Animal Health Research Journal

P-ISSN : 2356-7767 On Line-ISSN : 2535-1524
Journal Homepage: https://animalhealth.ahri.gov.eg/ Research Paper

Prevalence and survival of Methicillin Resistant *Staphylococcus aureus* (MRSA) during production and preservation of traditional and bifidus yoghurt

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Received in 19/3/2025
Received in revised from 28/4/2025
Accepted in 17/6/2025

Keywords:
S. aureus,
yoghurt,
MRSA,
mecA gene,
traditional,
bifidus.

Abstract

Methicillin-resistant *Staphylococcus aureus (S. aureus)* (MRSA) contaminating milk and its products, particularly yoghurt, poses great health hazards to consumers. So, this study detected *S. aureus* and MRSA incidence in traditional yoghurt in Assiut Governorate, Egypt, also compared the effect of two starter culture types on survived MRSA during manufacturing and storage of traditional and bifidus yoghurt with the commercial types. The incidence of *S. aureus* was 56.36% (31/55) in yoghurt samples. The 23S rRNA specific to it was detected in 6/10 isolates. These isolates showed high MAR index (0.75) and were resistant to amoxicillin and novobiocin (90%), methicillin and oxacillin (80 %), and erythromycin (70%). This was confirmed by finding the *mecA* genes specific amplicon (310 bp) by PCR in 18/20 isolates. Using one of these isolates, the inhibitory potential of traditional and bifidus yoghurt types against MRSA during manufacturing and storage following the addition of some starter cultures was investigated. Results revealed a strong reducing efficacy of the used starter cultures on MRSA count after the storage period ended from $2.1\pm0.13x10^6$ CFU, recording the highest reduction percentage in commercial Bifidus yoghurt ($0.00021\pm0 \times 10^6 = 2.1 \times 10^2$) CFU followed by traditional yoghurt prepared in the laboratory, reaching $0.00042\pm0.0 \times 10^6$CFU. The pH decreased significantly from 6.58±0.04 to 3.8±0.01, 3.96±0.02, 4.12±0.01, and 4.16±0.01 for the prepared bifidus, prepared traditional, commercial bifidus, and commercial traditional yoghurts, respectively, after storage period ended. Conclusively, *S. aureus* is prevalent among traditional yoghurt and combining the traditional starter culture with *B. actiregularis* in yoghurt manufacturing under sanitary conditions is beneficial.

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Introduction

Yoghurt is the most common fermented dairy product in Egypt and many countries. It is a healthy food particularly due to the potential risk of consuming raw milk or its raw products in pathogens transmission. Daily yoghurt consumption is increasingly welcomed habit due to its rich nutrients, assistance in nutrients absorption, beneficial probiotics, and avoidance of lactose intolerance. It provides a favorable media for growth of wide variety of bacteria due to its nutritional contents. In developing areas, traditional yoghurt is commonly to be produced by poor practices from raw milk under unsanitary conditions and easily contaminated by milk-borne bacterial pathogens such as *S. aureus*. Staphylococci can grow over a wide temperatures and pH ranges (7 - 48 °C and pH 4 – 10, respectively) **(Abdolshahi *et al*., 2018, Asfaw *et al*., 2023, and Wörmann *et al*., 2024)**.

S. aureus is a zoonotic bacterium which causes bovine mastitis commonly and directly excreted in milk. Staphylococcal mastitis is difficult to be treated due to bacterial resistance to β-lactam antibiotics, which are the routine treatment for mastitis. The possible presence of *S. aureus* and its toxins pose in food is a significant health hazard for millions of consumers around the world. Food poisoning resulted from staphylococcal infection outbreaks is prevalent over the world implicating endocarditis, osteomyelitis, and soft tissue lesions **(Hu *et al.,* 2023)**.

It excretes numerous stable toxins (enterotoxins, lipases, hemolysins, and coagulases**)** under suitable temperature, pH, and moisture conditions. The level of this bacterium in yoghurt determines the degree of potential risks it poses. A bacterial number of about 10^5 CFU food can produce an amount of enterotoxins (less than 1.0 μg) which is enough for intoxication **(Asfaw *et al*., 2023 and Freitas *et al.,* 2023)**. Staphylococcal poisoning signs start with onset of nausea, intense vomiting, and severe enteritis and recover within 3 days, though; severe cases require hospitalization (**Nacer *et al*., 2024)**.

Antibacterial-resistant bacteria have widely emerged due to the abuse and prolonged application of antibiotics in humans and animals, creating significant challenges that threaten human and animal life and affect dairy product production. Methicillin-Resistant *S. aureus* (MRSA) have become a global health issue causing life-threatening infections **(Sajith *et al*., 2012)**.

The antibacterial resistance of MRSA is due to encoding penicillin binding protein (Pbp2a) by *mec*A genes. *mec*A gene of Staphylococcus spp. is highly conserved so its detection by PCR is gold standard for recognizing methicillin resistance. The resistance mechanism involves changes on *mec*A gene which induce organism's resistance to one or more antibiotics and hence multidrug resistance **(Adeyemi *et al*., 2024)**.

MRSA presence in traditional yoghurt was reported in several studies and it could survive fermentation for 8 - 10 days during which pH resistant-toxins could be produced. Using probiotic starter culture could be decrease the population of *S. aureus* within short storage time (**Soliman and Ahmed, 2019 and Feyissa *et al*., 2023**).

Starter cultures are critical food biopreservative in yoghurt production and improving its safety. Recently, the commonly used natural starters for yoghurt manufacturing are *Streptococcus thermophilus (S. thermophilus), Lactobacillus bulgaricus (L. bulgaricus),* and Bifidobacterium spp. In certain countries, other cultures that can grow well even at low pH, like *L. lactis* and *helveticus*, are also common **(Tufail *et al*., 2011 and Matela *et al*., 2019)**. They acidify milk rapidly (deliver pH value from 4.0 to 4.5) and provide it flavor by excreting a range of compounds included organic acids such as lactic acid in addition to ethanol, hydrogen peroxide, and bacteriocins that explain their antibacterial effect against the viability and multiplication of undesirable microorganisms in yoghurt. Moreover, these bacteria facilitate lactose assimilation and eradicate symptoms of lactose intolerance. Several studies proved the capability of *L. rhamnosus* to

antagonize *S. aureus* in yoghurt revealing its protective effect **(Kamal *et al*., 2018)**.

Wörmann *et al*. (2024) recorded that these starter cultures are promising interference approach to control pathogens in dairy products and to reduce the risk of their transmission to consumers. However, little is known about the effect of these cultures on MRSA in yoghurt.
Therefore, this study was designed to assess the prevalence and antibacterial resistance profile of MRSA contaminating yoghurt and the effect of different starter cultures on its growth.

Materials and Methods

Incidence of *S. aureus*

1. Sampling

Fifty-five (55) balady yoghurt samples were aseptically obtained from dairy markets in Assiut Governorate, Egypt. The samples were transported under health requirements to the laboratory and examined bacteriologically.

2. Isolation and identification of *S. aureus*

S. aureus was isolated from the yoghurt samples using the "direct broth and plate culture" method (**Feyissa *et al*., 2023)**. Briefly, 1 g of each sample was transferred to 10 ml Sodium chloride broth (7.5 %, Oxoid Company, Ltd., UK) and incubated at 37°C for 24 h. For bacterial isolation, the broth culture was plated onto Mannitol salt agar (Oxoid Company, Ltd., UK) at 37°C for 24 hrs. The produced colonies were examined for morphological characteristics, gram staining, catalase reactivity, and coagulase production **(Asfaw *et al*., 2023)**.

Coagulase test

Tube coagulase test was performed with a coagulase typing kit using commercially-prepared rabbit plasma (Difco, Detroit, U.S.A.). In sterile Wassernamn tubes, the suspected broth culture (5 drops) were added to 0.5 ml of sterile reconstituted rabbit plasma (10 folds- diluted by sterile normal saline) and gently mixed. The tubes were incubated at 37° C for 24 h. Tubes were examined every two hours. The reaction was considered positive, if any detectable clotting was seen inside the tube when it was slanted. Negative control was taken in account.
The coagulase reactive isolates were further identified by PCR using *S. aureus* specific-23S rRNA gene (**Prihandani *et al*., 2024)** according to **Elshereif *et al*. (2025)**.

3. The antibacterial sensitivity profile of the isolated *S. aureus*

The standard "Kirby–Bauer disk diffusion" method was followed as outlined by **CLSI (2022)** to evaluate the antibacterial sensitivity pattern of the obtained *S. aureus* isolates. The freshly cultured colonies were suspended in sterile saline (5 mL) with adjusting the turbidity to a 0.5 McFarland. The bacterial suspension was seeded over the dry surface of Mueller Hinton agar (Oxoid Ltd.) plates by using a sterile swab. Then, the antibiotic discs were applied onto the inoculated plates and incubated at 37°C overnight (18–24 hours). The zone diameter of inhibition (ZI) was estimated in millimeter (mm).The antibiotics tested were Methicillin (ME, 5 µg), Erythromycin (E, 15µg), Amoxicillin (AMX, 10µg), Novobiocin (NV, 30µg), Enrofloxacin (EX, 5µg), Sulfa/trimethoprim (SXT, 25µg), Streptomycin (S, 10µg), and Oxacillin (OX, 5µg). A strain was considered "multidrug resistant" if it is resistant to at least one antibiotic from three or more different antibiotic classes. The test was repeated triplicate.

4. Detection of the methicillin resistance gene (*mecA*)

Twenty (20) MRSA isolates were examined for the presence of the 310bp amplicon specific to the *mecA* gene by further subjecting them to PCR using the primer set GTA GAA ATG ACT GAA CGT CCG ATA A (forward) and CCA ATT CCA CAT TGT TTC GGT CTA A (reverse) (Metabion, Germany). The bacterial genome was extracted following QIAamp DNA Mini kit's guidelines with some modifications (Qiagen, Germany, GmbH). Briefly, 20µl of the bacterial suspension were lysed by 10 µl proteinase K and 200µl lysis buffer and incubated for 10 min at 56^{o}C. The lysate was mixed with 200µl ethanol (100%), washed, and centrifuged. Nucleic acid was eluted with 100 µl of elution buffer, and then amplified as a template using the primer set and EmeraldAmp Max PCR Master Mix (Takara, Japan), in applied biosystem 2720 thermal cycler following the cycling condition of **McClure *et al*. (2006)**. Specifically, a preliminary hot start at

94°C for 5 minutes, followed by 35 cycles, each consisting of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds, and the step of final extension at 72°C for 10 minutes. The amplicons (5µl) were seen on ethidium bromide-stained agarose gel (1.5%) (Applichem, Germany, GmbH) by using UV light and compared to a DNA ladder with a molecular size of 100-1500 bp (Qiagen, Gmbh, Germany).

Effect of yoghurt starter on Methicillin-resistant *Staphylococcus aureus* (MRSA)

1. Starter cultures

The starter bacteria used for yoghurt production were reference live cultures of 3 strains, including *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Bifidobacterium bifidum* (*B. bifidum*). They were obtained from the Faculty of Agriculture, Assiut University.

2. *S. aureus* culture

An identified MRSA strain of *S. aureus* from this study was diluted in nutrient broth and adjusted to 2.1 x 10^6 CFU/mL as described by **Göksoy *et al*. (2000)**.

3. Ultra-high temperature (UHT) milk

Fresh raw milk was obtained from a clinically healthy cow (Department of dairy production, Faculty of Agriculture, Assiut University). This milk was confirmed to be free from *S. aureus* by bacteriological examination trials. The milk was handled according to **Datta *et al*. (2002)** by heating at 135–145°C for 2–3 s for killing any spore-forming pathogens.

4. Yoghurt manufacturing and inoculation

"Traditional yoghurt" was manufactured by adding a blend of *S. thermophilus* and *L. bulgaricus* (1 % v/v each) to UHT cow's milk (38 -42 ^{o}C). The "bifidus yoghurt" was prepared by mixing a culture of *S. thermophilus*, *L. bulgaricus*, and *B. bifidum* (1 % v/v each) with the UHT cow's milk. The two yoghurt types were inoculated with *S. aureus* at 2.1 X 10^6 CFU/mL during preparation immediately after addition of the starter culture and thoroughly mixed. Then, the inoculated milk was incubated at 42°C till transforming into a gel-like creamy texture and reaching a pH of 4.5. The formed yoghurt was kept at 4^{o}C for 21 days. The *S. aureus* population count and pH were monitored 5 times during the experiment; at the 1st, 3rd, 5th, 7th, 14th, and 21st days following storage. The ability of commercially available starter cultures (*S. thermophilus, L. bulgaricus,* and *B. actiregularis*) in the commercial traditional and bifidus yoghurts to inhibit the MRSA multiplication through a simulated temperature and time profile of yoghurts-making process were evaluated, too. Non-inoculated negative control yoghurt for each type was considered. Each treatment was represented by 5 replicates and the whole experiment was repeated 3 times (n = 3) to calculate the values' mean for using in statistical analysis **(Rahman *et al*., 2024 and Yousefvand *et al*., 2024)**.

5. Determination of pH and titratable acidity

pH was determined using a digital pH meter (ADWA/ Romania). Briefly, 10 g of yoghurt sample was dissolved in 100 mL of distilled water. The mixture was allowed to equilibrate at room temperature and the pH was then estimated.

The titratable acidity was accessed as previously described by **A.O.A.C. (2005)** and expressed as percentage of lactic acid. It was determined by mixing 10 g of yoghurt with 20 mL of distilled water and titrating with 0.1 N NaOH using phenolphthalein as an indicator to an end-point of faint pink color. The measurements were done in triplicate.

Statistical analysis

Data were collected, arranged, and analyzed using a one-way ANOVA conjugated with Duncan's multiple comparison tests (SPSS Inc., Chicago, Illinois, USA) at a 0.05 P value (**Yousefvand *et al*., 2024**).

Results

S. aureus was isolated from 31 of the 55 examined yoghurt samples by bacteriological and biochemical analyses with a percentage of 56.36 %. The bacterial colonies were circular yellow surrounded by yellow zone diffused into the media which stained gram positive and arranged in clusters. All isolates were catalase and coagulase reactive. The gene encoding 23S rRNA was detected in 6 isolates out of ten strains **(Fig. 1)**.

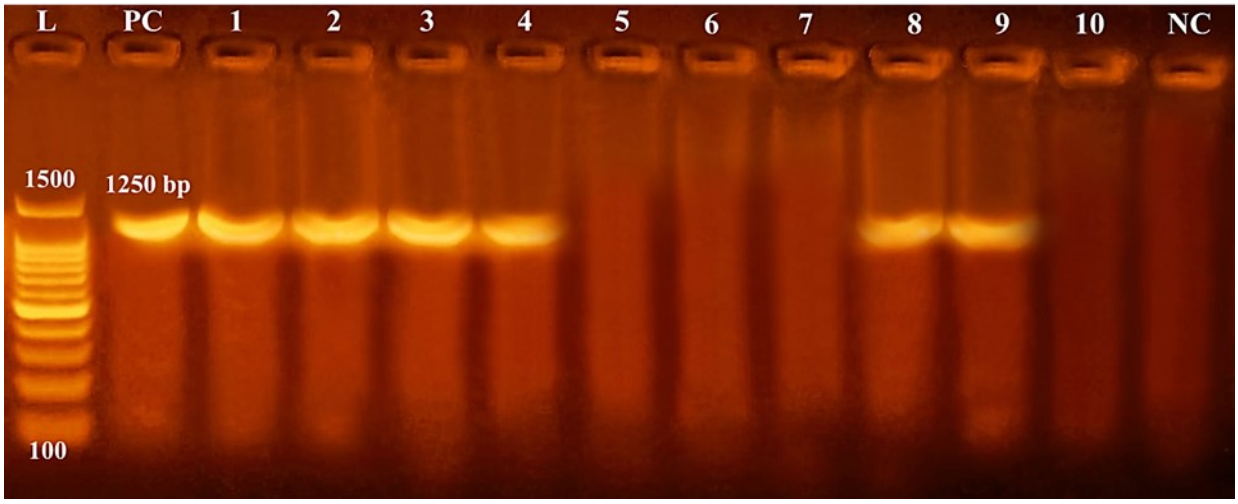

Fig. (1). Agarose gel electrophoresis image of the 23S rRNA amplicon specific to *S. aureus*. **L**: 1500 bp DNA marker; **PC**: A positive control for *S. aureus* with an amplicon size of 1250 bp; **Lanes 1-4, 8, and 9:** Positive samples. **NC:** A negative control of nuclease free water. **Lanes 5-7 and 10:** Negative samples.

The antibacterial sensitivity profile of the isolated *S. aureus*

S. aureus isolates expressed high MAR index (0.75) to the tested antibacterial drugs. The highest resistance level was observed against amoxicillin and novobiocin (90%) followed by methicillin and oxacillin (80%) and erythromycin (70%). Thirty percentages of these isolates showed resistance against streptomycin. These isolates had high sensitivity to Enrofloxacin, Sulfa/trimethoprim, and Streptomycin (100%, 100%, 70%, respectively) **(Table 1).**

Table (1). In vitro antibacterial susceptibility profile of *S. aureus* isolates (t=10)* and their multiple antibacterial resistance (MAR) index:

Antibiotic	conc.	Resistance (n/t) %	Sensitivity (n/t) %	Antibacterial resistance profile	MAR index
NV	30	(9/10) 90	(1/10) 10	AMX, NV, ME, OX, E, S.	**MAR**=6/8=0.75
Amx	10	(9/10) 90	(1/10) 10		
OX	5	(8/10) 80	(2/10) 20		
ME	5	(8/10) 80	(2/10) 20		
E	15	(7/10) 70	(3/10) 30		
S	10	(3/10) 30	(7/10)70		
Sxt	25	(0/10) 0	(10/10) 100		
Ex	5	(0/10) 0	(10/10) 100		

*t: Total tested isolates. **n (%)**: Data are expressed in number of reactive isolates (percentage). **MAR:** Multiple Antibiotic Resistance index is the ratio of the antibiotics number to which the bacterium is resistant to total the number of antibiotics to which the bacterium is exposed. **ME:** Methicillin (5 µg), **E**: Erythromycin (15µg), **AMX:** Amoxicillin (10µg), **NV:** Novobiocin (30 µg), **EX:** Enrofloxacin (5µg), **SXT:** Sulfa/trimethoprim (25µg), **S:** Streptomycin (10µg), and **OX:** Oxacillin (5µg)

Detection of methicillin resistance gene (*mecA*)

The 310 bp band specific to *mecA* gene was identified in 90 % (18/20) of MRSA isolates submitted for PCR **(Fig. 2)**.

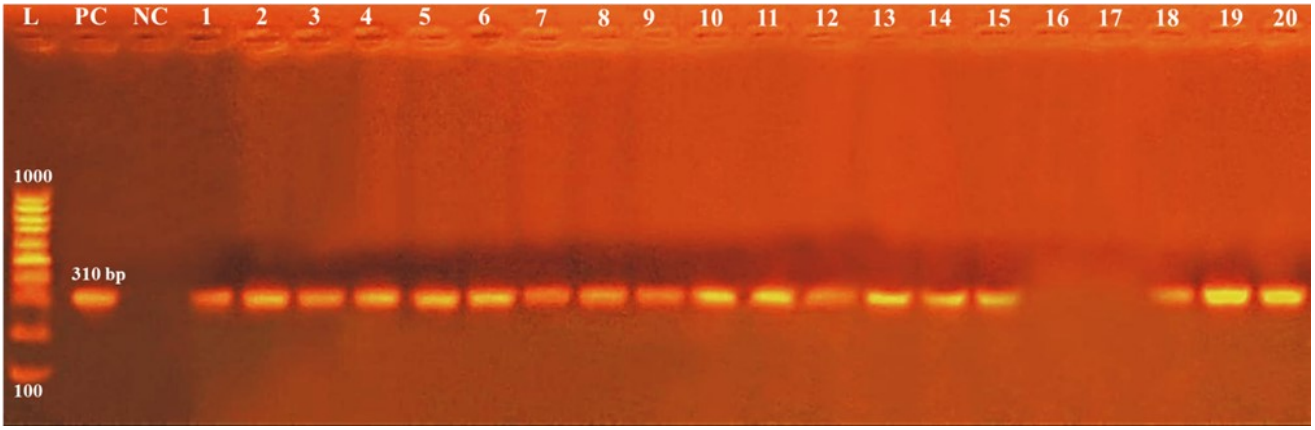

Fig. (2). Agarose gel electrophoresis image of the *mecA* gene amplicon specific to *S. aureus*. **L**: 1000 bp DNA marker; **PC**: A positive control for *mecA* gene with an amplicon size of 310 bp; **NC:** A negative control of nuclease free water; **Lanes 1-15 and 18-20:** Positive samples. **Lanes 16 and 17:** Negative samples.

Effect of different yoghurt starter on MRSA viability

MRSA counts decreased significantly (P value < 0.0001) up to the end of the storage **(Table 2)** and highly significant differences in survival of MRSA were noticed between the used starter strains. The decrease in MRSA counts started on the 1st day of storage. The prepared yoghurt containing *S. thermophilus* and *L. bulgaricus* (traditional yoghurt) showed a significant reduction in MRSA count on the 3rd and 21st days of storage in comparison to the commercial yoghurt containing the same starter culture type (from 2.1± 0.13 x 10^6 CFU to 0.15±0.01 and 0.00042 x 10^6 CFU versus 0.39±0.01 and 0.00051±0.0 x 10^6 CFU respectively). Combining *B. actiregularis* with the traditional starter culture (commercial bifidus yoghurt) decreased MRSA count from 2.1± 0.13 x 10^6 CFU to 0.00021±0.0 x10^6 CFU in the 21st of storage which was the lowest compared to the traditional cultures and that combined with *B. bifidum* (prepared bifidus yoghurt).

Table (2). MRSA counts (x10^6 CFU) in the inoculated prepared and commercial yoghurts during storage:

Days	Lab. prepared yoghurt		Commercial yoghurt		P value
	Traditional	Bifidus	Traditional	Bifidus	
	S. thermophilus* *L. bulgaricus	***S. thermophilus* *L. bulgaricus* *B. bifidum***	***S. thermophilus* *L. bulgaricus***	***S. thermophilus* *L. bulgaricus* *B. actiregularis***	
1st	0.62±0.02^b	0.84±0.01^a	0.59±0.011^b	0.0952±0.001^c	2.91E-17
3rd	0.15±0.01^c	0.77±0.01^a	0.39±0.01^b	0.064±0.0^d	1.36E-19
5th	0.078±0^b	0.69±0.01^a	0.075±0.0^c	0.031±0.0^d	3.40E-25
7th	0.03±0.0^b	0.042±0.0^a	0.027±0.0^b	0.0065±0.0^c	1.13E-13
14th	0.0027±0.0^b	0.0076±0.0^a	0.0047±0.0^b	0.00062±0.0^c	1.29E-19
21st	0.00042±0.0^c	0.0013±0.0^a	0.00051±0.0^b	0.00021±0.0^d	2.28E-12

Data are presented as average ± standard error from three independent experiments. **CFU:** Colony Forming Unit.
***S. thermophilus*:** *Streptococcus thermophilus,* ***L. bulgaricus*:** *Lactobacillus bulgaricus,* ***B. bifidum*:** *Bifidobacterium bifidum,* ***B. actiregularis*:** *Bifidobacterium actiregularis.*
[a-d:] Averages, within a row, followed by a different superscript are significantly different (p<0.0001).

Effect of starter cultures on yoghurt pH and titratable acidity

All starter cultures decreased the initial milk's pH with quite notable variations in the pH between the different used strains that were used **(Table 3)**. In the first day of storage, pH decreased from 6.58±0.04 (raw milk) to 4.59±0.01 and 4.58±0.0 in the prepared and commercial traditional yoghurts, respectively, but decreased to 4.46±0.03 and 4.32±0.01 in the bifidus yoghurt, respectively. This decrease in pH progressed throughout the storage till reaching 3.96±0.0 and 3.80±0.01 in the prepared yoghurt types, while both commercial ones showed higher pH (4.12±0.01 and 4.16±0.01) after 21 days.

The titratable acidity percentage increased gradually in all yoghurt types during the days of storage period. Both the prepared and commercial bifidus yoghurt have remarkably high titratable acidity relative to traditional **(Table 3)**.

Table (3). Values of pH changes in the MRSA inoculated prepared and commercial yoghurts during storage:

Time	Lab. prepared yoghurt				Commercial yoghurt				P value
	Traditional		Bifidus		Traditional		Bifidus		
	S. thermophilus *L. bulgaricus*		*S. thermophilus* *L. bulgaricus* *B. bifidum*		*S. thermophilus* *L. bulgaricus*		*S. thermophilus* *L. bulgaricus* *B. actiregularis*		
	pH	Acidity %	pH	Acidity %	pH	Acidity %	pH	Acidity %	
1st	4.59±.01[a]	0.52	4.46±.03[b]	0.58	4.58±.0[a]	0.52	4.32±.01[c]	0.58	8.36E-09
3rd	4.51±.01[a]	0.62	4.41±.01[b]	0.66	4.51±.01[a]	0.64	4.3±.03[c]	0.68	0.000001
5th	4.35±.0[b]	0.68	4.45±.0[a]	0.68	4.42±.01[a]	0.68	4.29±.02[c]	0.7	3.28E-09
7th	4.28±.01[b]	0.72	4.28±.02[b]	0.78	4.38±.02[a]	0.74	4.2±.01[c]	0.72	0.000002
14th	4.10±.01[c]	0.76	3.96±.01[d]	0.82	4.22±.01[a]	0.77	4.18±.01[b]	0.76	1.22E-11
21st	3.96±.02[b]	0.8	3.80±.01[c]	0.87	4.12±.01[a]	0.82	4.16±.01[a]	0.84	3.09E-12

Data are presented as average ± standard error from three independent experiments. a-d: Averages, within a row, followed by a different superscript are significantly different ($p<0.0001$).

Discussion

S. aureus is a food poisoning pathogen that contaminates raw milk. In addition to its pathogenicity, the danger of these bacteria lies in their ability to produce thermo- and proteases-stable toxins. Moreover, it was recorded to have multidrug resistance genes which hinder their control **(Abdolshahi *et al.*, 2018)**. The examined yoghurt samples in this study showed 56.36 % *S. aureus* detection rate by bacteriological and biochemical testing. All these isolates were coagulase reactive. Sixty percentages (60%; 6/10) of the isolated bacteria encoded the 23S rRNA which is a sensitive tool for recognizing *S. aureus* and related to its growing resistance to antibacterial drugs as previously explained by **Besier *et al.* (2008)**. The finding showed that the traditional yoghurt had a high prevalence of *S. aureus* which is alarming to the adverse health hazards which can be produced from its consumption **(Nacer *et al.*, 2024)**. This high prevalence might be attributed to contamination with wastes of infected animals, or cross-contamination during improper milk handling at collecting facilities (**Rahimi, 2013 and Ahmed *et al.*, 2019**). Moreover, the Egyptian traditional yoghurt is a traditional milk product that is processed by personnel who are not aware enough of the hygienic procedures for manufacturing and distributing such product in ill-qualified shops. Consequently, strict measures are required to decrease the access and growth of such bacterium to milk and its products, stressing the importance of checking *S. aureus* prevalence among Egyptian workers of dairy industry

(Badawy *et al*., 2022). In agreement with the current results, a high prevalence of *S. aureus* (62.2%) was observed in Port-said, Egypt **(Saad *et al*., 2023)**. Higher results were recorded by **Nwamaka and Chike (2010)** in Eastern Nigeria. Lesser prevalence rates were recorded by **Usman *et al*. (2016), Al-Ashmawy *et al*. (2016), Ahmed *et al*. (2019), Weldeselassie *et al*. (2020),** and **Feyissa *et al*. (2023)** in Nigeria, Egypt (Assiut and Mansoura), and Ethiopia (Tigray and West Showa Zone) (15.71%, 35%, 40%, 45.83% and 4.8%, respectively). The prevalence variation *S.aureus* from the previous reports might be due to differences in sample size, isolation techniques, husbandry practices, awareness and skill of the animal owners, animal health delivery systems, and geographic region of the sampled area (**Weldeselassie *et al*., 2020)**.

Pathogens' antibacterial resistance traits remains the main concern of the world and represent the primary worry in managing infections brought on by pathogenic strains of *S. aureus*. *S. aureus* resistance to antibacterial drugs has been developed since formulating the first antibiotic (penicillin) (**Guo *et al*., 2020)**. MRSA strain is widely distributed among food products and responsible for mortalities which exceed that of HIV-AIDS **(Peterson, 2010)**. In the current study, the detected *S. aureus* isolates expressed high MAR index (0.75) to the tested antibacterial drugs indicating a high-risk source of these bacterial isolates. This observation came in agreement with **Umaru *et al*. (2014)**, **Usman *et al*. (2016) and Weldeselassie *et al*. (2020)**. **Mir *et al*. (2022)** explained that MAR indices higher than 0.2 refer to that isolates come from a source where antibiotics are utilized extensively and/or in large quantities.

The dissemination of such resistant isolates can pose a serious public health problem **(Faghihi *et al*., 2019)**. The isolated bacteria in this study displayed a greatest resistance to amoxicillin and novobiocin (90 %) followed by methicillin and oxacillin (80 %), and erythromycin (70 %). Enrofloxacin, Sulfa/trimethoprim, and streptomycin showed relatively low levels of resistance (0 %, 0 %, and 30 %, respectively), suggesting that these medications would be good options for treating these MRSA strains. Susceptibility of isolates to sulpha/ trimethoprim could be a signs of community-associated MRSA. The resistance percentages to novobiocin and oxacillin is alarming and have been reported widely **(Abo-Shama *et al*., 2014**, **Idris *et al*., 2024 and Iancu *et al*., 2025)**. In agreement with our results, **Usman *et al*. (2016)** noticed that all *S. aureus* (100 %) they found in yoghurt produced from the fresh milk have been MRSA and over 90 % of these isolates are methicillin, penicillin, and oxacillin resistant by Kirby-Baeur disk diffusion. Also, **Feyissa *et al*. (2023)** reported that all *S. aureus* isolates detected in the traditionally processed yoghurt were resistant to penicillin, tetracycline, and oxytetracycline. Lower MRSA prevalence rate (35%, 14/40) was recognized by **Al -Ashmawy *et al*. (2016)** in Mansoura City, Egypt.

On the other hand, **Ahmed *et al*. (2019)** didn't recognize any MRSA strain in commercial yoghurt. They mentioned that many of the dairy products (including yoghurt) produced by the Egyptian companies are exported across the Middle Eastern countries (e. g. Saudi Arabia, Libya, Jordan, Lebanon, United Arab Emirates, and Qatar). The Egyptian government and the dairy industry must guarantee the quality and safety of these products before they may be exported as they are one of ready-to-eat food which its contamination any pathogenic organisms and toxins will prevent its exportation and this causes immense economic losses.

In this study, ninety percent (90 %) of the MRSA isolates possessed the *mec*A gene, which accounts for the isolates' strong resistance to methicillin and its closely related drugs. This gene is translated into β-lactamase enzyme that renders methicillin and its closely related antibiotics inactive. This result reflects the careless use of methicillins in the dairy animals to treat mastitis and prevent it. This finding was in line with **Lee (2006) and Al-Ashmawy *et al*. (2016)** who recognized *mec*A gene in 19 of 19 and 12 of 12 MRSA isolates, respectively. **Weldeselassie *et al*. (2020)** recorded fewer incidence of *mec*A gene among MRSA strains (37.5%). Conversely, the current result was inconstant with **Usman *et al*. (2016)** and **Feyissa *et al*. (2023)** who didn't found *mec*A gene in MRSA strains isolated

(0/4 isolates in both studies) from the traditionally processed yoghurt. They attributed MRSA resistance to factors other than *mec*A gene such as Penicillin binding protein 2a (Pbp2a), *mec*A homologues such as *mec*C, mobile elements, transposons, and phages which can carry other resistance genes.

Yoghurts production is based on using a starter culture of *S. thermophilus* and *L. bulgaricus* as a symbiotic combination. Initially, *S. thermophilus* release folic acid, formic acid, CO2, etc. These products promote *L. bulgaricus'*s multiplication. On the other side, *L. bulgaricus* produces small peptides and free amino acids, which potentiate *S. thermophilus'*s growth. This symbiotic combination facilitates lactic acid production faster than in a single culture strain alone. Lately, *Bifidobacterium* proved its significance in yoghurt synthesis due to its production of beneficial short chain fatty acids (SCFAs) **(Matela *et al*., 2019)**.

Regardless of type, starter cultures in this study decreased MRSA count significantly up to the end of the cold storage. Highly significant differences in survival of MRSA were noticed between the used starter strains. This inhibitory outcome may be accredited either to the growing starter bacteria itself or to their antibacterial byproducts**.** This agreed with **Soliman and Ahmed (2019)**. In the current study, *S. thermophilus* and *L. bulgaricus* showed a significant reduction in MRSA count on the 3rd and 21st days of storage in comparison to the commercial yoghurt containing the same starter culture type. Combining *B. actiregularis* with the traditional starter culture (commercial bifidus yoghurt) decreased MRSA in the 21st of storage which was the lowest compared to the traditional cultures and that combined with *B. bifidum* (prepared bifidus yoghurt).

The different starter cultures reduced the initial milk's pH with quite notable variations in the pH of the yoghurts made by the starter strains that were used from 6.58±0.04 to 4.59±0.01 and 4.58±0.0 in the prepared and commercial traditional yoghurts, respectively, but decreased to 4.46±0.03 and 4.32±0.01 in the bifidus yoghurts, respectively. Notably, the pH in the bifidus yoghurt was slightly high acidic compared to the traditional that may be due to increased acids production.

The reduction of pH progressed throughout the storage till reaching 3.96±0.0 and 3.80±0.01 in the prepared yoghurts types, while both commercial ones showed higher pH after 21 days. This reduction in pH, particularly, in the late stages of yoghurt's cold storage can be explained by increased lactic acid accumulation resulted from anaerobic fermentation of milk lactose into lactic acid. This explains the reductive effect of starter cultures to the MRSA count. Moreover, the pH reduction is beneficial for calcium bioavailability for intestinal absorption due to its conversion to its ionic form and inhibition of phytic acid effect in the low pH **(Adolfsson *et al*., 2004)**.

Conversely, the titratable acidity percentage increased gradually in all yoghurt types during the storage period in this study. Both the prepared and commercial bifidus yoghurts have remarkably high titratable acidity relative to traditional this indicates to more availability of the fermenting starter cultures' strains. The pH and titratable acidity percentages of all yoghurt the current study were complying in accordance with FDA specifications **(Weerathilake *et al*., 2014)**. Our results came in line with other previous studies. **Matela *et al*. (2019)** when found that the pH and titratable acidity of yoghurt samples were in the range of 3.94-4.22 and 0.69-1.81, respectively.

Conclusion

A total of 55 traditional balady yoghurt samples were analyzed for their contamination by *S. aureus* and its MRSA strains. It was found that 56.36 % of these samples were positive for *S. aureus* where 90 % of these isolates were MRSA. The isolated MRSA strains were highly resistant to amoxicillin, novobiocin, methicillin, and erythromycin. Additionally, the effect of different starter culture strains on the growth of MRSA was studied. The used starter cultures showed a promising effect on the behavior of MRSA survival and yoghurt's pH and titratable acidity during storage at 4˚C for 21 days. Regardless of type, there was statistically significant effect of the used starter cultures on reducing MRSA count and inhibition of its growth. Combining the traditional starter cultures *S. thermophilus* and *L. bulgaricus* with *B. actiregularis* for yoghurt production showed

the most synergistic action against MRSA. Hence, the traditional balady yoghurt can be a source of MRSA infection and using *B. actiregularis* with the traditional starter culture in yoghurt production increased its safety and probiotics spectrum which is helpful in blocking dangerous infectious organisms and enhancing consumer immunity.

References

Abdolshahi, A.; Naybandi-Atashi, S.; Heydari-Majd, M.; Salehi, B.; Kobarfard, F.; Ayatollahi, S.A.; Ata, A.; Tabanelli, G.; Sharifi-Rad, M.; Montanari, C. and Iriti, M., (2018). Antibacterial activity of some Lamiaceae species against *Staphylococcus aureus* in yoghurt-based drink (Doogh). Cellular and Molecular *Biology*, *64* (8), pp.71-77.

Abo-Shama, U.H. (2014). Prevalence and antimicrobial susceptibility of *Staphylococcus aureus* isolated from cattle, buffalo, sheep and goat's raws milk in Sohag Governorate, Egypt. Assiut Vet Med J 2014; 60(141): 63-72.

Adeyemi, F.M.; Oyedara, O.O.; Yusuf-Omoloye, N.A.; Ajigbewu, O.H.; Ndaji, O.L.; Adegbite-Badmus, M.K.; Olumakinde, T.S. and Oluokun, T.E. (2024). Guardians of resistance and virulence: detection of mec, femA, Van, pvl, hlg and spa genes in methicillin and vancomycin -resistant *Staphylococcus aureus* from clinical and food samples in Southwestern Nigeria. BMC microbiology, *24*(1), p.498.

Adolfsson, O.; Meydani, S.N. and Russell, R.M. (2004). Yogurt and gut function. *The* American Journal of Clinical Nutrition, 80 (2), 245-256.

Ahmed, A.A.H.; Maharik, N.M.S.; Valero, A. and Kamal, S.M. (2019). Incidence of enterotoxigenic *Staphylococcus aureus* in milk and Egyptian artisanal dairy products. Food Control, 104, pp.20-27.

Al-Ashmawy, M.A.; Sallam, K.I.; Abd-Elghany, S.M.; Elhadidy, M. and Tamura, T. (2016). Prevalence, molecular characterization, and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolated from milk and dairy products. Foodborne pathogens and disease, *13* (3), pp.156-162.

Asfaw, T.; Genetu, D.; Shenkute, D.; Shenkutie, T.T.; Amare, Y.E.; Habteweld, H.A. and Yitayew, B. (2023). Pathogenic bacteria and their antibiotic resistance patterns in milk, yoghurt and milk contact surfaces in Debre Berhan town, Ethiopia. Infection and drug resistance, pp.4297-4309.

Association of the Official Analytical Collaboration (AOAC) International (2005). Official Methods of Analysis of AOAC International. 481. North Fredrick Avenue Gaithersburg, Maryland, USA. (18th edition).

Badawy, B.; Elafify, M.; Farag, A.M.; Moustafa, S.M.; Sayed-Ahmed, M.Z.; Moawad, A.A.; Algammal, A.M.; Ramadan, H. and Eltholth, M. (2022). Ecological distribution of virulent multidrug-resistant *Staphylococcus aureus* in livestock, environment, and dairy products. Antibiotics, *11*(11), p.1651.

Besier, S.; Ludwig, A.; Zander, J.; Brade, V. and Wichelhaus, T.A. (2008). Linezolid resistance in *Staphylococcus aureus:* gene dosage effect, stability, fitness costs, and cross-resistances. Antimicrob Agents Chemother. 52(4):1570-2.

Clinical and Laboratory Standards Institute (CLSI) (2022). Performance standards for antimicrobial susceptibility testing. In: CLSI Supplements M100S. 32th ed. Wayne, PA: Clinical and Laboratory Standards Institute.

Datta, N.; Elliott, A.J.; Perkins, M.L. and Deeth, H.C. (2002). Ultra-High Temperature UHT treatment of milk: Comparison of direct and indirect modes of heating. Aust J Dairy Technol, 57, 211-227.

Elshereif, L.M.; El Nady, E.A.M. and Girgis, A.I. (2025). Occurrence of *Staphylococcus aureus* in chicken breast meat and the influence of different thawing techniques on its survival rate. Egyptian Journal of Animal Health 5, 1.

Faghihi, S.M.; Rassouli, A. and Bahonar, A. (2019). A survey on the status of antibacterial drug use in dairy cattle farms in Qom province. Journal of Veterinary Research, 74 (4), 574–582.

Feyissa, N.; Alemu, T.; Birri, D.J. and Dessalegn, A. (2023). Isolation, identification, and determination of antibiogram characteristics of *Staphylococcus aureus* in cow milk

and milk products (yoghurt and cheese) in West Showa Zone, Ethiopia. International Dairy Journal, *137*, p.105503.

Freitas, J.K.G.R.; Assis, C.F.D.; Oliveira, T.R.M.D.; Maia, C.M.D.M.; de Sousa, B.J.; Medeiros, G.C.B.S.D.; Seabra, L.M.A.J. and Chaves Damasceno, K.S.F.D.S. (2023). Prevalence of staphylococcal toxin in food contaminated by Staphylococcus spp.: Protocol for a systematic review with meta-analysis. Plos one, *18*(2), p.e0282111.

Umaru, G.A.; Kabir, J.; Umoh, V.J.; Bello, M. and Kwaga, J.K.P. (2014). Occurrence of vancomycin-resistant *Staphylococcus aureus* (VRSA) in fresh and fermented milk in Nigeria: A preliminary report. Int J Public Health and Epidemiol, 3, pp.054-058.

Göksoy, E.O.; James, C. and Corry, J.E.L. (2000). The effect of short-time microwave exposures on inoculated pathogens on chicken and the shelf-life of uninoculated chicken meat. Journal of Food Engineering, *45*(3), pp.153-160.

Guo, Y.; Song, G.; Sun, M.; Wang, J. and Wang, Y. (2020). Prevalence and therapies of antibiotic-resistance in *Staphylococcus aureus.* Frontiers in cellular and infection microbiology, *10*, p.107.

Hu, J.; Chen, L.; Li, G.; Pan, Y.; Lu, Y.; Chen, J.; Xiong, W. and Zeng, Z. (2023). Prevalence and genetic characteristics of fosB-positive *Staphylococcus aureus* in duck farms in Guangdong, China in 2020. Journal of Antimicrobial Chemotherapy, *78*(3), pp.802-809.

Iancu, I.; Igna, V.; Popa, S.A.; Imre, K.; Pascu, C.; Costinar, L.; Degi, J.; Gligor, A.; Iorgoni, V.; Badea, C. and Nichita, I. (2025). Etiology and antimicrobial resistance of subclinical mastitis pathogens *Staphylococcus aureus*, Streptococcus spp. and Enterococcus spp. in sheep milk. Veterinary Research Communications, *49*(1), p.30.

Idris, M.U.; Daniyan, S.Y.; Bala, J.D. and Chinma, C.E. (2024). January. Phenotypic detection and molecular identification of Methicillin-resistant Staphylococcus aureus from dairy products in pastoral communities in Niger State, Nigeria. In Journal of Pure and Applied Sciences (Science Forum) (Vol. 24, No. 1, pp. 447-458).

Kamal, R.M.; Alnakip, M.E.; Abd El Aal, S.F. and Bayoumi, M.A. (2018). Biocontrolling capability of probiotic strain *Lactobacillus rhamnosus* against some common foodborne pathogens in yoghurt. Int. Dairy J. 85, 1–7.

Lee, J.H. (2006). Occurrence of methicillin-resistant *Staphylococcus aureus* strains from cattle and chicken, and analyses of their mecA, mecR1 and mecI genes. Veterinary Microbiology, *114*(1-2), pp.155-159.

Matela, K.S.; Pillai, M.K. and Thamae, T. (2019). Evaluation of pH, titratable acidity, syneresis and sensory profiles of some yoghurt samples from the kingdom of Lesotho. Food Research, *3*(6), pp.693-697.

McClure, J.A.; Conly, J.M.; Lau, V.; Elsayed, S.; Louie, T.; Hutchins, W. and Zhang, K. (2006). Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. J Clin. Microbiol 44: 1141-114.

Mir, R.; Salari, S.; Najimi, M. and Rashki, A. (2022). Determination of frequency, multiple antibiotic resistance index and resistotype of Salmonella spp. in chicken meat collected from southeast of Iran. Veterinary medicine and science, *8*(1), pp.229-236.

Nacer, S.; Nassik, S.; El Ftouhy, F.Z.; Derqaoui, S.; Mouahid, M. and Lkhider, M. (2024). Detection of Staphylococcal Enterotoxins A and E and Methicillin Resistance in *Staphylococcus aureus* Strains From Moroccan Broiler Chicken Meat. International Journal of Food Science, (1), p.2790180.

Nwamaka, N.T. and Chike, A.E. (2010). Bacteria population of some commercially prepared yoghurt sold in Enugu State, Eastern Nigeria. African Journal of Microbiology Research 4, 984-988.

Peterson, L.R. (2010). To screen or not to screen for Methicillin- resistant Staphylococcus aureus. Journal of Clinical Microbiology, 48(3), 683–689.

Prihandani, S.S.; Wibawan, I.W.T.; Noor, S.M.; Putri, A.F. and Nuradji, H. (2024). June. Phenotypic and Genotypic Identification to *S. aureus* isolates from Cattle in Bogor. In IOP Conference Series: Earth and Environmental Science (Vol. 1359, No. 1, p.

012145). IOP Publishing.

Rahimi, E. (2013). Enterotoxigenicity of *Staphylococcus aureus* isolated from traditional and commercial dairy products marketed in Iran. Brazilian Journal of Microbiology, 44(2), 393–399.

Rahman, M.N.; Islam, M.N.; Mia, M.M.; Hossen, S.; Dewan, M.F. and Mahomud, M.S. (2024). Fortification of set yoghurts with lemon peel powders: an approach to improve physicochemical, microbiological, textural and sensory properties. Applied Food Research, *4*(1), p.100386.

Saad, A.H.; El-kosi, O.H.R. and Salma, E.M. (2023). Detection of some Food Poisoning Bacteria from Milk Utensils and Dairy Products in Port-said Governorate, Egypt. Journal of Advanced Veterinary Research, *13*(10), pp.2047-2050.

Sajith Khan, A.K.; Shetty, P.J.; Lakshmi Sarayu, Y.; Chidambaram, A. and Ranganathan, R. (2012). Detection of mecA genes of methicillin-resistant *Staphylococcus aureus* by polymerase chain reaction. International Journal of Health and Rehabilitation Sciences, *1*(2), pp.64-8.

Soliman, N.S. and Ahmed, L.I. (2019). Survival of *Staphylococcus aureus* in Bio-yoghurt. Open Journal of Applied Sciences, *9* (7), pp.564-572.

Tufail, M.; Hussain, S.; Malik, F.; Mirza, T.; Parveen, G.; Shafaat, S.; Wajid, A.; Mahmood, R.; Channa, R.A. and Sadiq, A. (2011). Isolation and evaluation of antibacterial activity of bacteriocin produced by *Lactobacillus bulgaricus* from yogurt. African Journal of Microbiology Research, *5*(22), pp.3842-3847.

Usman, R.Z.; Mustapha, B.M. and Mohammed, F.I. (2016). Isolation and identification of methicillin resistant *Staphylococcus aureus* (MRSA) from traditionally fermented milk "nono" and yoghurt in Kaduna Metropolis, Nigeria. Food Science and Quality Management, *2*(2), pp.1-21.

Weerathilake, W.A.D.V.; Rasika, D.M.D.; Ruwanmali, J.K.U. and Munasinghe, M.A.D.D. (2014). The evolution, processing, varieties and health benefits of yogurt. International Journal of Scientific and Research Publications, 4(4), 1-10.

Weldeselassie, M.; Gugsa, G.; Kumar, A.; Tsegaye, Y.; Awol, N.; Ahmed, M.; Abebe, N. and Taddele, H. (2020). Isolation and characterization of *Staphylococcus aureus* from food of bovine origin in Mekelle, Tigray, Ethiopia. The Open Microbiology Journal, 14(1).

Wörmann, M.E.; Pech, J.; Reich, F.; Tenhagen, B.A.; Wichmann-Schauer, H. and Lienen, T. (2024). Growth of methicillin-resistant *Staphylococcus aureus* during raw milk soft cheese-production and the inhibitory effect of starter cultures. Food microbiology, *119*, p.104451.

Yousefvand, A.; Pham, Q.H.; Ho, T.M.; Amiri, S.; Mäkelä-Salmi, N. and Saris, P.E. (2024). Bifidobacterium animalis subsp. lactis BB12-Derived Postbiotic Powders Enhance Antioxidant and Physicochemical Properties of Low-Fat Yoghurt. Food and Bioprocess Technology, pp.1-17.