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Enhancing Probiotic Stability against Methicillin Resistant *Staphylococcus Aureus* In Milk Product Industry

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Abstract

AS HEALTH awareness rises among consumers, there is an increasing demand for nutritious probiotic options. Dairy products serve as effective carriers for these beneficial probiotics, enhancing colonic microflora, and offering various health benefits. A total of ninety random samples of Kareish cheese, Damietta cheese, and Yogurt (30 samples each) collected from retail outlets were bacteriologically tested to determine the presence of *Staphylococcus aureus.* Of the 90 *S. aureus* isolates, 22 (24.4%) were isolated from the examined products. *S. aureus* isolates exhibited high resistance levels to oxacillin (81.8%) and penicillin (77.3%). Polymerase chain reaction (PCR) was utilized to indicate the presence of the *mec*A gene in *S. aureus*, which was detected at a percentage of 100%. *Lactobacillus acidophilus* and *Bifidobacterium lactis* exposed to UV radiation demonstrated significant antimicrobial effects against *S. aureus* in the agar well diffusion assay. Additionally, these probiotic strains were utilized in the Kareish cheese production, which was injected with *S. aureus* as an in vitro experiment and then kept at 4 ± 1°C for 14 days. The treated groups were assessed to identify the presence of *S. aureus* counts, which established a significant reduction in counts during the periods of incubation in the treated groups compared to the control group. RT-qPCR findings revealed a down-regulation of *mec*A gene expression in the treated groups G2, followed by G4 and G3, respectively when compared to the control group.

Keywords: Dairy products, *mec*A, Probiotics, *Staphylococcus aureus*, UV Radiation.

Introduction

Milk products are considered highly nutritious particularly beneficial for young individuals. Their rich content of proteins, minerals, fats, and vitamins is irrefutable [1]. In Egypt, milk products such as Kareish cheese, Damietta cheese, and yogurt are widely popular due to their health and nutritional benefits. Kareish cheese is regarded as the most prevalent white soft cheese because of its lower fat content, affordability, and high protein levels compared to other cheeses [2]. Moreover, milk products can be a primary source of potentially pathogenic bacteria for humans [3]. Conversely, milk and its products provide an optimal environment for the proliferation of various microorganisms that can pose public health risks to consumers [4]. *S. aureus* represents a significant foodborne pathogen that can cause serious diseases in people. It is generally present in milk products, and milk, especially in fresh brined cheese [5].In recent years, Methicillin-resistant *S. aureus* (MRSA) has emerged as a rising pathogen in companion animals, livestock, and several other farm animal species [6][7]. MRSA infections are notably challenging to treat as they are resistant to numerous antibiotics, particularly beta-lactams like penicillin, methicillin, and oxacillin, which target bacterial cell wall synthesis. MRSA carries the *mec*A gene, which encodes Penicillin Binding Protein 2A (PBP 2A). This altered protein interferes with the action of beta-lactam antibiotics, rendering them ineffective [8].The occurrence of multidrug-resistant pathogens is rising globally, presenting distinct challenges to global healthcare systems. Innovative strategies are required to address infections spread by these organisms, including the use of prebiotics and probiotics [9].Probiotics are one type of product whose use is growing, primarily available as dietary supplements and food, and in medicinal forms. [10].

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Antibiotic-resistant bacteria are an escalating global issue. Organizations such as the Centers for Disease Control and Prevention, the Infectious Diseases Society of America, and the World Health Organization have all recognized increasing antimicrobial resistance as a major health threat. In the United States, it is estimated that 2.8 million antibiotic-resistant infections and 35,000 deaths occur each year [11]. Despite ongoing investigations into new antimicrobial treatments the development of novel drugs is highly constrained, and the number of options under evaluation remains limited. Innovative strategies will be necessary to tackle multidrug-resistant (MDR) bacteria [12].

Lactic acid bacteria and Bifidobacteria are the primary probiotic strains used in dairy products. They establish a stable presence in the gastrointestinal tract and can withstand acidic conditions in gastric fluids, as well as bile acids and salts. They also inhibit the growth of other bacteria in the intestinal tract by producing lactic acid [13]. They reduce the proliferation of pathogenic microorganisms by lowering the intestinal pH and secreting substances such as bacteriocins, hydrogen peroxide, antimicrobial peptides, diacetyl, and organic acids [14]. It has been noted that there is a lack of research in the available studies regarding dairy products solely fermented by *Bifidobacteria*. Typically, *bifidobacteria* are included as adjuncts in fermentation rather than being the primary agents of fermentation. One challenge in using bifidobacteria for fermentation is that they tend to produce a significant amount of acetic acid through the glycolytic pathway, which can complicate the production of dairy products. Exposing bifidobacteria to UV radiation can help in reduction of their acetic acid production, thereby mitigating some of the difficulties associated with their use in dairy fermentation [15]. Recently, functional food products, such as probiotic-enriched products, are gaining increased attention due to growing consumer awareness of nutritious food options that promote good health and protect against several diseases [16].The purposes of this study were to calculate the incidence of *S. aureus* in some dairy products, assess the antimicrobial resistance of isolated strains using the well diffusion method, then detect the resistance gene (*mec*A) in some antibiotic resistant isolates by PCR, estimate of the antimicrobial effect of probiotics (*Lactobacillus acidophilus* and *Bifidobacterium lactis* exposed to UV radiation) on the growth of *S. aureus* (MRSA) during the storage of Kareish cheese at 4^{o}C, along with pH changes of the cheese and evaluate the effect of the probiotics on the level of the *mec*A gene using the RTq-PCR in the Kareish cheese samples.

Material and Methods

Sample collection and preparation

A total of 90 random samples of Kareish cheese, Damietta cheese, and yogurt (30 each) were obtained from various shops and supermarkets in Gharbia Governorate, Egypt. The collected samples were transported directly to the Microbiology Department at the Animal Health Research Institute (Tanta Branch) under strict aseptic conditions in a chilled container to be prepared and analyzed as soon as possible for detection of *S. aureus*.

Isolation and identification of S. aureus

S. aureus identification and detection were performed according to [17, 18], utilizing nutrient broth as the enrichment medium and Baird Parker agar, Mannitol salt agar (Oxoid) as selective media. Twenty five gram from each collected and prepared sample were added to sterilized flask containing 225 ml buffered peptone water (BPW) and incubated aerobically at 37˚C for 24 hrs. One ml of prepared sample was inoculated into nutrient broth and incubated aerobically at 37 ^{o}C for 24 hrs. A loopful from incubated nutrient broth was streaked on Baired parker agar plates and incubated at 37 ^{o}C for 24 -48 hr. Positive samples (Black shiny convex colonies surrounded by halo zone). Also a loopful from incubated nutrient broth was streaked on Mannitol salt agar and incubated at 37 ^{o}C for 24 -48 hr Positive samples (yellow colonies). These colonies were picked up and kept in semisolid agar for biochemical identification and in Brain Heart Infusion broth for PCR examination.

Phenotypic characterization of S. aureus

Films were prepared from a pure culture of isolated organism stained with Gram's stain and examined microscopically. *S. aureus* cocci appeared as grape's like clusters of Gram positive cocci. For the biochemical identification of *S. aureus*, coagulase, oxidase, catalase, and hemolysis tests were performed according to [19].

Coagulase test:

Fresh pure culture were inoculated into tubes containing 5 mL of BHI broth and incubated at 37^{o}C overnight, 0.5 mL was transferred to tube containing 0.5 mL of sterile rabbit plasma. Inoculated tubes were incubated at 37ºC and examined for clot formation through 4 hr. In case of negative result, incubate the sample at room temperature and re-examined after 24 hrs. A distinct clot indicate positive coagulase activity.

Oxidase test:

A piece of filter paper was soaked with few drops of oxidase reagent. A colony of the tested organism was then smeared on the filter paper. In positive results the phenylene diamine in the reagent will be oxidized into a deep purple within few seconds (10 sec), while negative result is detected by no change in the color.

Catalase activity test:

Few drops of 3% hydrogen peroxides were placed on to a clean dry slide before being rubbed with a loopful of suspected pure culture. Formation of gas bubbles indicates a positive result.

Detection of haemolysis:

A loop full from inoculated BHI broth were streaked on the surface of sheep blood agar plates and incubated at 37ºC for 24 hrs for detection of hemolysis. *S.aureus* colonies are beta-hemolytic.

Antibiotic sensitivity test of S. aureus isolates

The bacterial isolates were assessed in vitro for their sensitivity to the following antimicrobial discs: penicillin (P) 10 mcg, flucloxacillin (FL) 5 mcg, erythromycin (E) 15 mcg, vancomycin (VA) 30 mcg, clindamycin (DA) 2 mcg, cefotaxime (CTX) 30 mcg, and linezolid (LNZ) 30 mcg, according to [20]. An inoculum for each isolate was prepared by emulsifying colonies from an overnight pure culture in sterile normal saline then adjust the suspension turbidity to 0.5 McFarland standard. The bacterial suspension was uniformly streaked on dried surface of Mueller Hinton agar plates using sterile swabs and left for 3 min prior to introduction of the antibiotic disks. Plates were incubated at 35 °C for 24 h, and the degree of sensitivity was interpreted according to [21], through measuring the diameters of the inhibition zone in millimeters.

Molecular identification of S. aureus isolates and detection of mecA.

DNA extraction

The manufacturer's instructions were followed to extract DNA from six *S. aureus* isolates using the QIAamp DNA Mini Kit (Qiagen, Germany).

Polymerase chain reaction amplification using specific oligonucleotide primer

The PCR Master Mix and cycling conditions for the primers were prepared in accordance with the Emerald Amp GT PCR Mastermix (Takara) kit. According to [22] [23], oligonucleotide primers used in PCR have specific sequences and amplify a specific product, as illustrated in Table 1. DNA samples for uniplex PCR were amplified in a total of 25µl as follows: 12.5µl of Emerald Amp GT PCR mastermix Code No.RR310Akit, 1µl of each primer of 20 pmol concentrations, 5.5 µl of grade water and 5µl of template DNA.The reaction was performed in a Biometra thermal cycler. Temperature and time conditions of the primers during PCR were applied. Electrophoresis grade agarose (1 g) was prepared in 100 ml TBE buffer in a sterile flask, it was heated in microwave to dissolve all granules with agitation, and allowed to cool at 70˚C, then 0.5µg/ml ethedium bromide was added and mixed thoroughly. For gel analysis, 20 µl of PCR products were loaded in each gel slot. A 100 bp DNA ladder (QIAGEN Inc، Valencia, CA, USA) was used to determine the fragment sizes. The run was stopped after about 30 min and the gel was transferred to UV cabinet.The gel was photographed by a gel documentation system and the data was analyzed through computer software.

The used primers for detection *S. aureus* isolates and *mec*A gene in Table (1).

Preparation of probiotics and MRSA inoculum.

MRSA isolates from Kareish cheese, Damietta cheese, and yogurt were identified and then confirmed by PCR at the Animal Health Research Institute (AHRI), Dokki, Egypt. One strain was adjusted to a concentration of 10^6 cfu/ml [24]. Two Lactic acid bacterial strains *L. acidophilus* DSM 20079 (ATCC 4356), and *B. lactis* DSM 10140=CIP 105265 were obtained from the Faculty of Agriculture Ain Shams University in Cairo, Egypt's Microbial Resources Center (Cairo MIRCEN) was subcultured on de Man, Rogosa and Sharpe (MRS) agar as well as in MRS broth media (Oxoid) at 37°C in an anaerobic jar and adjusted to 10^8cfu/ml [25].

The effect of B. lactis exposed to UV radiation

Activated *B. lactis* was propagated in MRS broth with 0.05% (wt/vol) l-cysteine added and incubated at 37 °C in anaerobic jar. The cells were collected from the MRS broth by centrifugation at 3,500 r.p.m for 10 minutes. The cell pellets were resuspended and harvested and washed two times in sterile phosphate-buffered saline (PBS, pH 7) then spread onto a petri dish, and irradiated with UV light (254nm) (ABS class 2 cabinet, BIOQUELL, UK) for 3 minutes according to[15].

Antibacterial activity of B. lactis and L. acidophilus against MRSA.

Neutralized cell-free culture supernatants (CFCS) preparation of B. lactis and L. acidophilus

CFCS was prepared by taking 1 ml of *L. acidophilus, B. lactis* exposed to UV radiation, and a mix of *L. acidophilus* and *B. lactis* also exposed to UV radiation), which were cultured in MRS broth for 24 hours at 37 °C in an anaerobic jar. Centrifugation at 14,000 r.p.m. for 5 minutes was employed to remove the cells (Sorvall RC6 PLUS, Thermo-Electron Corporation, Asheville, NC, USA). The supernatant was then sterilized using a single-sheet Seitz filter with a 0.45 µm pore size, the supernatant was sterilized to eliminate any viable bacterial cells and to obtain CFCS. [26, 27]

Agar well diffusion method.

The well diffusion method was employed to estimate the antimicrobial activity of the CFCS from the tested probiotics against MRSA. After culturing the target bacteria in broth and adequately diluting

the culture, 100 µl of the bacterial suspension was inoculated onto agar plates under aerobic conditions. The plates were divided into wells with a diameter of 5 mm, and 10 µl of the cell-free culture supernatant was added to each well. The plates were incubated anaerobically at 37°C for 24 hours then incubated anaerobically for 1 hour. [26, 27] The diameter of the inhibition zone surrounding the wells was used to evaluate the antimicrobial activity. The inhibition zones was categorized as follows: (-) non-visible inhibition, (+) 0.5-6 mm inhibition zone, (++) 7-12 mm inhibition zone, and (+++) more than 12 mm inhibition zone size, as per [28].

In vitro antibacterial effect of L. acidophilus and B. lactis against MRSA experimentally inoculated in Kareish cheese

Preparation of Kareish cheese

Fresh skimmed milk was sourced from a local producer and transported to the laboratory within 30 to 40 minutes. It was then warmed in a water bath at 80 °C while stirring for 10 minutes. After cooling the milk to 40 °C, calcium chloride and sodium chloride were added at concentrations of 0.02% and 7% w/w, respectively. Additionally, rennet was introduced at a rate of 1.5 g per 100 kg of milk to act as a coagulating agent. [29]

Experimental design.

The milk was categorized into five groups (200g each) arranged as follows:

G1: Control positive inoculated with MRSA
G2: inoculated with MRSA and *L. acidophilus*.
G3: inoculated with MRSA and *B. lactis* exposed to UV
G4: inoculated with MRSA, and a mix of *L. acidophilus* and *B. lactis* exposed to UV radiation
G5: control negative, not inoculated with MRSA or probiotics strains.

Bacterial enumeration.

MRSA count were determined for all experiment groups throughout the storage period at 0, 3, 7, 10, and 14 days according to [30].

Determination of pH value:

The pH values of the Kareish cheese samples were measured using a digital pH meter at 0, 3, 7, 10 and 14 days [31].

Statistical analysis

The data are presented as the mean ± SE (standard error). The mean values of the different groups were compared using Duncan's post hoc test in a one-way analysis of variance (ANOVA) at a significance level of $P \leq 0.05$. The method cited in [32] was employed to conduct the statistical analysis, which was then computerized using SPSS version 20 [33].

Molecular identification depending on RT-qPCR

Expression of 16S rRNA and mecA

Kareish cheese samples from the different experimental groups were analyzed using RT-PCR to determine whether the presence of *L. acidophilus, B. lactis*, and *B. lactis* exposed to UV in co-culture with MRSA influenced on the expression of the 16S rRNA and *mec*A genes. The primers used in RT-qPCR according to [23] [34] are presented in Table 3. The Ct of each sample was compared to that of the control group utilizing the ""ΔΔ Ct" method, as outlined in [35], to estimate the variation in gene expression across different samples.

Primer sequences and target genes for SYBR green RT-qPCR in table (2).

Results

Incidence % of S. aureus isolated from examined samples

According to phenotypic and biochemical identification, 22/90 (24.4%) *S.aureus* were isolated from Kareish cheese, Damietta cheese and Yoghurt samples with the isolation rates of 36.6, 20% and 16.66% respectively.(Table, 3)

In vitro anti-microbial sensitivity test for isolated S. aureus.

The in- vitro sensitivity tests for 22 isolates of *S.aureus* in Table (4) revealed that, the isolated *S.aureus* were highly resistant for Oxacillin (81.8%) followed by Pencillin, (77.3%) Cefotaxim (68.2%), Erythromycin(59.1%), Clindamycin (50%), vacomycin (36.4%) and Linzolid (31.8%), respectively.

Detection of mecA gene using PCR

A total of six *S. aureus* isolates were confirmed positive for 23S rRNA gene, but all the tested *S. aureus* isolates were observed positive for *mec*A gene using PCR (100%).

Diameter of the inhibition zone (mm) of L. acidophilus and B. lactis by agar well diffusion test.

The inhibition zone diameter of *L. acidophilus* was 17 mm followed by *L . acidophilus* and *B. lactis* exposed to UV radiation (15mm) and *B. lactis* exposed to UV radiation (11mm). (Table 5).

Impact of L. acidophilus and B. lactis on survival of MRSA as log cfu/g in Kareish cheese during refrigeration storage:

In comparison to G1 the impact of *L. acidophilus* and *B. lactis* on survival of *S. aureus* in G2, G3 and G4 showed significant decrease in the log cfu/g in Kareish cheese during 3rd, 7th and 10th and 14th day during refrigeration storage at 4±1°c.

pH of controls and inoculated groups with probiotics and bacterial strains in Kareish cheese during refrigerator storage at 4±1°c:

In comparison to G1 and G5 the pH of G2 and G4 showed significant decrease during 3rd, 7th and 10^{th} and 14^{th} day but G3 showed significant decrease during 7th and 10^{th} and 14^{th} day in Kareish cheese during refrigerator storage at 4±1°c. (Table, 7).

Impact of L. acidophilus and B. lactis on mecA gene expression using RT-qPCR

The expression levels of *mec*A gene in MRSA isolates was examined using the RTq-PCR method under the influence *l.acidophilus* and *B. lactis*. Results showed that mecA gene expression in G2was reduced to 0.24 fold change of the value in control group G1 followed by treated groups G4 and G3 which decreased by 0.31 and 0.61 fold change respectively.

Discussion

S. aureus is a pathogenic bacterium that resides on the skin and in the nasal passages of both animals and human. It's one of the most common hospital-acquired infections [36]. *S. aureus* was isolated from Kareish cheese, Damietta cheese, and yogurt by percentages of 36.6%, 20%, and 16.66%, respectively (Table 3). Higher results (52.5%) were obtained by [37] for Kareish cheese, and by [38] for Damietta cheese (30%)and Yogurt (38%), while lower results were found by [39], who confirmed the contamination of soft cheese samples with *S. aureus* (8.3%) , and [40] who isolated *S. aureus* from cheese and yogurt (14.9% and 4.8%, respectively). The high incidence of *S. aureus* in Kareish cheese may be due to the undergone heat treatment. Street vendors often display Kareish cheese in open pans, exposing it to dust and flies [41]. Antimicrobials are extensively employed in veterinary practice today. However, unrestricted use or administration of these drugs in sub-therapeutic doses can lead to the emergence of antimicrobial-resistant strains, which is a serious concern not only for animal health but also for human health [42]. Table 4 shows that most of the tested *S. aureus* isolates demonstrated resistance against oxacillin at a rate of 81.8%, followed by penicillin (77.3%), cefotaxim(68.2),erythromycin(59.1), clindamycin(50), vancomycin(36.4%), and linezolid (31.8%). Nearly similar results were obtained by [43] for cefotaxim (60%), [44] for erythromycin (73.6%) and [45] for oxacillin (73.3%), while these results conflicted with those of [46] for erythromycin (14.8%), [44] for vancomycin (5.9%) and [47] for cefotaxime (10%). The reduced susceptibility of *S. aureus* to *β*-lactam antibiotics detected in this study could be attributed to the production of *β*-lactamase enzymes [48].

PCR has become a highly sensitive and specific technique for pathogen identification [49]. In the following study, all tested isolates of *S. aureus* (100%) carried the *mec*A gene. This result is consistent with those reported by [43] and [50], who found the *mec*A gene in 100% and 82.92% of their isolates, respectively. However, this finding conflicts with [51], who were unable to detect the *mec*A gene. The detection of MRSA in food products in markets confirms that MRSA poses challenges beyond hospitals and has also entered the food chain [52,53].

Probiotics are described as "live microorganisms" provided in adequate quantities to confer a health benefit on the host. These microbes are beneficial to the host by enhancing the population of beneficial microorganisms, which may be reduced by antibiotic use. They are also referred to as good bacteria or friendly bacteria. [54]. Table (5) illustrated that *L. acidophilus, B. lactis* both exposed to UV radiation, as well as *B. lactis* mixed with *L. acidophilus* exhibited inhibitory effects on the growth of *S. aureus* with inhibition zones (measured by the agar well diffusion method) of 17 mm, 11 mm, and 15 mm, respectively. This result is consistent with those reported by [55] and [56] who classified inhibition zone diameters as very strong (20–30 mm), strong (10–20 mm), moderate (5–10 mm), and weak (5 mm). Several previous studies have reported that Lactic Acid Bacteria (LAB) possess antimicrobial properties due to their production of various antimicrobial compounds, including bacteriocin, hydrogen peroxide, organic acids, diacetyl, and reuterin [57].

People are progressively seeking out foods that offer benefits beyond basic nutrition, knownas functional foods. This growing interest has driven the development of numerous products, particularly dairy items, enriched with probiotic bacteria [58].

Results in Table (6) explain the *B. lactis* and *L. acidophilus* effects exposed to UV radiation on the growth pattern of MRSA in experimentally inoculated Kareish cheese samples. On the zero day, no significant differences were detected between all examined groups (G1, G2, G3, and G4), while from the 2nd day until the storage period end there were a significant difference ($p< 0.05$). MRSA counts in Kareish cheese samples declined to (6.4±0.1 to 3.33±0.01), (6.39±0.1 to 4.77±0.01), and (6.39±0.01 to 3.91±0.01) log cfu/g in treated probiotics groups G2, G3, and G4, respectively, when compared to the control group (G1) during the storage period. These results corresponded with [59], who reported that *L. acidophilus* strain had inhibitory effect on *S. aureus* viability in Tallaga cheese reducing its counts from 6.7×10^8 to 9.0×10^3 cfu/g through refrigeration compared to the control and *B. bifidum* decline *S. aureus* from 6.8×10^8 to 1.6×10^4 cfu/g through storage in the refrigerator than control one, and corresponded with the results obtained by El Kholy et al.[60], who found that *L.*

acidophilus had remarkable effect in preventing survival of *S. aureus* in cheese during storage, while Hemmat et al. [61] found that *L. acidophilus* mitigate *S. aureus* count from 4.26 at zero day of storage to 1.72 log10 cfu/g at 6th day of storage , and *B. lactis* also mitigate *S. aureus* count from 4.26 at zero day to 1.49 log10cfu/g for at 6th day of storage, respectively. We noticed from the result of this experiment that MRSA count in G3 was higher than G2, this may be attributed to the impairment of cellular functions of *B. lactis* due to exposure to UV radiation that causes the formation of reactive oxygen species which react with lipids, proteins, and nucleic acid leading to oxidative stress [62]. The exposure of proteins residing in the membrane lipid bilayer to oxidative stress can result in amino acid side chains alterations and changes in the protein structure [63]. Thus, UV irradiation induced a change in the carrier protein that produced inefficient acid bacteria secretion.

Inspection of Table (7) illustrated that the pH values of incubated Kareish cheese samples on day zero were very similar, and no significant differences ($P > 0.05$) were among the groups. Nevertheless, by the end of the storage period, pH had significantly decreased ($P< 0.05$) compared to those of controls one (G1, G5) which may be attributed to that probiotic bacteria produce acetic and lactic acids as metabolic byproducts.

Probiotic strains (*L. acidophilus* and *B. lactis* exposed to UV radiation) were employed to reduce antibiotic resistance in MRSA by targeting the *mec*A gene. RT-qPCR Figure 1 showed that G2 reduced *mec*A gene expression in MRSA to a 0.24 fold change from its original value, representing a downregulation of 76%.while *mec*A gene expression in G4 was reduced to 0.31 fold change, representing a 69% decrease, while *mec*A gene expression in G3 was declined to 0.61 fold change, representing a 39% decrease in *mec*A gene expression. These results are consistent with [64] who stated that probiotics are effective for reducing MRSA growth and antibiotic resistance through down-regulation of *mec*A.

Conclusion

Some of dairy products in this study, as Kareish cheese, Damietta cheese, and Yogurt were highly contaminated with MRSA which represents a public health hazard to humans. Therefore, incorporation of some probiotics like *Lactobacillus acidophilus* and *Bifidobacterium lactis* exposed to UV radiation suppressed *S. aureus* (MRSA) count in the Kareish cheese due to the combined effects of decreased pH and antimicrobial activity leading to improved product safety. Dairy products' industries should not rely solely on pasteurization and fermentation but permissible additives, such as probiotics, should also be used during various stages of production and storage to enhance product quality.

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Declaration of Conflict of Interest
There is no conflict of interest.

Author's contribution
All authors contributed equally to the manuscript, including the experimental design, conducting the experiments, and preparing and reviewing the manuscript prior to submission.

TABLE 1. The used primers for detection *S. aureus* isolates and *mec*A gene.

Target gene	Sequences	Amplified segment (bp)
23S rRNA	F-AC GGAGTTACAAAGGACGAC	1250 bp
	R- AGCTCAGCCTTAACGAGTAC	
***mec*A**	F-GTAGAAATGACTGAACGTCCGATAA	310 bp
	R- CCAATTCCACATTGTTTCGGTCTAA	

TABLE 2. Primer sequences and target genes for SYBR green RT-qPCR.

Target gene	Primers sequences
16S rRNA	F- CCTATAAGACTGGGATAACTTCGGG
	R- CTTTGAGTTTCAACCTTGCGGTCG
***mec*A**	F- GTA GAA ATG ACT GAA CGT CCG ATA A
	R- CCA ATT CCA CAT TGT TTC GGT CTA A

TABLE 3. Incidence of *S. aureus* isolates from examined samples (n=30)

Samples	Number of examined samples	Number of positive samples	% of positive samples
Kareish cheese	30	11	36.7
Damietta cheese	30	6	20
Yoghurt	30	5	16.67
Total	90	22	24.4

N. B: % was calculated according to the total number of examined samples

TABLE 4. In vitro antimicrobial sensitivity testing of *S. aureus* isolates

Antimicrobial disc	Disk concentration	Number of Sensitive Strains (%)	Number of Intermediate Strains (%)	Number of Resistant Strains (%)
Pencillin	10 µg	1(4.5%)	4(18.2%)	17(77.3%)
Oxacillin	1 µg	0 (0%)	4(18.2%	18 (81.8)
Clindamycin	2 µg	8 (36.36%)	3(13.64%)	11(50%)
Vancomycin	30 µg	12 (45.5%)	2(9.1%)	8(36.4)
Erythromycin	15 µg	6 (27.3%)	3(13.6%)	13(59.1%)
Cefotaxim	30 µg	2 (9.1%)	5 (22.7%)	15 (68.2%)
Linzolid.	30 µg	13 (59.1%)	2(9.1%)	7 (31.8%)

% Percentage in relation to 22 isolates of *S. aureus.*

TABLE 5. Diameter of the inhibition zones (mm) of *L. acidophilus* and *B. lactis* by agar well diffusion test.

Probiotics	*L. acidophilus*	*B. lactis* exposed to UV radiation	*L. acidophilus* and *B. lactis* exposed to UV radiation
Inhibition zones (mm)	17	11	15

TABLE 6. Impact of *L. acidophilus* and *B. lactis* on the survival rate of MRSA **as log cfu/g in Kareish cheese during refrigeration storage.**

Days Groups	Zero day	3 days	7 days	10 days	14 days
G1	$6.41 \pm .01^{a}$	$6.46 \pm .01^{a}$	$6.91 \pm .2^{a}$	$7.22 \pm .02^{a}$	$7.27 \pm .01^{a}$
G2	$6.40 \pm .01^{a}$	$5.82 \pm .03^{d}$	$4.92 \pm .02^{c}$	$3.87 \pm .01^{e}$	$3.33 \pm .01^{e}$
G3	$6.39 \pm .01^{a}$	$6.31 \pm .01^{b}$	$5.78 \pm .02^{b}$	$4.93 \pm .02^{c}$	$4.77 \pm .01^{c}$
G4	$6.39 \pm .01^{a}$	$5.97 \pm .02^{c}$	$5.43 \pm .01^{bc}$	$3.97 \pm .01^{d}$	$3.91 \pm .01^{d}$

The values represent Mean ± SE of three experiments. Means within a column followed by different letters are significantly different ($P < 0.05$).

TABLE 7. The pH records of control and inoculated groups with probiotics and bacterial strains in Kareish cheese during refrigeration storage at 4±1°c

Days Groups	Zero day	3 days	7 days	10 days	14 days
G1	$6.12 \pm .02^{a}$	$5.46 \pm .03^{a}$	$5.43 \pm .02^{a}$	$5.20 \pm .01^{a}$	$5.1 \pm .03^{a}$
G2	$6.09 \pm .05^{a}$	$5.16 \pm .06^{b}$	$5.00 \pm .06^{c}$	$4.93 \pm .02^{d}$	$4.85 \pm .03^{c}$
G3	$6.08 \pm .04^{a}$	$5.57 \pm .03^{ab}$	$5.28 \pm .04^{c}$	$5 \pm .03^{d}$	$4.95 \pm .05^{c}$
G4	$6.13 \pm .01^{a}$	$5 \pm .06^{c}$	$4.98 \pm .03^{d}$	$4.90 \pm .01^{e}$	$4.80 \pm .01^{d}$
G5	$6.07 \pm .01^{a}$	$5.44 \pm .02^{a}$	$5.4 \pm .01^{a}$	$5.30 \pm .03^{a}$	$5.1 \pm .06^{a}$

Means within a column followed by different letters are significantly different ($P < 0.05$).

a: Indicates groups have no significant difference in their means.
b: Indicates that there is a significant difference between the groups marked with "b" and those marked with a.
c: Indicates a greater significant difference, where groups labeled "c" differ from those labeled "b" and "a."
d and **e**: Indicate even larger significant differences compared to the previous letters.

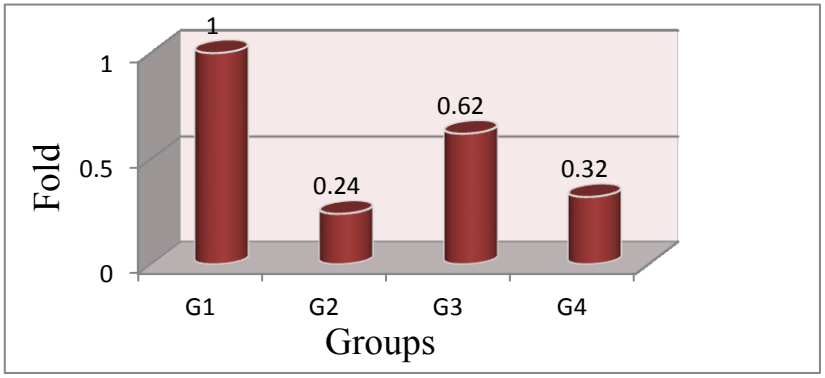

Fig. 1. Relative expression (fold change) of MRSA *mec*A gene of treated groups in comparison to control group

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تعزيز ثبات البروبيوتيك ضد المكورات العنقودية الذهبية المقاومة للميثيسيلين في صناعة منتجات الألبان.

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الملخص

مع ارتفاع الوعي الصحي بين المستهلكين، هناك طلب متزايد على خيارات البروبيوتيك المغذية. تعمل منتجات الألبان كحامل فعال لهذه البروبيوتيك المفيدة، مما يعزز البكتيريا القولونية ويقدم فوائد صحية متنوعة. تم فحص تسعين (90) عينة عشوائية من جبن القريش، وجبن الدمياطى، والزبادي (30 لكل منهما) تم الحصول عليها من منافذ البيع المختلفة وتم فحصها من الناحية البكتريولوجية لوجود الميكروب العنقودى الذهبى. وتم عزل 22/ 90 بنسبة (24.4%) من ميكروب المكورات العنقودية الذهبية من المنتجات المفحوصة. وكانت المكورات العنقودية الذهبية المعزولة شديدة المقاومة للأوكساسيللين (81.8%)، يليها البنسلين (77.3%). تم تطبيق تفاعل البلمرة المتسلسل (PCR) على المكورات العنقودية الذهبية للكشف عن جين mecA، والذي تم اكتشافه بنسبة (100%) من العترات التى تم فحصها. فيما يتعلق بالنشاط المضادة للميكروبات لبكتيريا Lactobacillus acidophilus وBifidobacterium lactis المعرضة للأشعة فوق البنفسجية باستخدام (well diffusion method) ضد MRSA، فكان لديها نشاط قوي مضاد للميكروب. تم استخدام سلالات البروبيوتيك في إنتاج جبن القريش، والتي تم حقنها بمادة MRSA كتجربة في المختبر وتم تخزينها عند درجة حرارة 4 ± 1 درجة مئوية لمدة 14 يومًا. تم بعد ذلك تقييم المجموعات المعالجة من حيث عدد MRSA بشكل تجريبي، مما أظهر انخفاضًا كبيرًا في العدد خلال فترات الحضانة في المجموعات المعالجة مقارنة بالمجموعة الضابطة. كشفت نتائج RT-qPCR عن انخفاض تنظيم التعبير الجيني لـ mecA في المجموعات المعالجة G2، تليها G4، وG3، على التوالي عند مقارنتها بمجموعة التحكم.

الكلمات الدالة: منتجات الالبان ، جين ال *mec*A ، البروبيوتيك ، المكورات العنقودية الذهبية، الأشعة فوق البنفسجية.