staphylococcus aureus* isolates and determination of the Enterotoxin productivities of *S. aureus* isolates harboring seg, seh, or sei Genes. J Clin Microbiol, 2002, 40(3): 857-862.
52. Omoe K., Hu D.L., Takahashi-Omoe H., Nakane A., Shinagawa K., Identification and characterization of a new staphylococcal enterotoxin related putative toxin encoded by two kinds of plasmids. Infection and Immunity, 2003, 71(10): 6088-6094.
53. Seo K.S., Bohach G.A., Foodborne pathogenic bacteria. *S. aureus.* In M. P. Doyle, & L. R. Beuchat (Eds.), Food microbiology: fundamentals and frontiers, 2007, (pp. 493-518). Washington: ASM Press.
54. Le Maréchal C., Thiéry R., Vautor E., Le Loir Y., Mastitis impact on technological properties of milk and quality of milk products-a review. Dairy Sci. Technol., 2011c, 91, 247-282.
55. Berdgoll M.S., *S. aureus.* In: Doyle, M.P. (Ed.), Food- Borne Bacterial Pathogens. Marcel Dekker, New York, pp., 1989, 464-523.
56. Holecková B., Holoda E., Fotta M., Kalinácova V., Gondol J., Grolmus J., Occurrence of enterotoxigenic *Staphylococcus aureus* in food. Annals of Agricultural and Environmental Medicine, 2002, 9(2): 179-182.

57. Rall V.L., Vieira F.P., Rall R., Vieitis R.L., Fernandes A.Jr., Candeias J.M., Cardoso K.F., Araújo J.P. Jr., PCR detection of staphylococcal enterotoxin genes in *Staphylococcus aureus* strains isolated from raw and pasteurized milk. Vet. Microbiol., 2008, 132(3-4): 408-413.

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