International Journal of Innovative Research in Medical Science (IJIRMS)
Volume 11, Issue 02, February 2026,
https://doi.org/10.23958/ijirms/vol11-i02/2165

Original Article

Detection of *Staphylococcus aureus* Enterotoxins in Raw and Processed Milk in Khartoum State, Sudan

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Received: 04 January 2025; Accepted: 26 January 2026; Published: 01 February 2026

Abstract

Staphylococcus aureus is a leading cause of foodborne illness due to its production of heat-stable enterotoxins. This study investigated the presence of enterotoxigenic *S. aureus* in raw, pasteurized, and ultra-high temperature (UHT) processed milk at Khartoum State, Sudan in October 2019. A total of 56 milk samples were collected randomly by purchasing the raw milk from some public markets, meanwhile, different manufacturing batches of pasteurized and UHT milk samples were obtained from the outlets of dairy factories and supermarkets. After isolation and identification of *S. aureus*, a multiplex PCR assay was used to simultaneously detect *sea*, *seb*, *sec* and *sed* enterotoxins genes. The *S. aureus* was found in 48.2% of the milk samples investigated, with isolation rates of 29.6, 14.8 and 55.6% in raw, pasteurized and UHT milk, respectively. The targeted enterotoxin genes revealed positive detection only in the UHT milk as 15 samples were found to harbored enterotoxigenic *S. aureus*: 8 (14.3%) sea, 5 (8.9%) sec and 2 (3.6%) seb. These findings demonstrate that even commercially processed fluid milks can be contaminated with enterotoxigenic *S. aureus* and that the direct detection of the classical enterotoxins from fluid milk was possible. The present study discusses and underscore the public health risks of staphylococcal enterotoxins in dairy products and highlight the urgent need for improved hygiene and monitoring for milk safety by the starting routine screening and examination programs for milk and dairy products before offering them for sale. This will help in safeguard the public health from the risks of *S. aureus* enterotoxins and other pathogens associated with food poisoning in the country.

Keywords: ***Staphylococcus aureus*, occurrence, classical enterotoxins, fluid milks, PCR, Sudan.**

Introduction

Staphylococcus aureus is a Gram-positive bacterium frequently implicated in food-borne diseases worldwide as it is a versatile pathogen carried by approximately one-third of the human population, often residing on the skin or nasal mucosa without symptoms (Phiri *et al*., 2022). Moreover, dairy products, including milk and cheese, are common vehicles for staphylococcal food poisoning (SFP) outbreaks when they become contaminated with enterotoxigenic *S. aureus* and are improperly handled (Kou *et al*., 2021).

The *S. aureus* produces a variety of virulence factors, most notably the staphylococcal enterotoxins (SEs). These toxins are potent gastrointestinal exotoxins that function as superantigens, capable of triggering massive T-cell activation and cytokine release at picomolar concentrations (Benkerroum, 2018). The five "classical" enterotoxins; SEA, SEB, SEC, SED, and SEE; are responsible for the majority of SFP cases worldwide (Argudin *et al*., 2010). Among these, staphylococcal enterotoxin A (SEA) is the most commonly implicated in food poisoning, while SEB has also gained attention as a potential bio-weapon (Ler *et al*., 2006). Several newer enterotoxins (SEG, SEH, SEI, and others) also possess emetic activity and a remarkable property of SEs is their resistance to inactivation as they remain stable at high temperatures and low pH, and are not easily destroyed by proteolytic enzymes (Argudin *et al*., 2010). Thus, even if *S. aureus* cells are killed by pasteurization, pre-formed enterotoxins can retain their biological activity in milk and dairy products (Janstova *et al*., 2012). Indeed, classical SEs retain activity after typical pasteurization and account for over 90% of documented SFP outbreaks (Cha *et al*., 2006).

Contamination of milk with *S. aureus* can occur at multiple points from farm to table. Raw milk can be contaminated directly from cows with subclinical mastitis shedding *S. aureus*. Moreover, inadequate farm hygiene or improper milking practices can introduce *S. aureus* into milk, where it may proliferate if refrigeration is insufficient (Hennekinne *et al*., 2012). Milk and dairy products are often associated with staphylococcal food poisoning, which is caused by heat-stable staphylococcal enterotoxins that are produced by the bacterium inside the food (Abril *et al.*, 2020 and Benkerroum, 2018). Pasteurized milk, while initially free of viable *S. aureus*, can become re-contaminated post-pasteurization through contact with contaminated equipment or handling by food workers (Von Eiff *et al*., 2001; Havelaar *et al*., 2010). If such post-process contamination is followed by temperature abuse (storage at ambient temperatures), the surviving *S. aureus* can grow and produce enterotoxins in the product (Rall *et al*., 2008). The UHT milk is generally shelf-stable and sterile when packaged, but any breach in package integrity or aseptic handling can similarly lead to introduction of *S. aureus* (Peles *et al*., 2007). Notably, the highest risk of enterotoxins production is associated with pasteurized or UHT milk that is contaminated after processing and then stored improperly; e.g., at room temperature (Rall *et al*. 2008). Generally, the food safety associated with food-borne pathogens during storage at low temperature draw more attention globally (Liu *et al.*, 2023).

The prevalence of enterotoxigenic *S. aureus* in dairy products is a concern for public health authorities (Akindolire *et al*., 2015). A systematic review reported that on average 11-33% of raw milk samples worldwide harbor *S. aureus*, with considerable regional variability (Hoteit *et al*., 2022). In particular, Shalaby *et al.* (2024) found a within-herd, prevalence of 11.6% for enterotoxigenic *S. aureus* in raw milk. Global studies have shown variable contamination rates: for example, in Northern Portugal 53% of bulk tank raw milk samples contained *S. aureus*, whereas a study in Xinjiang, China found *S. aureus* in 43% of retail raw milk samples (Kou *et al.*, 2021). The proportion of *S. aureus* isolates carrying enterotoxin genes also varies (Kou *et al.*, 2021). Surveys in Europe and Asia indicate that roughly 40-60% of dairy *S. aureus* isolates carry one or more enterotoxin genes, Shuiep *et al*. (2009) found 46 (41%) of camels milk samples fromm Sudan were positive for *S. aureus*. The milk samples positive for *S. aureus* were from Omdurman (n= 25; 54.3%), Eastern Nile (n= 21; 45.7%) and El Obeid (n= 4; 14.3%). Meanwhile, Mansour *et al*. (2017) found 23 isolates of *S. aureus* (16.3%) associated with cow's and buffalo's milk in Egypt, and the most frequent gene was SEE (100%), followed by SEB (8.7%) and SEC (8.7%). For instance, Oliveira *et al.* (2022) detected at least one enterotoxin gene in 46.8% of *S. aureus* isolates from raw milk, Jung and Lee (2022) also reported 43% of bulk tank milk isolates in Korea were contaminated with enterotoxigenic *S. aureus*. In contrast, only 12.9% of *S. aureus* from raw milk in one Chinese study carried classical enterotoxin genes, highlighting geographical differences (Jung and Lee 2022). Moreover, the frequent presence of enterotoxigenic strains in milk is alarming because consumption of contaminated dairy products can lead to staphylococcal food poisoning (Jung and Lee 2022). Symptoms of SFP (nausea, vomiting, abdominal cramps, and diarrhea) can onset rapidly after ingestion of contaminated food, and severe cases may require hospitalization (Argudin *et al*., 2010).

Sudan has a large dairy industry, and milk is a staple food in Khartoum State. However, dairy safety can be challenging due to the warm climate, intermittent refrigeration, and varying practices in milk production and distribution (Mohamed and El Zubeir, 2018). Previous studies in Sudan have isolated *S. aureus* from raw milk and dairy products such as white cheese (Mohamed and El Zubeir (2018). Thus, there it is of concern that enterotoxigenic *S. aureus* could be present in the milk supply, posing a risk of SFP outbreaks (Bystron *et al*. 2009). Nevertheless, data on the current prevalence of *S. aureus* enterotoxins in Sudanese milk are scarce. This study was undertaken to detect genes encoding classical staphylococcal enterotoxins in raw and processed milk from Khartoum State and to evaluate potential differences in contamination between raw, pasteurized, and UHT milk. The current study hypothesized that while pasteurization or UHT treatment should eliminate *S. aureus*, poor post-processing handling could lead to contamination of processed milk with enterotoxigenic strains. By combining culture and PCR-based toxin gene detection, the main objective of this study was to determine the levels and to identify the main types of *Staphylococcus aureus* enterotoxins in raw and processed milk in Khartoum State, Sudan and to propose strategies for improving milk safety.

Materials and Methods

Sample collection

A total of 56 milk samples were collected during October 2019 from Khartoum State, Sudan. The samples comprised raw milk (n=20) that obtained from public markets (small-scale vendors and farms), pasteurized milk (n=12) from retail outlets and UHT milk (n=24) from supermarkets. Each sample (~500 mL) was collected aseptically into sterile containers and transported to the laboratory under chilled conditions.

Examination of milk samples

Culture media

Isolation and identification of *S.* aureus

Mannitol salt agar (Hi-Media, MH118) and other used media were prepared according to the manufacturing company instructions. Meanwhile, sterilization was done according to Marshall (1992).

Milk samples were enriched and cultured for *S. aureus*. Ten milliliters of each sample were incubated in tryptic soy broth (Fisher Scientific, 11409658) at 37°C for 18–24 hours. A loopful of enriched broth was streaked onto mannitol salt agar and Baird-Parker agar (Fisher Scientific, 12972588) and incubated at 37°C for 24-48 hours. Suspected *S. aureus* colonies (yellow with halo on mannitol salt agar, or black with halo on Baird-Parker) were sub-cultured for purity (Flowers *et al*., 1992). For all bacterial isolates, smears were prepared from young culture suspensions and stained by Gram stain. Then Gram stain, shape, size, and arrangement of cells were determined under the light microscope (Benson *et al.*, 2001). The criteria outlined in the flow chart developed by El Sanousi *et al.* (2015) for the identification of *S. aureus* was followed. All isolates shown growth in mannitol salt agar were suspended in nutrient broth (Fisher Scientific, 10679125) until transferred to PCR laboratory. Confirmation of *S. aureus* using PCR amplification of the species-specific *nuc* gene (thermostable nuclease) was also performed. Confirmed *S. aureus* isolates were stored in tryptic soy broth with 25% glycerol at -20°C for further analysis.

DNA extraction

Genomic DNA was extracted from each confirmed isolate (or directly from enriched broth if isolates were sparse) using a commercial bacterial DNA kit (Bioneer, D-1040) or a boiling lysis method. Briefly, a single colony was suspended in lysis buffer, heated (≈80°C), then centrifuged to obtain a clear lysate. DNA concentration and purity were checked using a spectrophotometer (MSE supplies, USA), and DNA was stored at -20°C until PCR analysis.

PCR detection of enterotoxin genes

A multiplex PCR assay was employed to simultaneously detect four classical staphylococcal enterotoxin genes: sea, seb, sec, and sed. Primer sequences and PCR conditions were based on previously published protocols (Ref), with minor modifications. Each 25 µL PCR reaction contained 2-3 µL template DNA, 0.5 µM of each primer pair, 1× PCR buffer, 2.0 mM $MgCl_2$, 200 µM of each dNTP, and 1 U Taq DNA polymerase. Thermal cycling consisted of an initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 45 sec; and a final extension at 72°C for 7 min. The PCR products were analyzed by 1.5% agarose gel electrophoresis with ethidium bromide staining. A 100 bp DNA ladder was used as a size marker. Positive controls using reference *S. aureus* strains (Bioneer, B-1030) carrying known sea, seb, sec, and sed genes and a no-template negative control were included in each run.

DNA extraction from milk samples directly

All milk samples that did not show *S. aureus* growth were subjected to direct DNA extraction using a modified boiling method according to Fusco *et al*. (2011). Both DNA and RNA were successfully extracted. The DNA samples were analyzed by agarose gel electrophoresis (0.8% agarose, 120-volt, 30 min) (MSE supplies, USA).

Data analysis

The prevalence of *S. aureus* in different milk types (raw, pasteurized, and UHT) was calculated as the percentage of samples yielding confirmed *S. aureus*. The detection of enterotoxin genes was recorded for each isolate and summarized by milk type. Owing to the exploratory nature and modest sample size, formal statistical comparisons were not performed; results were interpreted descriptively.

Results

Occurrence of *S. aureus* in raw and processed milk

Out of 56 total samples, 27 (48.2%) were positive for *S. aureus* (**Table 1**) by culture (**Figure 1**) and confirmation tests (**Figure 2**). Table 1 also shows the distribution of *S. aureus* in raw, pasteurized, and UHT milk. *S. aureus* was isolated from 8 (29.6%) raw milk samples, 4 (14.8%) pasteurized milk samples, and 15 (55.6%) UHT milk samples. Moreover, the samples contaminated with non-S. *aureus* revealed 7 (24.1%), 11 (37.9%) and 11 (37.9%) isolates, respectively (**Table 1**). The *S. aureus* contamination corresponds to roughly one-third of the raw milk tested, one-seventh of the pasteurized milk, and over half of the UHT milk. The high occurrence of UHT products was unexpected given that thermal processing should eliminate vegetative bacteria, indicating that either insufficient heat treatment was used or contamination likely occurred after heat treatment.

Detection of enterotoxin genes

None of the *S. aureus* isolates from raw or pasteurized milk carried the four classical enterotoxin genes (*sea, seb, sec, sed*) targeted in this study. In contrast, a subset of *S. aureus* isolates from UHT milk was positive for the investigated enterotoxin genes (**Figure 3 and Table 2**). Among the 15 UHT isolates, 8 carried the sea gene (*sea*), 5 carried the sec gene (*sec*), and 2 carried the seb gene (*seb*). No isolate was positive for the sed gene (*sed*) as shown in Figure 2. Several UHT isolates carried more than one enterotoxin gene; notably, two isolates harbored both sea and sec genes (**Table 2**).

Comparison between milk types

The absence of enterotoxin genes in raw and pasteurized milk samples suggests that the *S. aureus* strains contaminating those samples were non-enterotoxigenic (with respect to SEA-SED) or present below detection limits. By contrast, UHT milk showed a considerable incidence of enterotoxigenic *S. aureus*, with over half of the *S. aureus*-positive UHT samples carrying one or more classical toxin genes (**Figure 3 and Table 2**). This finding is striking, given that UHT processing (typically 135-150°C for a few seconds) should destroy all vegetative bacteria, implying that contamination occurred after heat treatment (during packaging or storage).

Direct DNA extraction from milk samples

All milk samples that did not show *S. aureus* growth, as indicated in Table 3, were subjected to direct DNA extraction. Non visible DNA bands were observed in raw and pasteurized milk samples following direct extraction. In contrast, UHT milk samples showed the presence of *sea* in 3 samples (27.2%) and *sec* in 2 samples (18.1%) out of the 11 tested, as presented in Table 3.

Table 1: Distribution and prevalence of Staphylococcus aureus and other staphylococcal species in raw, pasteurized, and UHT milk samples collected in Khartoum State, 2019.

Milk type	No. of samples	*S. aureus*-positive samples	Non *S. aureus*-positive samples
Raw milk	27	8 (29.6%)	7 (24.1%),
Pasteurized milk	12	4 (14.8%)	11 (37.9%)
UHT milk	27	15 (55.6%)	11 (37.9%)
Total	56	27 (48.2%)	29 (51.8%)

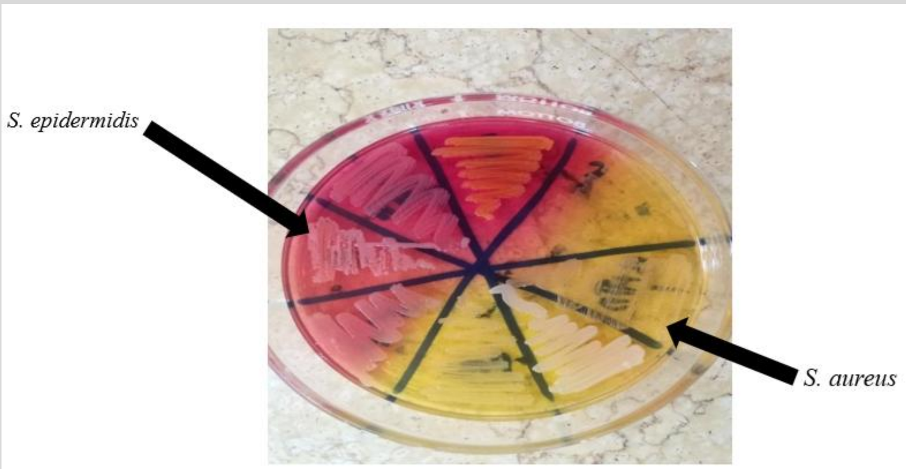

Figure 1: Growth of Staphylococcus spp. in mannitol salt agar

- Mannitol non fermentor colonies of *S. epidermidis* (upper left).
- Mannitol fermentor colonies of *S. aureus* (down right)

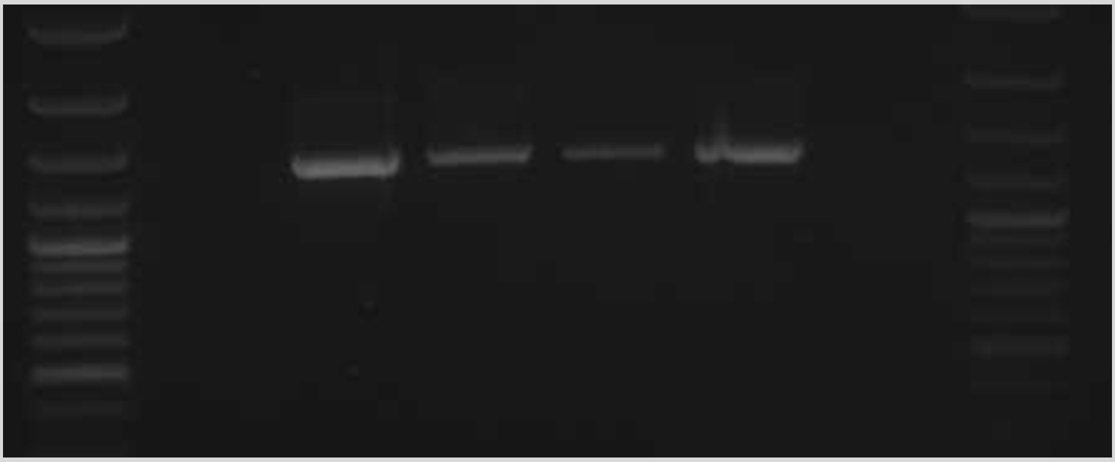

Figure 2: Gel electrophoresis showing 16s rDNA PCR products.

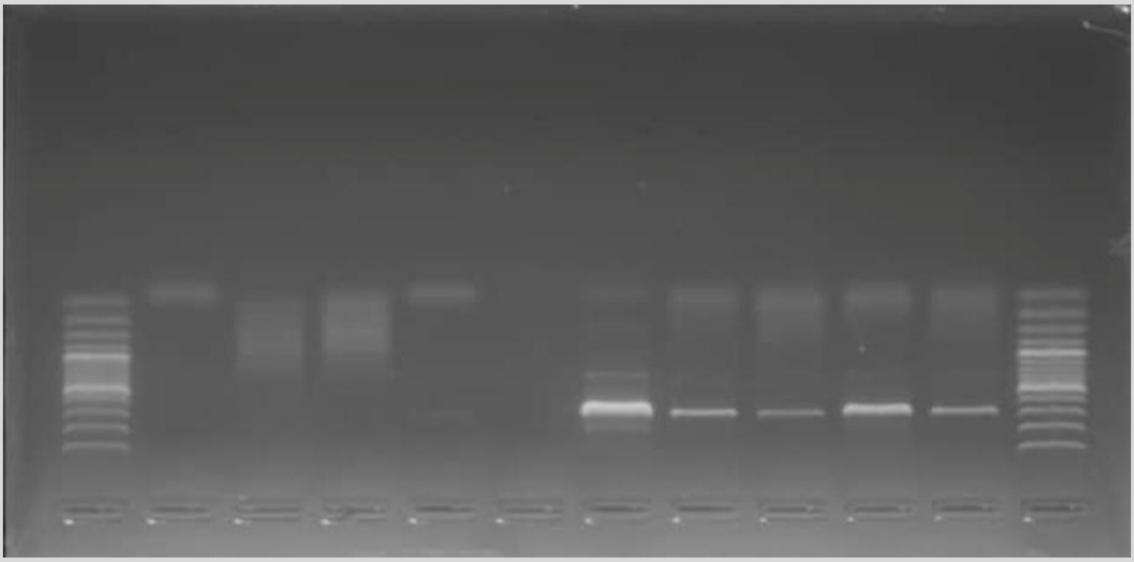

Figure 3: Gel electrophoresis of amplified PCR products of 16s rDNA gene (228 bp) from staphylococci isolates by monoplex PCR technique

Agarose (1.5%), TBE buffer (1X), 5V/Cm for 1 hr stained with ethidium bromide:

M: The DNA molecular weight marker (100 bp ladder)

N: Control negative

Lane 4, Lane 5, Lane 6, Lane 7, Lane 8 Positive amplification of 228 bp for 16s rDNA gene

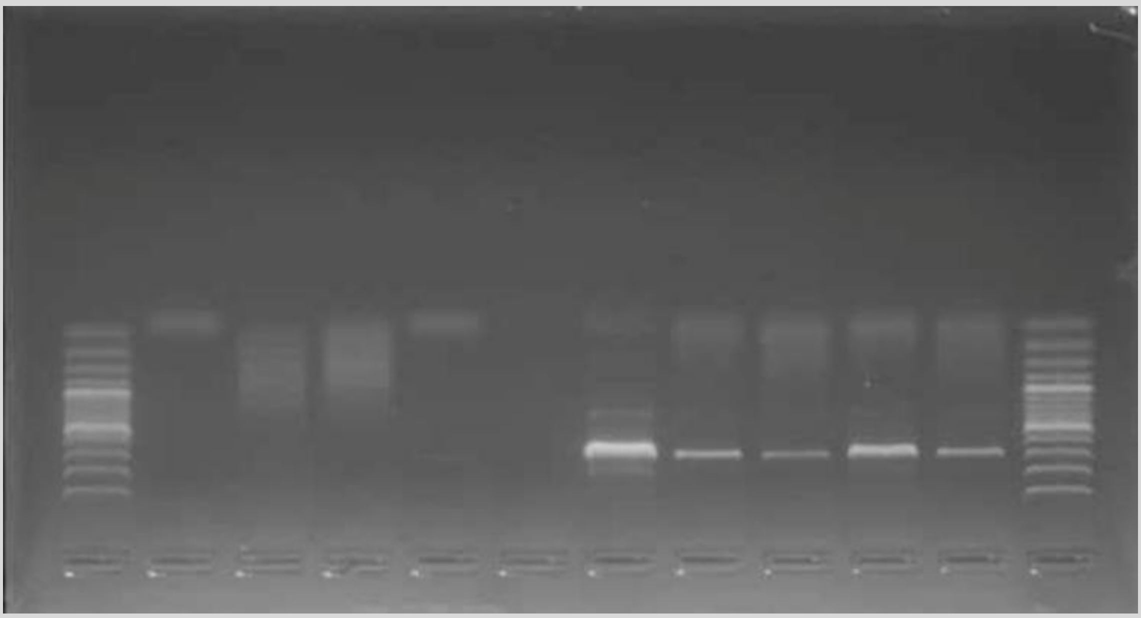

Figure 4: Gel electrophoresis of enterotoxin genes (sea, seb, sec, sed).

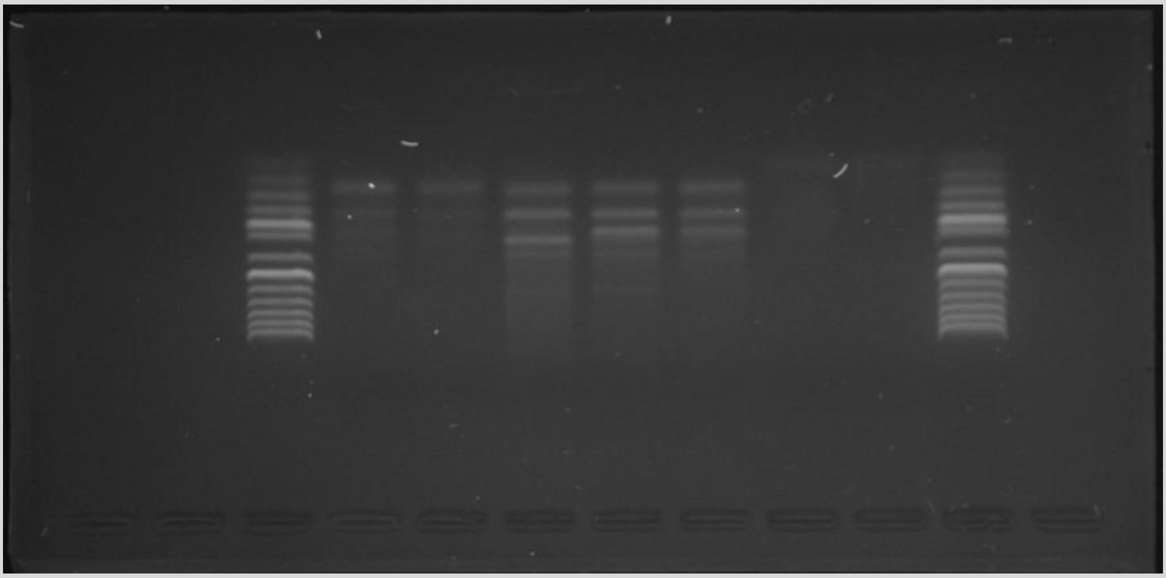

Figure 5: Gel electrophoresis of amplified PCR products of staphylococcal enterotoxin genes (sea, seb, sec, and sed) in multiplex PCR technique

Agarose (1.5%), TBE buffer (1X), 5V/Cm for 1 hr stained with ethidium bromide
M: The DNA molecular weight marker (50 bp ladder).
N: Control negative.
Lane 4, 5, 6, 7, 8 positive amplification of 120 bp enterotoxin (A).
Lane 4, 5, 6: Positive amplification of 257 bp for enterotoxin ©.
Lane 5, 6: Positive amplification of 478 bp for enterotoxin (B).

Table 2: Occurrence of Staphylococcus aureus enterotoxins in fluid milk samples collected from Khartoum State, 2019

Result	**Milk types**			**Total**
	Raw	**Pasteurized**	**UHT**	
Negative	15 (36.6%)	15 (36.6%)	11 (26.8%)	41 (73.2%)
SEA	0 (0.0%)	0 (0.0%)	8 (100%)	8 (14.3%)
SEB	0 (0.0%)	0 (0.0%)	2 (100%)	2 (3.6%)
SEC	0 (0.0%)	0 (0.0%)	5 (100 %)	5 (8.9%)
SED	0 (0.0%)	0 (0.0%)	0 (0%)	0 (0%)
Total	15 (26.8%)	15 (26.8%)	26 (46.4%)	56 (100%)

Table 3: Direct extraction from milk samples directly

Samples	**Milk Type**		
	Raw	**Pasteurized**	**UHT**
Gene types	7 (100%)	11 (100%)	11 (37.9%)
sea	0	0	3 (27.2%)
seb	0	0	0
sec	0	0	2 (18.1%)
sed	0	0	0
Total positive	0	0	5 (45.3%)

Discussion

The grape like clusters of Staphylococci shown in Figure 1, they occur because the bacterial cells divide into three planes in an irregular pattern producing branches of cocci, which are characteristics to *Staphylococcus spp*. (Brooks *et al*., 2007). The *S. aureus* enterotoxins (SEs) are potent gastrointestinal exotoxins synthesized by *S. aureus* throughout the logarithmic phase of growth or during the transition from the exponential to the stationary phase (Derzelle *et al*., 2009). The SEs is resistant to conditions (heat treatment, low pH) that easily destroy the bacteria that produce them, and to proteolytic enzymes, hence retaining their activity in the digestive tract after ingestion (Argudin *et al*., 2010). The *S. aureus* is one of the most prevalent potential pathogenic bacteria found in pasteurized donor human milk (DHM) and some strains produce heat stable enterotoxins that are able to survive pasteurization. These enterotoxins have been associated with gastritis and potentially necrotizing enterocolitis in preterm infants (Almutawif *et al*., 2019).

This study provides insight into the contamination of both raw and commercially processed milk with *S. aureus* in Khartoum State (**Table 1**) and highlights the risk of staphylococcal enterotoxins in the dairy supply. Similarly, Shuiep *et al*. (2009) found 46 (41%) of camels milk samples were positive for *S. aureus* in Sudan and that 95% of the *S. aureus* isolates were found to be positive for three or more SEs genes by PCR amplification. Although pasteurization kills *S. aureus* cells, thermostable SEs generally retain their biological activity even after pasteurization (Janstova *et al*., 2012).

Nearly half of the milk samples contained *S. aureus*, underscoring the ubiquity of this pathogen in the local dairy chain (**Table 1**). The highest contamination rate was observed in UHT milk (55.6%), which is noteworthy since UHT products are generally assumed to be sterile after processing. This findings suggest that post-processing contamination occurred in the UHT milk samples, allowing *S. aureus* to enter the product. Similar instances of *S. aureus* contamination in pasteurized or UHT milk have been reported elsewhere, often linked to failures in hygienic handling. Zhang *et al.* (2022) found *S. aureus* in pasteurized milk in China and reported that 17.24% of those isolates contained the sea enterotoxin gene. Post-pasteurization contamination can occur if milk contacts unsanitary equipment or packaging materials, or if personnel introduce bacteria during filling operations. Once a few *S. aureus* cells are present in heat-treated milk, they can multiply rapidly if the cold chain is broken (Zhang *et al*., 2022). Temperature abuse (e.g., leaving milk at room temperature) greatly increases the risk, as *S. aureus* can grow and produce enterotoxins even in sealed containers given sufficient time (Derzelle *et al*., 2009). Moreover, at low-temperature, *S. aureus* cells did not enter into the viable but non-culturable state (Liu *et al.*, 2023).

The detection of classical enterotoxin genes (*sea, seb, sec*) exclusively in UHT milk isolates is intriguing (Figure 3 and Table 2). The detection of multiple enterotoxin genes within individual *S. aureus* isolates in this study corroborates previous reports indicating that enterotoxigenic strains often carry a diverse repertoire of toxin-encoding genes. Interestingly, none of the *S. aureus* isolates is recovered from raw milk contained these genes, which contrasts with the findings from other regions where a substantial proportion of raw milk isolates have been reported as enterotoxigenic (Jung and Lee, 2022). This discrepancy may reflect regional differences in herd management, milk handling practices, or the prevalence of specific *S. aureus* lineages. One explanation could be the relatively small sample of raw isolates; with only eight raw *S. aureus* strains, it is possible that by chance none harbored sea-sed. Another factor is that the PCR used in this study as screening was limited to four toxin genes. *S. aureus* can carry a variety of enterotoxin genes, including the "non-classical" types (e.g., see, seg, seh, sei). This study did not test for these, so it is possible that some raw milk isolates might carried genes such as see or seg-sei. Indeed, a study from China found that while only about 13% of raw milk *S. aureus* carried classical SE genes, many had other virulence factors and the most common toxin gene was see (Jingsha *et al*., 2019). However, Jingsha *et al*. (2019) found that *seg*, *sei* and *sem* were most frequently detected in pasteurized milk in China. This suggested that future investigations in Sudan should include a broader panel of enterotoxin genes and phenotypic assays to detect actual toxin production. It is also possible that many *S. aureus* strains from raw milk were of bovine origin (mastitis-associated), which sometimes have lower prevalence of classical enterotoxins compared to human-origin strains (Kou *et al*., 2021). Moreover *sen*, *sec*, *sel*, *seo*, *sep* and *she* were detected as 25%, 16.7%, 16.7%, 8.3%, 8.3%, 8.3% respectively (Kou *et al*., 2021). Luca *et al*. (2019) found *sea* (35.29%), *seb* (5.88%) *sec* (5.88%) *sed* (29.41%) and *see* (47.06%) in *Staphylococcus aureus* isolates from mastitic cows. The dairy products made from pasteurized milk or raw milk derived from animals with subclinical mastitis are intrinsically contaminated, which may lead to staphylococcal outbreaks (Hennekinne *et al*., 2012). Moreover, the numerous examples of *S. aurues* causing bacteremia were reported in human with predisposing conditions of dairy farms (Vahedi *et al*., 2013).

In contrast, the contamination in UHT milk might have originated from human sources (e.g., handlers or environment), as sea is often linked to human carriers (Mansour *et al*., 2017). The dominance of *sea* and *sec* genes in UHT isolates in the present study aligns with the fact that *sea* is the most common cause of staphylococcal food poisoning globally (Hennekinne *et al*., 2012). The presence of *sec* is notable as well, since *sec* is typically associated with dairy outbreaks and is produced by some mastitis strains; its detection here may indicate a dairy source contamination too (Hennekinne *et al*., 2012). Shuiep *et al*. (2009) was able to detect *sec* in raw camels milk samples. Interestingly, *seb* was found in two UHT isolates; *seb* is less frequently implicated in food poisoning but is a powerful superantigen that has been studied as an inhaled bioweapon. Similarly, enterotoxins A and B were detected after 9 hours of pasteurized DHM kept at at 37°C (Almutawif *et al.*, 2019). On the other hand, the *seb* gene was found in most of rice samples with difference in their continuous expression level (Liu *et al.*, 2023). The co-occurrence of multiple toxin genes in single isolate (e.g., sea and sec) agrees with other research (Almutawif *et al*., 2019) indicating that enterotoxigenic *S. aureus* often carry multiple toxins, which can enhance their pathogenic potential. The presence of multiple enterotoxin genes was reported for enterotoxigenic *S. aureus* isolated during veterinary routine diagnostic in Germany (El Zubeir *et al*., 2007), mastitic cows (Luca *et al*., 2019), ovine mastitis (Achek *et al*., 2020; Azara *et al*., 2017), ovine milk (Merz *et al*., 2016), bovine milk (Peles *et al*., 2007; Merz *et al*., 2016) and raw camel milk (Shuiep *et al*., 2009). In addition, raw milk (Kou *et al*., 2021; Rall *et al*., 2008) and pasteurized milk (Jingsha *et al*., 2019; Rall *et al*., 2008) also showed the presence of some enterotoxigenic *S. aureus*.

When contextualised with recent literature, the prevalence of *Staphylococcus aureus* in raw milk (29.6%) in Khartoum, although slightly lower than that reported in several developing regions, remains notably high and of epidemiological concern. A recent meta-analysis conducted in Ethiopia documented a comparable prevalence of 30.7% in raw cow milk (Deddefo *et al.,* 2022), while studies from West Africa have reported rates exceeding 40% (Akkou *et al*., 2018).

The detection of *S. aureus* in 14.8% of pasteurized milk samples in the present study is particularly alarming, as contamination in heat-treated products should ideally be absent. This observation points to potential lapses in post-pasteurization handling or hygiene during packaging and distribution. In well-regulated industrial settings, thermal processing effectively eliminates *S. aureus* from milk and dairy products; however, inadequate sanitation practices can lead to recontamination following heat treatment (Phiri *et al.,* 2022). Consistent with this, Oliveira *et al.* (2022) reported *S. aureus* in 53% of raw milk samples in Portugal but none in pasteurized milk, reflecting the efficacy of stringent hygienic and processing controls.

The presence of *S. aureus* in pasteurized retail milk in this study therefore underscores critical weaknesses in the local dairy processing and supply chain. These findings highlight the urgent necessity for strict enforcement of Good Manufacturing Practices (GMPs) and comprehensive application of Hazard Analysis and Critical Control Point (HACCP) systems to ensure microbial safety and protect public health within the dairy sector.

Perhaps the most critical implication of this study lies in the public health threat posed by enterotoxin-producing *Staphylococcus aureus* in ready-to-consume milk. Although staphylococcal food poisoning (SFP) is typically self-limiting, it can rapidly affect large populations during outbreaks and may cause severe illness in vulnerable groups. The staphylococcal enterotoxins (SEs) are of particular concern for food safety due to their direct involvement in SFP and their role in life-threatening toxic shock syndrome (Benkerroum, 2018; Abril *et al*., 2020). Moreover, *S. aureus* is capable of producing a wide array of extracellular toxins such as exfoliative toxins, hemolysins, and leukocidins that can contribute to SFP outbreaks through contaminated dairy products (Abril *et al*., 2020). This risk is further amplified in the context of Khartoum, where the consumption of raw or inadequately refrigerated milk remains common due to cultural practices and limited access to proper cold storage (Mohamed and El Zubeir, 2007). Such conditions create an optimal environment for *S. aureus* growth and enterotoxin production, substantially increasing the likelihood of foodborne poisoning incidents (Phiri *et al*., 2022).

The present study revealed that a considerable proportion of UHT milk samples harboured enterotoxin genes a finding of particular concern given that consumers generally regard UHT milk as a safe and long shelf-life product. When such products are stored at ambient temperatures in inadequately regulated markets lacking consistent cooling systems, any contaminating *S. aureus* may actively produce toxins. Notably, the classical staphylococcal enterotoxins (SEs) do not cause detectable changes in the taste or odour of milk, yet even trace (nanogram) concentrations can induce illness (Oliveira *et al*., 2022). Consequently, routine quality control protocols should encompass not only bacterial enumeration but also screening for enterotoxins whenever *S. aureus* is detected particularly in products vulnerable to temperature abuse.

Synthesizing the present results with some recent evidence (Phiri *et al.,* 2022) underscores the critical need for continuous surveillance of *S. aureus* and its enterotoxins across the dairy production chain. The emergence and broader implementation of advanced rapid detection technologies particularly multiplex PCR, as applied in this study, alongside high-sensitivity immunoassays have revolutionised post-2020 monitoring practices, enabling prompt identification and mitigation of contamination risks (Fusco *et al.,* 2011). The development of a multiplex PCR assay constitutes a powerful and efficient tool for the direct detection of *S. aureus* in milk and other food matrices, substantially reducing analysis time and enhancing diagnostic accuracy (Fusco *et al.,* 2011). Applied for the first time in Sudan, this molecular approach enables rapid identification of enterotoxigenic *S. aureus* strains in dairy products, addressing a critical gap in national food safety monitoring. Notably, many countries have established strict guidelines for permissible *S. aureus* counts in milk and require testing for enterotoxins when bacterial loads exceed defined thresholds, particularly in cheese and dried milk products. In Sudan, however, specific regulatory frameworks are still under development. This underscores an urgent need for implementing proactive food safety measures and establishing evidence-based standards, as highlighted by the findings of the present study, to prevent contamination, safeguard public health, and align national dairy practices with international standards.

Limitations

This study had a limited sample size and geographic scope (one month, selected urban areas). Prevalence figures should therefore be interpreted with caution and not generalized to all seasons or regions. Additionally, it was only screened for four classical enterotoxin genes; the toxin production was not confirmed e.g., via ELISA or test for genes like *see* or *seg-sei*. Despite these limitations, the use of PCR provides a sensitive indicator of enterotoxigenic potential. Future studies should include more samples over time, a broader array of enterotoxin genes, and quantification of any produced toxins. Investigating sources of post-process contamination in dairy factories (through environmental sampling or tracing specific points of entry) would also be valuable.

Conclusion

Contamination of milk with *Staphylococcus aureus* and its enterotoxins continues to represent a pressing food safety challenge in Khartoum State, Sudan. The frequent detection of *S. aureus* in raw milk from local markets underscores the risks associated with consumption without adequate heat treatment. Alarmingly, a notable proportion of UHT milk samples were also found to harbour enterotoxin-producing *Staphylococcus aureus*, likely resulting from post-processing contamination. Given that these enterotoxins can withstand thermal treatments and cause illness even in the absence of viable bacteria, the findings highlight a serious public health concern. This study underscores the urgent need for comprehensive hygiene management across the dairy production and distribution chain. Implementation of strict sanitation protocols, aseptic packaging, and rigorous cold chain maintenance by producers, retailers, and consumers is essential to prevent bacterial proliferation, safeguard consumer health, and maintain confidence in dairy products. Such measures have critical implications not only for local food safety but also for broader regional public health protection. The present findings underscore the critical need for public health authorities to implement systematic and routine screening of milk and dairy products for *Staphylococcus aureus* and staphylococcal enterotoxins using rapid and sensitive detection methods. In line with recent evidence, these results highlight that proactive surveillance, coupled with stringent quality control measures, is pivotal for preventing staphylococcal foodborne outbreaks and ensuring the safety of the dairy supply chain. Such strategies are essential not only for protecting consumer health but also for strengthening public confidence in dairy products.

Declarations

Ethics approval and consent to participate

Not applicable

Data Availability

All data is available from the corresponding author on reasonable request.

Conflicts of Interest

None

Funding Statement

None

Acknowledgments

Not applicable

References

[1] Abril, A.G., Villa, T., Barros-Velázquez, J., Cañas, B., Sánchez-Pérez, A., Calo-Mata, P. and Carrera, M. (2020). *Staphylococcus aureus* exotoxins and their detection in the dairy Industry and mastitis. *Toxins*, *12*(9), 537. DOI: 10.3390/toxins12090537

[2] Achek, R., El-Adawy, H., Hotzel, H., Tomaso, H., Ehricht, R., Hamdi, T. M., Azzi, O., & Monecke, S. (2020). Short communication: Diversity of staphylococci isolated from sheep mastitis in northern Algeria. *Journal of Dairy Science*, **103**(1), 890–897. DOI: 10.3168/jds.2019-16583

[3] Akindolire, M, A., Babalola, O, O., Ateba, C, N., (2015). Detection of Antibiotic Resistant *Staphylococcus aureus* from Milk: A Public Health Implication. *Int. J. Environ. Res. Public Health*, 12(9), 10254-10275. DOI: 10.3390/ijerph120910254

[4] Akkou, M., Bentayeb, L., Ferdji, K., Medrouh, B., Bachtarzi, M. A., Ziane, H., Kaidi, R., & Tazir, M. (2018). Phenotypic characterization of Staphylococci causing mastitis in goats and microarray-based genotyping of *Staphylococcus aureus* isolates. *Small Ruminant Research*, 169, 29–33. DOI: 10.1016/j.smallrumres.2018.10.015

[5] Azara, E., Longheu, C., Sanna, G., & Tola, S. (2017). Biofilm formation and virulence factor analysis of *Staphylococcus aureus* isolates collected from ovine mastitis. *Journal of Applied Microbiology*, 123(2), 372–379. DOI: 10.1111/jam.13502

[6] Almutawif, Y., Hartmann, B., Lloyd, M., Lai, C.T., Rea, A. and Geddes, D. (2019). *Staphylococcus aureus* enterotoxin production in raw and pasteurized milk: The effect of selected different storage durations and temperatures. *Breastfeeding Medicine*, 14(4):256-261. DOI 10.1089/bfm.2018.0227

[7] Argudin, M. A., Mendoza, M. C. and Rodicio, M. R. (2010). Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins*, *2*(7), 1751-1773. DOI: 10.3390/toxins2071751

[8] Benson, E., Melissa, S. H., Gil, H. C., Joyce, I. C., John, T. H., Joseph, P. M. and Eric, T. B. (2001). A structural variation for murb: X-ray crystal structure of *Staphylococcus aureus* UDP-N-Acetylenolpyruvyglucosamine reductase. *Biochemistry* 2001, 40, 8, 2340–2350. DOI: 10.1021/bi002162d

[9] Benkerroum, N. (2018). Staphylococcal enterotoxins and enterotoxin-like toxins with special reference to dairy products: An overview. *Food Science and Nutrition*, *58*(12): 1943-1970. DOI:10.1080/10408398.2017.1289149

[10] Brooks, G. F., Caroll, K. C. , Butel, J. S., Morse, S. A. (2007). *Staphylococcus* (Jawetz). Melnick and Adelberg's, Medical Microbiology, 24th edition. *The McGraw-Hill. New York*.

[11] Bystron, J., Bania, J., Lis, E., Molenda, J. and Bednarski, M. (2009). Characterization of *Staphylococcus aureus* strains isolated from cows' milk. *Bull Vet Inst Pulawy*, 53: 59-63.

[12] Cha, J. O., Lee, J. K., Jung, Y. H., Yoo, J. I., Park, Y. K., Kim, B. S. and Lee, Y. S. (2006). Molecular analysis of *Staphylococcus aureus* isolates associated with staphylococcal food poisoning in South Korea. *J. Appl. Microbiol*., 101: 864-871. DOI: 10.1111/j.1365-2672.2006.02957.x

[13] Deddefo, A., Mamo, G., Leta, S., and Amenu, K. (2022). Prevalence and molecular characteristics of *Staphylococcus aureus* in raw milk and milk products in Ethiopia: a systematic review and meta-analysis. *International Journal of Food Contamination*, 9, 8 DOI: 10.1186/s40550-022-00094-5

[14] Derzelle, S., Dilasser, F., Duquenne, M. and Deperrois, V. (2009). Differential temporal expression of the staphylococcal enterotoxins genes during cell growth. *Food Microbiology,* 26: 896–904. DOI: 10.1016/j.fm.2009.06.007

[15] El Sanousi, S.M.; Said, K.B.; Elbager, S.; Awad, A.; Rodwan, K. and Eltom, K.H. (2015). A flow chart for the identification of *Staphylococcus* species. *U. of K. J. Vet. Med. Anim. Prod*., 6 (2), 93-97. DOI: https://onlinejournals.uofk.edu/index.php/vet/article/view/443

[16] El Zubeir, I. E. M.; Kanbar, T.; Alber, J.; Lämmler, C.; Akineden, O.; Weiss, R. and Zschöck, M. (2007). Phenotypic and genotypic characteristics of methicillin resistance from *Staphylococcus intermedius* isolated from clinical specimens during routine veterinary microbiological examinations. *Veterinary Microbiology*, 121: 170-176. DOI: 10.1016/j.vetmic.2006.11.014

[17] Flowers, S.R.; Andrews, W.; Donnelly, C.W. and Koenig, E.K. (1992). Pathogens in Milk and Milk Products. In: Standard Methods for the Examination of Dairy Products, Marshall R.T. (Ed), 16th Ed., American Public Health Association, Washington, DC, USA, 103-212.

[18] Fusco, V., Quero, D. M., Morea, M., Balliotta, D. and Angelo, V. (2011). Rapid and reliable identification of *Staphylococcus aureus* harbouring the enterotoxin gene cluster (egc) and quantitative detection in raw milk by real time PCR. *International Journal of Food Microbiology,* 144: 528-537. DOI: 10.1016/j.ijfoodmicro.2010.11.016

[19] Havelaar, A. H., Brul, S., de Jong, A., de Jonge, R., Zwietering, M. H. and Ter K. B. H. (2010). Future challenges to microbial food safety. *Int. J. Food Microbiol*., 139:79-94. DOI: 10.1016/j.ijfoodmicro.2009.10.015

[20] Hennekinne, J. A.; De Buyser, M. L; and Dragacci, S. (2012). *Staphylococcus aureus* and its food poisoning toxins: Characterization and outbreak investigation. *FEMS Microbiology Reviews,* 36: 815-836. DOI: 10.1111/j.1574-6976.2011.00311.x

[21] Hoteit, M., Yaghi, J., El Khoury, A., Daou, R., Hindieh, P., Assaf, J, C., Al Dawi, J., El Khoury, Al Jawaldeh, A. (2022). Prevalence and antibiotic resistance of *Staphylococcus aureus* and *Escherichia coli* isolated from bovine raw milk in Lebanon: A study on antibiotic usage, antibiotic residues, and assessment of human health risk using the One Health Approach. *Antibiotics*, 11(12):1815. DOI: 10.3390/antibiotics11121815

[22] Janstova, B., Necidova, L. and Bohumíra, J. (2012). Comparing the growth of *Staphylococcus aureus* and production of staphylococcal enterotoxin C in sheep's and goat's milk. *Journal of Microbiology, Biotechnology and Food Sciences*, (8): 758-768. https://www.proquest.com/scholarly-journals/comparing-growth-s-aureus-production/docview/1648115827/se-2

[23] Jingsha, D., Shi, W., Jiahui, H., Qingping, W., Feng, Z., Jumeni, Z., Juan, W., Yu, D., Shuhong, Z., Xiaojuan, Y., Tao, L., Liang, X. and Haomind W. (2019). Prevalence and characterization of *Staphylococcus aureus* isolated from pasteurized milk in China. *Front. Microbiol.*, 10: 3389. DOI: 10.3389/fmicb.2019.00641

[24] Jung, H. R., and Lee, Y. J. (2022). Characterization of virulence factors in enterotoxin-producing Staphylococcus aureus from bulk tank milk. *Animals,* 12(3), 301. DOI: 10.3390/ani12030301

[25] Kou, X., Cai, H., Huang, S., Ni, Y., Luo, B., Qian, Y., Ji, H., and Wang, X. (2021). Prevalence and characteristics of *Staphylococcus aureus* isolated from retail raw milk in

Northern Xinjiang, China. *Frontiers in Microbiology*, 12, 638707. DOI: 10.3389/fmicb.2021.705947

[26] Luca, G., Massetti, L., Paola, S., Maria, F. L., Margherita, C., Musafiri, K., Paul, A., Francesco, P. and Beniamino, T. (2019). Characterization of enterotoxin-producing *Staphylococcus aureus* isolated from mastitic cows. *Journal of Dairy Sci*., 102:1059-1065. DOI: 10.3168/jds.2018-15373

[27] Ler, S.G., Lee, F.K. and Gopalakrishnakone, P. (2006). Trends in detection of warfare agents: detection methods for ricin, staphylococcal enterotoxin B and T-2 toxin. *Journal of chromatography A*, *1133*(1-2),.1-12.DOI: 10.1016/j.chroma.2006.08.078

[28] Liu, J., Huang, T., Soteyome, T., Mao, Y., Luo, Y., Yuan, L., Ma, Q., Liu, G., Qu, C., Seneviratne, G. and Kjellerup, B.V. (2023). The survival and enterotoxin gene expression of *Staphylococcus aureus* planktonic and biofilm cells in quick-frozen food. *Food Science & Technology*, 187, 115354. DOI: 10.1016/j.lwt.2023.115354.

[29] Mansour, A. S., Gad Elsaid, W. and Elgabry, E. A. (2017). Detection of *Staphylococcus aureus* enterotoxigenic strains in bovine raw milk by reversed passive latex agglutination and multiplex polymerase chain reaction. *Veterinary World*, (10): 2231-2244. DOI: 10.14202/vetworld.2017.843-847

[30] Marshall, R.T. (1992). Standard Methods for the Examination of Dairy Products, 16th edition, Ed., Marshall, R.T., American Public Health Association. Washington, DC, USA.

[31] Merz, A., Stephan, R., & Johler, S. (2016). *Staphylococcus aureus* isolates from goat and sheep milk seem to be closely related and differ from isolates detected from bovine milk. *Frontiers in Microbiology*, **7**, 319. DOI: 10.3389/fmicb.2016.00319

[32] Mohamed, O. A. K. and El Zubeir, I. E. M. (2018). Comparative study on chemical and microbiological properties of white cheese produced by traditional and modern factories. Annals. *Food Science and Technology*, 19: 111-112.

[33] Shuiep, E. S., Kanbar, T., Eissa, N., Alber, J., Lammler, C., Zschck, M., El Zubeir, I. E. M. and Weiss, R. (2009). Phenotypic and genotypic characterization of *Staphylococcus aureus* isolated from raw camel milk samples. *Research in Veterinary Science,* DOI: 10.1016/j.rvsc.2008.07.011

[34] Oliveira, R., Pinho, E., Almeida, G., Azevedo, N. F., and Almeida, C. (2022). Prevalence and diversity of *Staphylococcus aureus* and staphylococcal enterotoxins in raw milk from Northern Portugal. *Frontiers in Microbiology*, *13:* 846653. DOI: 10.3389/fmicb.2022.846653

[35] Peles, F., Wagner, M., Varga, L., Hein, I., Rieck, P., Gutser, K., Kereszturi, P., Kardos, G., Turcsanyi, I., Beri, B. and Szabo, A. (2007). Characterization of *Staphylococcus aureus* strains isolated from bovine milk in Hungary. *Int. J. of Food Microbiol*., 118: 186-193. DOI: 10.1016/j.ijfoodmicro.2007.07.010

[36] Phiri, B.S., Hang'ombe, B.M., Mulenga, E., Mubanga, M., Maurischat, S., Wichmann-Schauer, H., Schaarschmidt, S. and Fetsch, A. (2022). Prevalence and diversity of *Staphylococcus aureus* in the Zambian dairy value chain: A public health concern. *International Journal of Food Microbiology*, 375: 109737. DOI: 10.1016/j.ijfoodmicro.2022.109737

[37] Shalaby, M., Reboud, J., Forde, T., Zadoks, R. N., and Busin, V. (2024). Distribution and prevalence of enterotoxigenic *Staphylococcus aureus* and staphylococcal enterotoxins in raw ruminants' milk: A systematic review. *Food Microbiology*, 118: DOI: 10.1016/j.fm.2023.104405

[38] Shuiep, E. S.; Kanbar, T.; Eissa, N.; Alber, J.; Laemmler, C.; Zschǫck, M.; El Zubeir, I.E.M. and Weiss, R. (2009). Phenotypic and genotypic characterization of *Staphylococcus aureus* isolated from raw camel milk samples. *Research in Veterinary Science*, 86: 211-215. DOI: 10.1016/j.rvsc.2008.07.011

[39] Mohamed, N. I. and El Zubeir, I. E. M (2007). Comparison of some compositional quality measurement of market milk in Khartoum state, Sudan. *International Journal of Dairy Science*, 2: 42-49. DOI: 10.3923/ijds.2007.42.49

[40] Vahedi, M., Nasrolahei1, M., Sharif, M. and Mirabi, A. M. (2013). Bacteriological study of raw and unexpired pasteurized cow's milk collected at the dairy farms and super markets in Sari city in 2011. *J. Prev. Med. Hyg*., 54: 120-123.

[41] Von Eiff, E. C., Becker, K., Machka, K., Stammer, H. and Peters, G. (2001). Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. *N. Engl. J. Med*., 344: 11–16. DOI: 10.1056/NEJM200101043440102

[42] Rall, V. L. M., Vieira, F. P., Rall, R., Vieitis R. L., Fernandes, A. J., Candeias, J. M. G., Cardoso, K. F. G. and Arau´jo, J. P. J. (2008). PCR detection of staphylococcal enterotoxin genes in *Staphylococcus aureus* strains isolated from raw and pasteurized milk. *Veterinary Microbiology*, 132:408–413. DOI: 10.1016/j.vetmic.2008.05.011

[43] Zhang, Z., Song, Y., Ma, L., Huang, K., & Liang, Z. (2022) Co-occurrence of *Staphylococcus aureus* and Ochratoxin A in pasteurized milk. *Toxins*, *14*(10): 718. DOI: 10.3390/toxins14100718