Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

MRSA in kareish cheese: Role of probiotic (yoghurt starter) in controlling of *S. aureus* growth and SEb gene expression.

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Received in 4/2/2025
Received in revised from 3/3/2025
Accepted in 18/3/2025

Keywords:
Kareish Cheese
MRSA
Staphylococcus Enterotoxins
Yoghurt Starter.

ABSTRACT

This study intended to evaluate the microbial, chemical, and nutritional quality of kareish cheese through the analysis of 100 samples obtained from different retail markets in Fayoum Governorate. Incidence of *Staphylococcus spp.*, *Staphylococcus aureus* (*S. aureus*) and Methicillin-resistant *S. aureus* (MRSA) were 61%, 54% and 11%, respectively. Isolated MRSA strains sensitivity against some antibiotics commonly used in animal treatment (Cefotaxime, Oxytetracycline, Vancomycin, Flucloxacillin, Oxacillin, Ampicillin, Erythromycin, Chloramphenicol, Gentamycin, Tobramycin and Streptomycin) was investigated. All MRSA isolates (100%) showed high resistance to Vancomycin, Flucloxacillin, Oxacillin and Erythromycin, while 90.91% of isolates showed resistance to Streptomycin and 81.82% were resistant for Cefotaxime, Ampicillin and Chloramphenicol. The moderate resistance of isolates was for Tobramycin (54.55%) and Oxytetracycline (45.45%) and finally, Gentamycin (27.27%). Multiplex PCR analysis of the isolated *S. aureus's* enterotoxin genes showed that 7 isolates (77.78%) of the tested isolates (9) found enterotoxigenic, SEc gene was the highest frequency incidence (5 isolates, 55.56 %) followed by SEb gene (2 isolates, 22.22%). Yoghurt starter's antimicrobial activity against MRSA and its impact on *Staphylococcus* enterotoxin gene expression patterns during the production of Kareish cheese demonstrated positive

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DOI: 10.21608/ejah.2025.429724

outcomes, as it decreased *S. aureus* count and down-regulated the expression of the SEb gene. Therefore, in order to prevent *S. aureus* and its enterotoxins production, we recommend that probiotics (yoghurt starter) be incorporated into the production of dairy products.

INTRODUCTION

Kareish cheese is considered the commonly consumed soft white cheese in Egypt, a native dairy product. It is a generation-to-generation carryover from the era of the ancient Egyptians. Since Kareish cheese manufactured from skim milk, it contains low fat, low-cost source of protein, minerals (particularly calcium, phosphorous, sodium, and potassium), and vitamins. If produced under hygienic control, it is regarded as a good choice for healthy diet programs (**Food composition tables, 1998; Deeb et al. 2004; Todaro et al. 2013**).

Staphylococcus aureus produces Staphylococcal enterotoxins (SEs), it is found in animal and human noses, throats, and skin. Using unprocessed milk, improper processing or post-processing managing are the primary causes of cheese contamination with foodborne pathogens, including *S. aureus* (**Kousta et al. 2010**). External animal surfaces, the surrounding environment, the milking process, utensils, or human workers can all transfer *S. aureus* into milk (**Jamali et al. 2015**). *Staphylococcus aureus* is considered to be the third cause of most common foodborne pathogens. Its presence suggests inadequate hygienic practices throughout the manufacturing, handling, and managing of milk and its products. In addition, mastitic milk and diseased food handlers can be sources of contamination (**Eid et al. 2022**).

Unprocessed milk, cheeses, ice cream, and yoghurt, tend to be contaminated with Methicillin-resistant *S. aureus* (MRSA) (**Peton and Le Loir, 2014; Al-Ashmawy et al. 2016; Schnitt and Tenhagen, 2020**). MRSA was first discovered in healthcare settings, it has since been connected to infections in the community and in cattle (**Scientific Report of EFSA and ECDC, 2015**). As MRSA shows resistance to β-lactam antibiotics, which are frequently used for treatment of mastitic cases, so a condition of cure difficulty takes place (**Holmes and Zadoks 2011; Haenni et al. 2017**). Direct contact between farm animals and humans could transmit MRSA (**Goerge et al. 2017**), consumption of raw milk or unprocessed products may increase the opportunity of contracting the illness (**Al-Ashmawy et al. 2016; Basanisi et al. 2017**).

After Salmonellosis, Staphylococcal food poisoning disease (SFP) occurs worldwide, SFP is a disease resulted from ingestion of foods containing preformed Staphylococcal enterotoxins. 20-100 ng of toxins can cause SFP, and the sickness severity bases on the individual's health and the amount of consumed toxin (**Asao et al. 2003; Orwin et al. 2003; Aycicek et al. 2005; Schelin et al. 2011**).

Dizziness, emesis, diarrhea, and abdominal discomfort are among the signs that commonly show up rapidly, in a few hours of consumption. The disease is mild, self-curing, and cured in one or two days. However, hospitalization may be required for some susceptible groups, including newborns, elders, those with immunologic deficiencies, and people with underlying medical conditions (**Bergdoll, 1989; Murray, 2005**).

Staphylococcal Enterotoxins (SEs) are super antigenic, pyrogenic exotoxins that are soluble in water and saline. They have a strong resistance to proteolytic enzymes, low pH, drying, freezing, and heat (**Bergdoll, 1989; Krakauer, 2012**). Available commercial assays can characterize and detect just five classical enterotoxins (SEa, SEb, SEc, SEd, and See), out of the twenty-four SEs that have been found in the literature. Classic food poisoning is most frequently linked to SEb in particular. The pathogenesis of ulcerative colitis, chronic rhinitis, and chronic atopic dermatitis has also been connected to SEb (**Bozek et al. 2012; Hedayati et al. 2016** and **Nia et al. 2016**).

Generally, risk of toxi-infection takes place if *S. aureus* count in cheese is more than 10^5 cfu/gram during manufacture as SEs can be

formed. Temperature, acidity, salinity, activity of water, and existence of competitive bacteria are some of the extrinsic elements that affect *S. aureus's* capacity to produce these enterotoxins (**Le Loir et al. 2003; Ostyn et al. 2010; Masoud et al. 2012; Johler et al. 2015; Carfora et al. 2016**).

Adding starter cultures is a common way to enhance the commercial shelf life of cheese. Primarily used as starters in dairy products, lactic acid bacteria (LAB) are essential for fermentation, acid generation, and the synthesis of additional chemicals that enhance flavor and aroma. Along with these advantages, LAB also has an inhibitory effect on and *S. aureus* growth and therefore, enterotoxins production (**Vernozy-Rozand et al. 1998; Padhi et al. 2022**).

MATERIALS and METHODS

Staphylococcus aureus isolation and identification in the surveyed part*:*

Samples collection

One hundred randomly selected Kareish cheese samples were gathered from several retail market places in Fayoum Governorate, Egypt. In order to minimize any delays, the specimens were promptly brought to the lab in a sterile ice box. Bacteriological investigation of samples takes place once they arrived at the lab for counting, isolation and identification of *S. aureus*.

Sample preparation, inoculation and *S. aureus* count according to (FDA, 2001)

The cheese samples were prepared by making ten-fold serial dilutions to determine *S. aureus* existence. This was accomplished under sanitized conditions by mixing 25g. of the sample with 225 mL of 0.1% peptone water for 1.5 minutes. Ten-fold serial dilutions were made up to 10^{5}, followed by surface spreading of Baired Parker agar plates with 0.1 ml of each dilution, inoculated plates with control were incubated at 37º±C for 24 hours. Suspected colonies (black, shining with a narrow white rim and a clear area expending to the surroundings) were counted. For additional identification, suspected colonies were subsequently selected and streaked onto agar slants, which were then incubated for 24 hours at 37º±C.

Sensitivity test for *S. aureus* isolates

The Kirby-Bauer disk diffusion scheme was used to test antimicrobial sensitivity of isolated *S. aureus* strains on Mueller-Hinton agar plates (MH, Oxoid), following the **Clinical and Laboratory Standards Institute (CLSI), 2018**. The chosen antimicrobials were frequently used in veterinary medicine. In this investigation, antimicrobial disks used (Oxoid, U.K.) included: Cefotaxime (CTX) 30 µg, Oxytetracycline (OTC) 30 µg, Vancomycin (VAN) 30 µg, Flucloxacillin (FLX) 5 µg, Oxacillin (OXA) 10 µg, Ampicillin (AMP) 20 µg, Erythromycin (E) 15 µg, Chloramphenicol (C) 30 µg, Gentamicin (CN) 10 µg, Tobramycin (TOB) 10 µg, and Streptomycin (S) 10 µg. After 24 hours of incubation at 37°±C, CLSI guidelines were followed in recording and interpreting the results (**Weinstein and Lewis, 2020**). According to **Waters et al. (2011)**, resistance against three different classes of antibiotic means multidrug resistance of the isolate.

Detection of enterotoxin genes of isolated *S. aureus* by multiplex PCR:

DNA extraction*:* Using the QIAamp DNA Mini Kit (**Qiagen, Germany, GmbH**) and adjusting the manufacturer's recommendations.
Oligonucleotide Primer: Primers used were provided by Metabion (**Germany**) are in Table (A).

Table A. Sequences of forward and reverse primers for genes used in PCR system.

Target gene	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Reference
SEa	GGTTATCAATGTGC GGGTGG	CGGCACTTTTTTCT CTTCGG	Mehrotra et al., 2000
SEb	GTATGGTGGTGTAA CTGAGC	CCAAATAGTGACGA GTTAGG	
SEc	AGATGAAGTAGTTGATGTGTATGG	CACACTTTTAGAAT CAACCG	
SEd	CCAATAATAGGAG AAAATAAAAG	ATTGGTATTTTTTTT CGTTC	
SEe	AGGTTTTTTCACAG GTCATCC	CTTTTTTTTCTTCGG TCAATC	
16S rRNA	CCTATAAGACTGGGATAACTTCGGG	CTTTGAGTTTCAACCTTGCGGTCG	Mason et al., 2001

PCR amplification: For multiplex PCR, the reaction was performed according to (**Mehrotra et al. 2000**), using EmeraldAmp® MAX PCR Master Mix **(Takara, Japan)**.

Analysis of PCR Products**:** Using a voltage gradient of 5V/cm, the PCR products were partitioned by electrophoresis on a 1.5% agarose gel (**Applichem, Germany, GmbH**), the fragment sizes were estimated using a GeneRuler 100 bp ladder (**Fermentas, Thermo Fisher, Germany**). A gel documentation system (**Alpha Innotech, Biometra**) was used to shot the gel, and computer software was used to analyze the results.

Experimental design of laboratory manufacturing of kareish cheese: -

2.1. Inoculum preparation:

A previously isolated *S. aureus* strain having SEb gene (isolated from the survey and confirmed by PCR) inoculated into Brain Heart Infusion (BHI) broth **(BBL11407, Lansing, MI, USA)** and incubated for one 24 hours at 37°±C. The inoculum was re-suspended in skim milk after being twice rinsed with phosphate-buffered saline (PBS) (**Oxoid, Basingstoke, UK**) before being inoculated into milk.

Buffalo's Skim milk*:*

Three liters of buffalo's skim milk were pasteurized for 30 minutes at 63°C after being salted with 5% concentration. After that, the warm milk at 35-40°C was mixed with 3% local rennet. The warmed milk was mixed with the prepared inoculum, which had *S. aureus* count of 1.2×10^6 cfu/ml. For additional processing, the artificial contaminated milk was separated into two parts: part 1 (G1), which was the control and contained only *S. aureus*, and part 2 (G2) contained both *S. aureus* and a 7% active natural yoghurt starter culture. After that, Kareish cheese was manufactured as clarified by (**Effat et al. 2001**). After being weighed, the recovered cheese was chilled for ten days at 5°C in sterile containers for analysis.

Sampling

Samples of laboratory produced kareish cheese were taken from fresh (day zero) and chilled (day 10) for *S. aureus* count, chemical examination (protein %, pH value, moisture %, and total solids %), and detection of Staphylococcal Enterotoxin b (SEb). Chilled samples (day 5) for SEb gene expression of *S. aureus*.

Microbiological and chemical analysis of kareish cheese:

Total *S. aureus* count (**FDA, 2001**).

Protein was measured according to Kjeldahl method (**Lynch and Barbano, 1999**); pH value and Moisture were worked out following the AOAC guidelines (**Association of Official Analytical Chemists, 2000**). Total solids % (w/w) were estimated by subtract the resulted moisture % from 100%. Calculation of yield % takes place following the equation, yield % = amount of cheese (kg.)/the amount of skim milk (kg.) ×100.

Staphylococcal Enterotoxin b (SEb) analysis using ELISA technique:

According to **Rahimi et al. (2012)**, specimens from the control and starter cultured cheese were tested for the existence of SEb using an ELISA kit (**RIDASCREENÒ SET A, B, C, D, E Art. No: R4101, R-Biopharm**

AG, Germany). The samples were tested following the manufacturer's instructions and using an ELISA reader (**Start Fax 2100, Westport, UK**).

SEb gene expression:

RNA extraction: to protect RNA of isolates from degradation, RNA protector Bacteria Reagent **(Qiagen, Germany, GmbH)** was used. The procedures applied following QIAamp RNeasy Mini Kit's Enzymatic Lysis of Bacteria protocol (**Qiagen, Germany, GmbH**).

Oligonucleotide Primers*:* Primers used for *S. aureus* were provided by **Metabion (Germany)** as mentioned in Table (A).

SYBR green rt-PCR: The PCR reaction was carried out by a Stratagene MX3005P real-time PCR machine, using of QuantiTect SYBR Green PCR Master Mix **(Qiagen, Germany, GmbH)** and RevertAid Reverse Transcriptase (200 U/µL) **(Thermo Fisher)**.

Analysis of the SYBR green rt-PCR results*:* The stratagene MX3005P software was used for the Ct values and amplification curves determination. In accordance to **Yuan et al. 2006** "ΔΔCt" method, the Ct of each specimen was compared with that of the positive control to assess the variation of gene expression on the RNA of the various specimens using the following ratio: ($2^{-\Delta\Delta ct}$).

Whereas ΔΔCt = ΔCt reference – ΔCt target

ΔCt target = Ct control – Ct treatment and ΔCt reference = Ct control- Ct treatment

Statistical Analysis

The GraphPad InStat software (version 3, ISS-Rome, Italy) was used to conduct the statistical analysis. Unless otherwise noted, data of groups were compared using one-way analysis of variance (ANOVA) and Tukey-Kramer (TK) multiple comparison post-test. The results are given as mean ± standard error of the mean (SEM), and the microbial data are shown as log 10 cfu/g. Definition of significant at a p-value ≤ 0.05, and high significant as a p-value ≤ 0.001 **(Graph Pad InStat, 2017)**.

RESULTS

Results of *S. aureus* isolation and identification in the surveyed part*:*

Examination of 100 samples of Kareish cheese showed, 61 samples (61%) were positive for *Staphylococcus spp.*, 54 samples (54%) were positive for *S. aureus* (coagulase positive) and 11 samples (11%) were positive for MRSA (Fig. 1).

Sensitivity test for *S. aureus* isolates from surveyed part:

High resistance of MRSA isolates were 100% for Vancomycin, Flucloxacillin, Oxacillin and Erythromycin. Streptomycin (90.91%), (81.82%) for Cefotaxime, Ampicillin and Chloramphenicol. Lower resistances were recorded by Tobramycin (54.55%), Oxytetracycline (45.45%) and Gentamycin (27.27%). Results are summarized in Table (1).

Detection of enterotoxin genes of isolated *S. aureus* by multiplex PCR*:*

Multiplex PCR analysis of isolated *S. aureus* strains showed that 7 isolates (77.78%) of isolated *S.* aureus were enterotoxigenic, 5 isolates (55.56 %) was SEc gene which represents the highest frequency incidence followed by SEb gene which found in two isolates (22.22%). SEa, SEd and SEe genes could not be detected, Table (2) and Fig. 2.

Microbiological analysis of kareish cheese*:*

Yoghurt starter addition during experimental manufacturing of kareish cheese (G2) resulted in a significant reduction of total *S. aureus* counts in chilled cheese in comparison to the control (G1), as the mean counts were 6.18±0.02 and 6.35±0.02 log10 cfu/g. in G1 (fresh and chilled), which reduced to 5.91±0.01 and 5.64±0.15 log10 cfu/g. in G2 (fresh and chilled), Fig. 3.

Chemical analysis of kareish cheese*:*

Chemical analysis of surveyed and experimental kareish cheese results are shown in Table (3). Surveyed samples, the mean protein percent was 13.28±0.35. Experimental cheese samples in G2 group (Renin+yoghurt group) showed a significant increase of protein % in

fresh (day 0) and chilled (day 10) (14±0.13 and 17.2±0.09) compared to the control G1 group (Renin group) which recorded 12.6±0.18 and 13.3±0.13, respectively.

Regarding pH of surveyed samples, the mean value was 4.64±0.20. While in the experimental cheese samples, notable decrease in pH values of G2 group (6.25±0.14 and 6.18±0.08) in comparison to G1 group (6.66±0.20 and 6.56±0.20) in fresh and chilled cheese, respectively.

Moisture % and total solids % in surveyed samples were 72.03±1.39 and 27.98±1.39, respectively. Moisture % and subsequently total solids % showed a significant difference in the experiment between G1 and G2 in fresh and chilled cheese samples, where it decreased from 66.60±1.62 and 66.47±0.55 in (G1) to 60.22±1.06 and 59.93±0.85 in (G2) in fresh and chilled samples, respectively. On the other hand, total solids increased from 33.4±1.62 and 33.53±0.55 in (G1) to 39.78±1.06 and 40.07±0.85 in (G2) in fresh and chilled samples, respectively.

Experimental manufactured cheese yield % increased notably by addition of yoghurt starter from 19.33±0.38 % to 21.51±0.29 % (Fig. 4).

Staphylococcal Enterotoxin b (SEb) analysis using ELISA technique:

All samples either surveyed or experimental manufactured cheese were negative for Staphylococcal enterotoxins analysis.

SEb gene expression*:*

SEb gene expression analysis revealed that yoghurt addition (G2) caused a significant down-regulation of SEb gene to 0.309 in comparison to the control (G1), Fig. 5.

Table 1. Sensitivity test of MRSA isolates.

Antimicrobial Agent	Family	CPD	MRSA isolates (n. = 11)			
			Resistant		Sensitive	
			No.	(%)	No.	(%)
Cefotaxime (CTX)	Cephalosporins	30 µg	9	81.82	2	18.18
Oxytetracycline (OTC)	Tetracyclines	30 µg	5	45.45	6	54.55
Vancomycin (VAN)	Glycopeptides	30 µg	11	100	0	0
Flucloxacillin (FLX)	β-lactams	5 µg	11	100	0	0
Oxacillin (OXA)	β-lactams	10 µg	11	100	0	0
Ampicillin (AMP)	β-lactams	20 µg	9	81.82	2	18.18
Erythromycin (E)	Macrolide	15 µg	11	100	0	0
Chloramphenicol (C)	Phenicols	30 µg	9	81.82	2	18.18
Gentamycin (CN)	Aminoglycosides	10 µg	3	27.27	8	72.73
Tobramycin (TOB)	Aminoglycosides	10 µg	6	54.55	5	45.45
Streptomycin (S)	Aminoglycosides	10 µg	10	90.91	1	9.09

Table 2. PCR results for *S. aureus* enterotoxins genes.

Sample	SEa	SEb	SEc	SEd	SEe
1	-	+	-	-	-
2	-	-	+	-	-
3	-	-	+	-	-
4	-	+	-	-	-
5	-	-	-	-	-
6	-	-	+	-	-
7	-	-	+	-	-
8	-	-	-	-	-
9	-	-	+	-	-
No. of +ve samples	-	2	5	-	-
% of +ve samples	-	22.22%	55.56 %	-	-
Total +ve samples			7		
% of total +ve samples			77.78%		

Table 3. Chemical examination of surveyed and experimental kareish cheese.

Parameter Cheese	Protein %	pH value	Moisture %	Total solids %
Surveyed cheese				
minimum	11.58	4.14	67.2	23.8
maximum	15.06	5.44	76.2	32.8
Mean	13.28±0.35	4.64±0.20	72.03±1.39	27.98±1.39
Experimental cheese				
Day 0				
(G1) Renin group	12.6±0.18 ^{a}	6.66±0.20	66.60±1.62 ^{a}	33.4±1.62 ^{a}
(G2) Renin+yoghurt group	14±0.13 ^{b}	6.25±0.14	60.22±1.06 ^{b}	39.78±1.06 ^{b}
Day 10				
(G1) Renin group	13.3±0.13 ^{c}	6.56±0.20	66.47±0.55 ^{a}	33.53±0.55 ^{a}
(G2) Renin+yoghurt group	17.2±0.09 ^{d}	6.18±0.08	59.93±0.85 ^{b}	40.07±0.85 ^{b}

Mean values with different superscript letters in the same column are significantly different (P≤ 0.05).

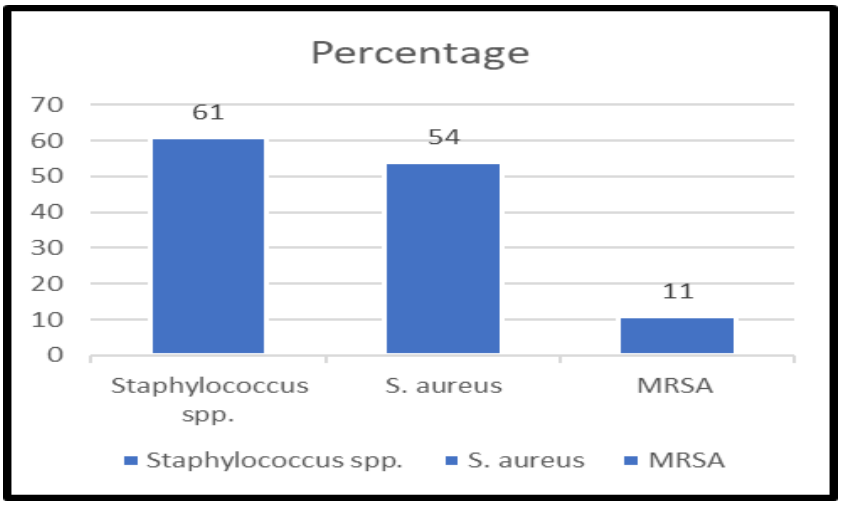

Fig. (1). The incidence of *Staphylococcus spp.* in surveyed kareish cheese samples.

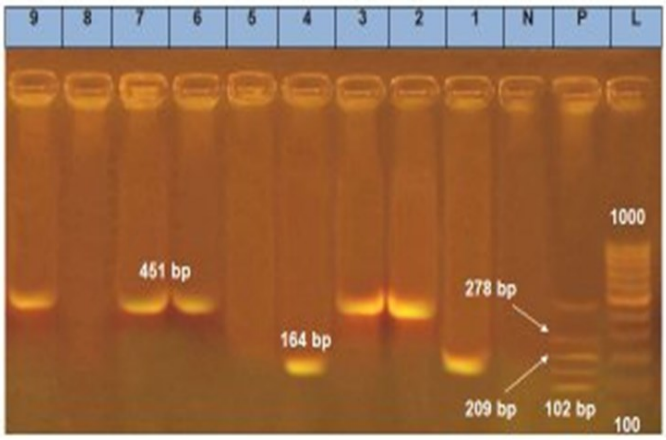

Fig. (2). PCR results for *S. aureus* enterotoxins genes.

L: 100-1000 bp DNA ladder
Lane 1 and 4: positive samples for SEb gene.
Lane 2, 3, 6, 7 and 9: positive samples for SEc gene
Lane 5 and 8: negative samples
Pos.: Positive control
Neg.: Negative control

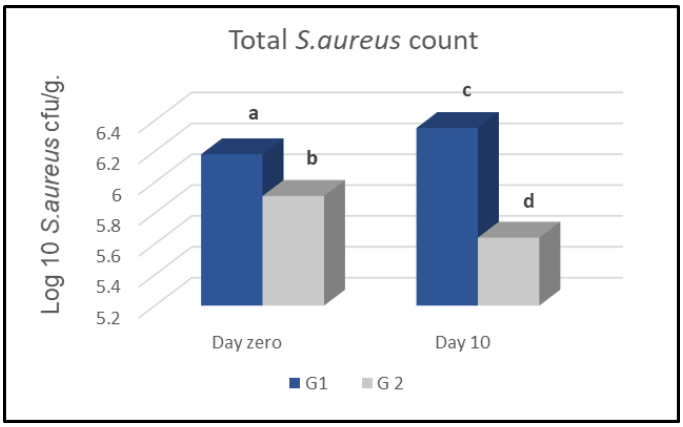

Fig. (3). Total *S. aureus* count of experimental cheese samples during cold storage.
G1 : Renin group
G2 : Renin + yoghurt group
Columns with different superscript letters are significantly different (P≤ 0.05).

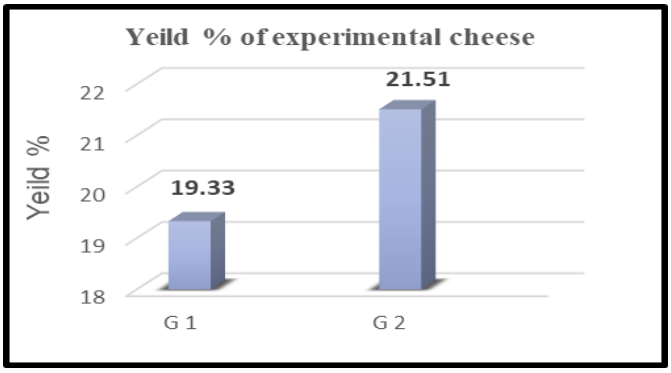

Fig. (4). Results of yield % of experimental cheese.
G1 : Renin group
G2 : Renin + yoghurt group

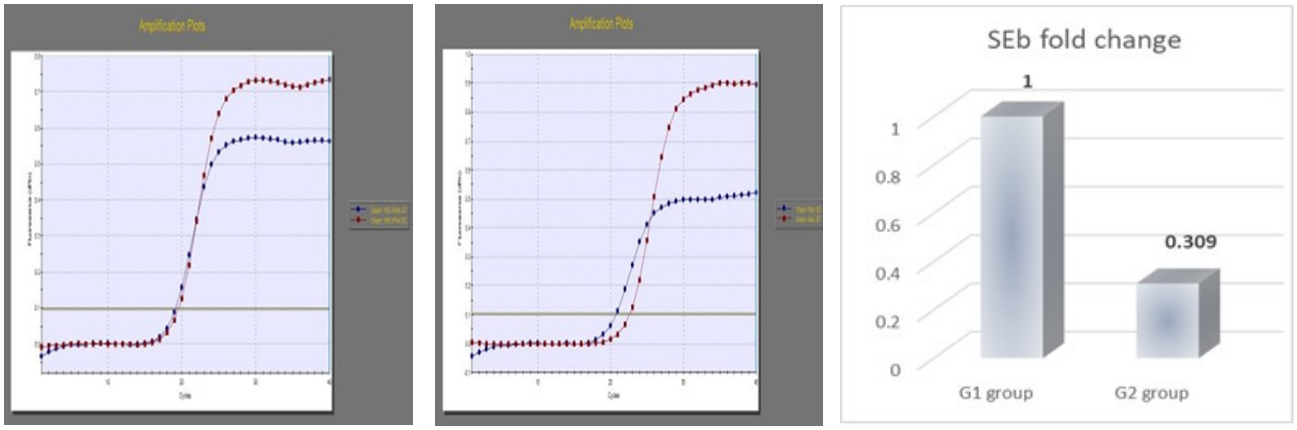

Fig. (5). Results for SEb gene expression of the experiment.
G1 : Renin group
G2 : Renin + yoghurt group

DISCUSSION

The samples reported as positive for the coagulase positive *S. aureus* were fewer than that mentioned by **Adame-Gomez et al. (2018)** and **Morar et al. (2021)**, as seen in Fig. (1). **Sadek and Koriem, 2020; Zeinhom and Abed, 2021; Elewa et al. 2024**, nonetheless, it was higher than others. The incidence rate of *S. aureus* varies depending on the production process, handling, distribution, and storage. However, the findings in Fig. (1) are in disagreement with those of **Zinke et al. (2012)**, who were unable to isolate MRSA from raw milk cheese, and **Adame-Gomez et al. (2018)**, who reported a lower percentage. Furthermore, a greater percentage of isolated MRSA from kareish cheese was noted by **Sadek and Koriem in 2020**. Kareish cheese manufactured from raw milk is contaminated with *S. aureus* and affected by environmental factors. It is thought that the main cause of contamination in cheese is cheese makers who have *S. aureus* on hands or in noses. According to **Andr´e et al. (2008)**, this contamination is believed to happen by respiratory secretions or direct physical contact.

In this research, high resistance of isolated MRSA strains to β-lactam and macrolide antibiotics was seen. These results align with **Sadat et al. 2022** and disagreed with **Sadek and Koriem, 2020; Morar et al. 2021; Ghanem et al. 2025** who stated low resistance to the same antibiotics. Regarding to aminoglycoside, tetracycline and phenicol antibiotics, this study agreed with **Sadek and Koriem, 2020; Morar et al. 2021; Ghanem et al. 2025** which report a lower resistance of MRSA strains to such antibiotics mentioned in Table (1).

Because it can be difficult to treat MRSA infections, they represent a major health threat, especially for those with deficient immune systems. According to the findings, isolates found in high-risk kareish cheese sources could potentially spread these bacteria to human, and the infection is hard to get rid of (**Pinchuk et al. 2010; Hammad et al. 2012**). Treatment is made more difficult by MRSA's tendency to create an exopolysaccharide barrier (**Gundogan et al. 2006**).

Additionally, *S. aureus* carries variety of resistant genes for multidrug on plasmids, which can be traded and dispersed among different species of *Staphylococci* (**Neihart et al. 1988**). *Staphylococcus aureus* strains are commonly resistant to antibiotic treatments, making antimicrobial resistance monitoring essential for assessing the effectiveness of new generations of antibiotics (**Pinchuk et al. 2010; Ghanem et al. 2025).**

The findings of **Nazari et al. (2014)** in comparison to this study regarding the incidence of enterotoxigenic *S. aureus* strains, they noted that 80.7% of *S. aureus* isolated from raw milk in Iran was enterotoxigenic, from which 26.9% had SEb gene. **Ahmed et al. (2019)** mentioned that 30% of isolated *S. aureus* from milk and various artisanal Egyptian

dairy products in Assuit city were enterotoxigenic, indicating a reduced incidence of enterotoxigenic *S. aureus*. Additionally, **El-Kholy et al. (2018)** reported a lower occurrence of SEc gene (20%) in certain dairy products from Beni-Suef City, Egypt.

Probiotic (yoghurt starter)-induced suppression of *S. aureus* growth concurs with **Misaghi et al. 2017; El-Kholy et al. 2018; Wormann, 2024.** The most well-known probiotic bacteria, *Lactobacillus* and *Bifidobacterium* which inhibited *S. aureus* proliferation in cheese (**Misaghi et al. 2017; El-Kholy et al. 2018**).

It is commonly known that dairy products, especially fermented milk, are efficient probiotic carriers (**Bergamini et al. 2005**). Probiotic bacteria have shown antagonistic activity against a variety of foodborne pathogens, including *Salmonella* species, *Clostridium perfringens*, *E. coli*, *L. monocytogenes*, and *S. aureus*. By generating lactic acid and other antimicrobial substances, lactic acid bacteria (LAB) aid in the restriction of microbial development (**Tadesse et al. 2005; Millette et al. 2007**). For instance, yoghurt's antibacterial properties are probably caused through the effect of lactic acid, which lowers pH, as well as other additional bioactive ingredients it might contain (**Hassan et al. 2013**).

The protein percentage and pH values of the surveyed Kareish cheese match those reported by **Allam et al. (2017)**. In comparison to the control G1 group (Renin group), experimental cheese demonstrated a significant rise in protein percentage in the G2 group (Renin+yoghurt group) in both fresh (day 0) and chilled (day 10) samples. In both fresh and chilled cheese, the G2 group's pH readings significantly decreased as compared to the G1 group. In fresh and chilled cheese samples, there is a notable difference between G1 and G2 in terms of moisture percentage and, consequently, total solids percentage. Chemical analysis confirmed that both cheese groups' pH levels and moisture percentage decreased as they chilled. On the other hand, chilling raised the percentage of total solids.

Fig. 4 shows the yield percentage of the experimentally produced cheese. The results revealed a significant rise combined to yoghurt starter inclusion which matching with **Ong et al. (2012)**. They stated that the pH of the milk at renneting can have an impact on the output of cheddar cheese, more dry matter (11–13%) produced in cheese produced at lower pH values (6.1 and 6.3) compared to those produced at 6.7 pH value. Thus, yoghurt's ability to lower pH may account for the impact of adding yoghurt starter on cheese production.

For Staphylococcal enterotoxins analysis, all samples of experimental and surveyed cheese tested was negative. Since *S. aureus's* capacity to produce its toxins counts on a number of extrinsic considerations, including temperature, pH, salt content, water activity, and competitive microbes' existence (**Johler et al. 2015; Carfora et al. 2016**). The manufacturing conditions of the kareish cheese used in the experiment may not have been ideal for the production of SEb enterotoxin so, no enterotoxin was found in this experiment. Other researchers as **Ahmed et al. (2023)** found SEa in experimentally produced Domiati cheese that had been inoculated with an enterotoxigenic strain of *S. aureus*, while by adding yoghurt starter culture they inhibited to a level as enterotoxins can't be detected.

Significant down-regulation of the SEb gene expression found associated to yoghurt addition (G2) in comparison to the control (G1). Indicating that addition of yoghurt starter was effective in lowering SEb gene expression. These findings coordinate with those of **Misaghi et al. (2017)**, they mentioned that *Lactobacillus* strains isolated from yoghurt have inhibitory effects on *S. aureus* proliferation, toxin production, and expression of the SEa gene. Gene expression studies investigating the effects of probiotic bacteria on Staphylococcal enterotoxins or other exotoxins, however, are rare (**Even et al. 2009**). According to **Laughton et al. (2006)**, the creation of a low molecular weight soluble chemical that can disrupt the expression of the *S. aureus* gene exotoxin may be the cause of the inhibitory actions of *Lactobacillus* strains. Results of SEb gene expression are seen in Fig. (5).

CONCLUSION

Using raw milk or poor hygiene during processing or post-processing are the main causes of cheese contamination with foodborne pathogens, like *S. aureus*, which increases the risk of food intoxication and poor-quality finished products. It was evident that adding yoghurt starter to kareish cheese production process had positive impacts. Therefore, from the obtained results, it could be recommend incorporating probiotics (such yoghurt starter) into the production of dairy products to prevent *S. aureus* and associated enterotoxins production, maintain customer safety and health, and produce high-quality products.

Conflicts of interest

There are no conflicts of interest.

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