Assiut University Journal of Multidisciplinary Scientific Research (AUNJMSR)
Faculty of Science, Assiut University, Assiut, Egypt.
Printed ISSN 2812-5029
Online ISSN 2812-5037
Vol. 2 (1): 99- 125 (2025)
https://aunj.journals.ekb.eg

Isolation and characterization of *Staphylococcus aureus* from dairy cow raw milk in Assiut, Egypt.

Thraa M. Ali[1], Mona M. Atia[2] and Naeima M. Yousef[1]

[1]Department of Botany & Microbiology, Faculty of Science, Assiut University, Assiut, Egypt.

[2]Department of Zoology & Entomology, Faculty of Science, Assiut University, Assiut, Egyp
[*]Corresponding Author: e-mail: Thraa.15242079@science.au.edu.eg

ARTICLE INFO

Article History:
Received: 2024-11-28
Accepted: 2025-01-09
Online: 2025-01-20

Keywords:
Staphylococcus aureus, cow milk, catalase test, multidrug resistant bacteria.

ABSTRACT

Milk provides an ideal medium for bacterial survival and growth due to its rich nutrient content. This study assessed the physical, chemical, and microbial quality of raw cow milk samples collected from various dairy markets in Assiut, Egypt. A total of 25 raw cow milk samples were obtained from Assiut city markets. The presence and diversity of *Staphylococcus aureus* were evaluated using Mannitol Salt Agar (MSA) medium. Chemical analyses revealed that the mineral content ranged as follows: total soluble salts (TSS%) from 0.72 to 3.96%, sodium (Na^+) from 367 to 598 mg/L, potassium (K^+) from 1.06 to 4.84 mg/L, and magnesium (Mg^{2+}) from 24.4 to 311.1 mg/L. Protein concentrations varied between 1.7 and 5.63 mg/L, while lipid and lactose concentrations fluctuated between 0.21–0.83 mg/L and 0.16–0.53 mg/L, respectively. The pH values of the milk samples ranged from weakly acidic to neutral (4.54–7.00). Microbial analysis indicated that *S. aureus* was detected in 92% of the milk samples, with total counts ranging from 2×10^2 to 78×10^4 CFU/mL. Antibiotic susceptibility testing of *S. aureus* isolates revealed that 47% (8/17) exhibited multidrug resistance (MDR). Notably, all tested isolates were resistant to cephazolin (30 µg), highlighting a growing challenge in managing infections in dairy animals. Morphological and biochemical identification confirmed the presence of *S. aureus* as Gram-positive, non-spore-forming cocci arranged in clusters, with yellow zone formation on MSA medium. Biochemical tests showed the isolates were catalase- and coagulase-positive. This study underscores the need for regular health monitoring of dairy animals, particularly for mastitis, and the implementation of appropriate treatment protocols to mitigate the risks posed by multidrug-resistant *S. aureus*.

INTRODUCTION

Milk is a highly nutritious and well-balanced food, offering a wide range of essential nutrients. As a result, it creates a favorable environment for the growth of various microorganisms, including harmful bacteria. Naturally, milk from healthy mammary glands is naturally free of bacteria. However, once it is secreted from the udder, it can be easily contaminated by spoilage and pathogenic bacteria from various sources, including feed, water, the dairy farm environment, udder surfaces, equipment, raw milk storage tanks, and handling by personnel. Additionally, milk can become contaminated before secretion due to the presence of mastitis-causing microorganisms [1, 2, 3]. Growth and multiplication of microorganisms in milk may be accelerated by high temperature [2]. *Staphylococcus aureus* is the most important pathogenic species of staphylococci in humans, responsible for a wide range of infections, from mild skin lesions to severe, life-threatening conditions. Animals serve as reservoirs for the transmission of this bacterium to humans. In dairy production, it can be excreted into milk from udders affected by mastitis, representing a serious public health concern [4, 5].

Staphylococcus aureus is a significant opportunistic pathogen found in raw milk and a known source of enterotoxins that cause food poisoning. Milk and its derived products serve as critical reservoirs for enterotoxin-producing *S. aureus*. Staphylococcal food poisoning (SFP) is one of the most common causes of gastroenteritis globally and occurs from the consumption of small amounts of preformed *S. aureus* enterotoxins in contaminated food [6, 7]. SFP typically manifests suddenly, with symptoms such as vomiting, abdominal pain, and nausea being the most frequent. Certain food items, including raw milk, dairy products, meat, poultry, egg-based products, and salads, are recognized as potential carriers for *S. aureus* transmission to humans [6]. Numerous outbreaks of SFP have been linked to the consumption of contaminated milk and dairy products. [8, 9, 10, 11, 12]. Contamination of dairy cows and raw milk with *S. aureus* continues to be a significant issue in the dairy industry. The frequency of foodborne disease outbreaks linked to contaminated dairy products highlights the public health importance of *S. aureus* [13]. Dairy animals are the primary source of *S. aureus*

contamination in raw milk, and the persistence of this problem remains a major concern in dairy production. The public health impact of *S. aureus* is underscored by numerous outbreaks of foodborne illness caused by tainted dairy products. Additionally, *S. aureus* is a leading cause of mastitis in dairy cows, resulting in substantial economic losses to the global dairy industry [16, 4].

Methicillin-resistant *S. aureus* (MRSA) refers to strains of *S. aureus* that are resistant to methicillin and all other β-lactam antibiotics, making it a significant global health concern. Due to its widespread presence, nosocomial infections caused by MRSA have been identified as one of the three most infectious diseases worldwide by the World Health Organization [17, 18, 19].

The objective of this study was to estimate the pooled prevalence of *Staphylococcus aureus*, evaluate the physical, chemical, and microbial quality of cow's milk, and investigate the biochemical characteristics and antimicrobial resistance profiles of the isolates

MATERIALS AND METHODS

1- Sample collection

Cow raw milk samples were collected from dairy markets Assiut, Egypt, transferred aseptically into sterile screw capped bottle and kept in an ice box, then transformed immediately to Microbiology laboratory for further analysis.

2- Chemical analysis of milk

Protein content of milk samples was determined according to Lowry’s method [20]. Lipid concentration was assessed using Sulfo-Phospho-Vanillin assay [21]. Lactose (reducing sugars) concentration using DNSA method [22], calcium and magnesium content by EDTA titration [23], sodium and potassium by flame emission technique [24], total soluble salt using Electrical Conductivity (EC) [25], chloride concentration by Mohr’s Method [26], bicarbonates by HCL [25] and pH values using pH meter were also investigated [27].

3- Isolation of *Staphylococcus aureus* from cow milk

Each milk sample was inoculated onto peptone water broth medium [28], incubated at 35-37°C for 20 hours, then streaked on Mannitol Salt Agar (MSA) plates (Oxoid, UK). All the plates were incubated at 37°C for 24 hours. The growing yellow colonies, that suggested to be *S. aureus* were picked up onto nutrient agar slants for further studies [29].

Standard plate count (SPC): To determine the total bacterial count in the milk sample, 1 ml of the milk was transferred into a sterile test tube containing 9 ml of peptone water broth medium. The mixture was thoroughly blended, followed by serial dilutions up to a dilution factor of $1:10^{-7}$. From these dilutions, duplicate 1 ml samples were introduced into 15-20 ml of standard plate count agar, thoroughly mixed, and allowed to solidify. The plates were then incubated at 37°C for 48 hours, and the resulting colony counts were recorded using a colony counter [30].

4- Characterization of *Staphylococcus aureus*

A. Microscopic examination:

Gram staining was used to confirm the *S. aureus* identification.

B. Biochemical characterization:

1. Catalase test:

A drop of 3% hydrogen peroxide was placed on a clean microscope slide. A noticeable amount of bacterial growth was then transferred using a sterile inoculating loop and mixed with the hydrogen peroxide. The reaction was observed for the formation of gas bubbles [32].

2. Coagulase Test:

A staphylococcal colony was emulsified in a drop of water on a clean, grease-free glass slide by gently spreading it (if the isolate does not form a smooth, milky suspension, the test should not be continued). Similar suspensions of other bacterial strains, serving as positive and negative controls, were also prepared to ensure the plasma's proper

reactivity. A flamed and cooled straight inoculating wire was then dipped into undiluted plasma at room temperature, and the adhering plasma (not a loopful) was mixed into the staphylococcal suspension on the slide. The wire was flamed again, and the process was repeated for the control suspensions. The test was considered positive if coarse clumping of cocci was visible to the naked eye within 10 seconds [28].

3. Growth at 15°C and 37°C:

Purified colonies of the bacterial strains were cultured in tubes of MRS broth medium (HiMedia, India) and their growth was monitored after incubation at 15°C and 37°C for 48 hours [28].

4. Sugar fermentation:

Through nutrient broth tubes containing 1% concentration of each of lactose, glucose, and sucrose as well as 0.05% phenol red (as an indicator), inoculate the broth with bacterial strain. The result was interpreted daily (for up to 7 days) by the presence of a yellow color, which indicates sugar assimilation, was observed following the incubation of the tubes at 37ºC [28].

5.Antibiotic sensitivity test The 23 isolates of *S. aureus* were tested for antibiotic sensitivity against seven commercially available antimicrobial discs (Oxoid, UK), selected based on the most commonly used drugs. The antimicrobial discs included clindamycin (2 µg), cephazolin (30 µg), amikacin (30 µg), amoxicillin/clavulanic acid (30 µg), gentamycin (10 µg), cefoxitin (30 µg), and erythromycin (15 µg). The test was performed using the Kirby-Bauer disc diffusion method [33], following the guidelines set by the Clinical and Laboratory Standards Institute (CLSI) [34]. To prepare the bacterial suspension, 2–3 fresh pure colonies from nutrient agar medium were transferred into saline (HiMedia, India). The turbidity of the cell suspension was then adjusted to match the 0.5 McFarland standard [35]. A sterile cotton swab was used to evenly spread the bacterial suspension onto nutrient agar plates (HiMedia, India). The antimicrobial discs were placed on the agar surface, spaced 3 cm apart, using sterile forceps, and the plates were incubated at 37°C for 20 hours. The results were recorded by measuring the

diameter of the clear zones around the discs, which were compared with the standard measurements provided by CLSI. *S. aureus* isolates resistant to three or more of the tested antimicrobial classes were classified as multidrug-resistant.

RESULTS

1- Physiochemical and biological properties of collected milk samples

The physiochemical properties (pH, total soluble salts, sodium, potassium, calcium, magnesium, chloride, bicarbonate, lactose, lipid, and protein) of twenty-five milk samples collected from markets were summarized in figure (1).

The results of this study revealed that, pH values of milk samples were slightly acidic to neutral, ranging from 4.54 - 7. Total Soluble Salt (TSS%) and mineral (Na, K, Ca, Mg, Cl, HCO_3), components of milk samples numbers (15, 16, 21) were found to be nearly similar to the permissible standard levels [36, 37]. Although, in other samples, they were either lower in samples 1, 8, 16, and 23 and higher samples 7,6 ,19. Protein content ranged from 1.7 - 5.63 mg/L. Lipid concentration varied between 0.21 - 0.83 mg/L. lactose concentration ranged between 0.16 and 0.53 mg/L and the average total plate counts of milk sampled were $2x10^2$ – $78x10^4$ CFU/ml (Tables 1&2).

Table:1 Protein, lipid and lactose contents of milk samples.

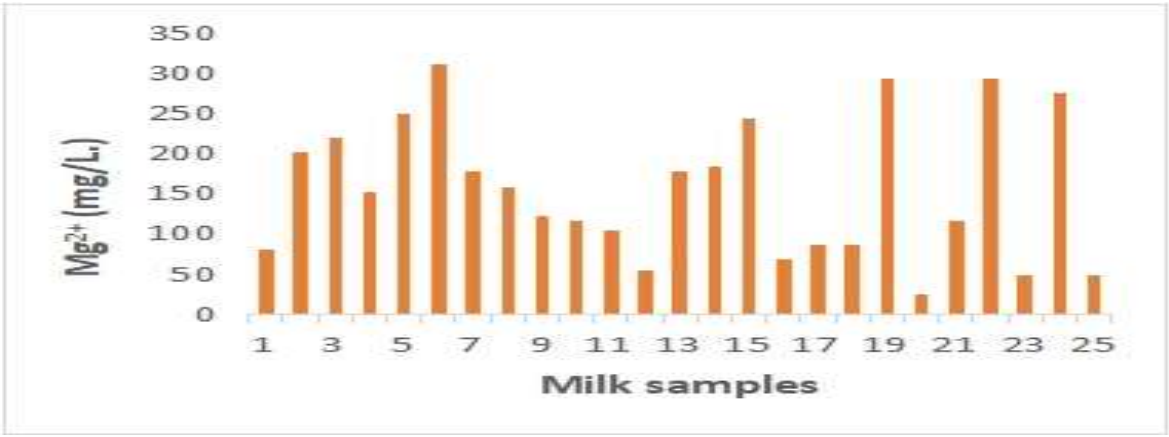
Milk samples	Protein (mg/L) Mean ±SD	Lipid (mg/L) Mean ±SD	Lactose (mg/L) Mean ±SD	Bacterial count CUF/ml
1	5.41±0.16	537±30.21	3.54±0.41	38x102
2	4.72±0.16	520±34.72	3.18±0.07	116x102
3	5.67±0.20	531±24.86	4.43±0.17	17x103
4	5.7±0.16	427±81.13	2.3±0.24	5x103
5	5.72±0.16	472±107.85	1.06±0.08	78x104
6	4.75±0.04	546±12.03	3.66±0.17	5x103
7	4.75±0.20	570±12.12	4.01±0.08	63x102
8	4.6±0.12	570±12.12	4.84±0.16	37x102
9	4.54±0.04	546±12.03	3.95±0.12	7x103
10	6.51±0.04	546±12.03	3.95±0.12	37x103
11	6.59±0.82	367±252.53	4.25±0.14	21x103
12	6.56±0.16	598±14.29	4.13±0.15	65x102
13	6.44±0.33	546±12.03	3.95±0.12	Negative
14	5.84±0.33	497±68.94	3.95±0.12	90x103
15	5.67±0.16	546±12.03	3.89±0.12	45x102
16	6.44±0.59	472±107.85	3.89±0.12	4x103
17	6.21±0.27	520±34.72	4.13±0.15	3x103
18	6.73±0.04	520±34.72	4.31±0.16	6x103
19	5±0.49	520±34.72	3.66±0.12	35x102
20	4.82±0.57	479±100.47	2.83±0.11	Negative
21	6.62±0.65	472±107.85	1.24±0.11	89x10

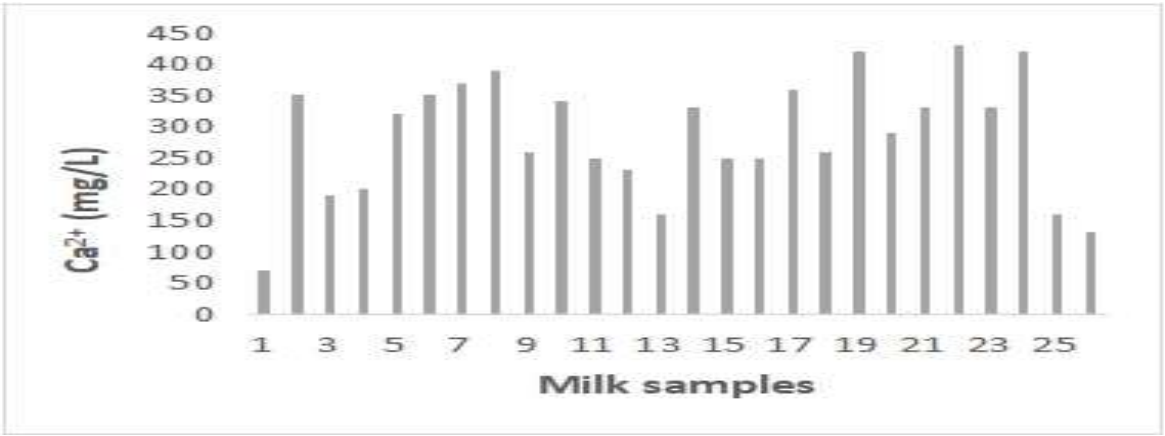
22	4.92±0.57	428±174.32	3.58±0.16	42x103
23	5.44±0.65	451±136.74	3.78±0.14	22x102
24	5.02±0.65	497±68.94	4.43±0.13	2x102
25	7±0.16	546±12.03	4.37±0.14	56x103

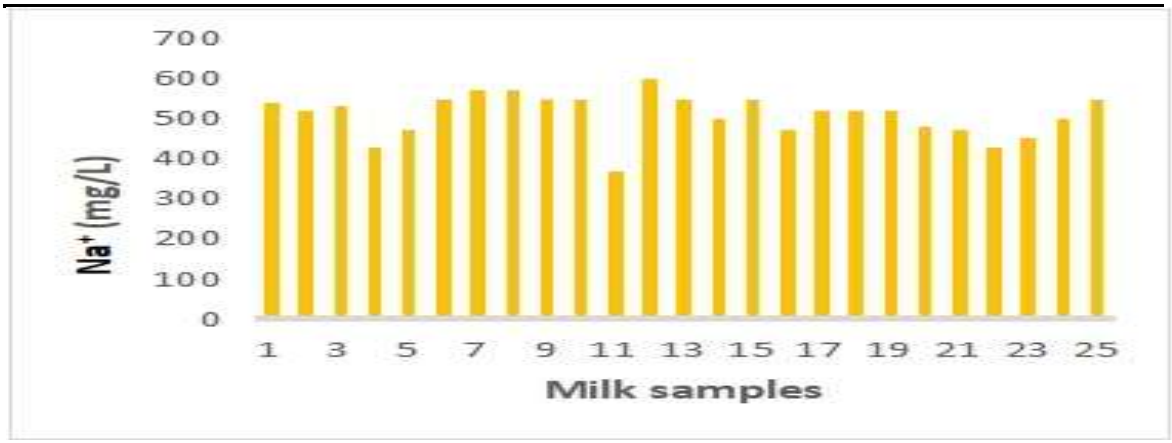
Table: 2 Mineral content of milk samples.

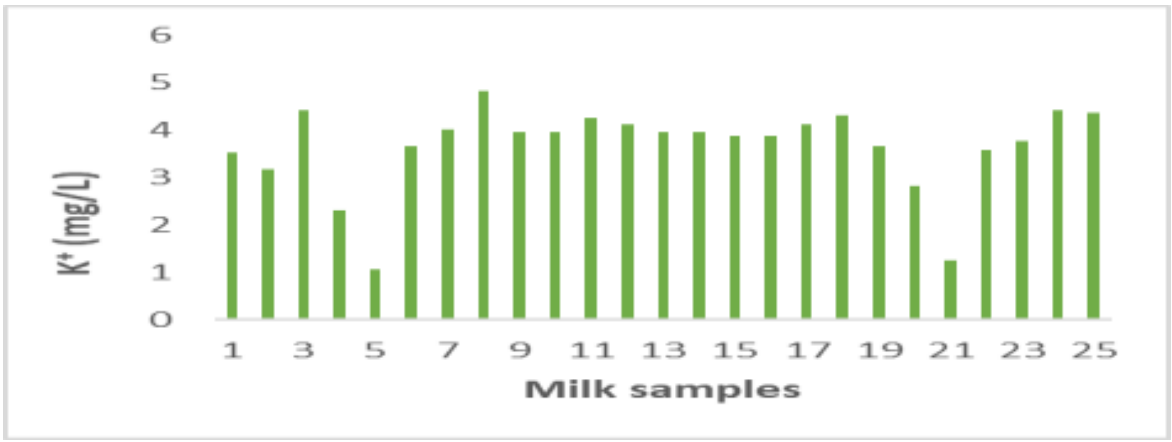
Milk samples	PH Mean ±SD	Na^{+}(mg/L) Mean ±SD	K^{+} (mg/L) Mean ±SD	Ca^{2+}(mg/L) Mean ±SD	Mg^{2+} (mg/L) Mean ±SD	Cl^{-} (mg/L) Mean ±SD	HCO^{3-}(mg/L) Mean ±SD	TSS% Mean ±SD
1	1.73±0.41	0.23±0.02	0.34±0.03	79.3±0.47	2.492±0.58	11.59±0.89	1.63±0.27	1.63±0.27
2	3.59±0.19	0.47±0.02	0.41±0.03	201±0.47	1.157±0.11	12.2±0.98	1.77±0.22	1.77±0.22
3	3.64±0.41	0.78±0.04	0.16±0.02	219.6±4.08	1.246±0.08	15.25±1.84	1.83±0.19	1.83±0.19
4	3.85±0.29	0.39±0.01	0.49±0.03	152±4.08	0.996±0.10	14.64±0.47	1.43±0.19	1.43±0.19
5	3.11±0.32	0.73±0.02	0.18±0.04	250.1±0.47	0.89±0.08	12.81±1.07	1.59±0.16	1.59±0.16
6	5.05±0.78	0.7±0.02	0.41±0.03	311.1±4.08	1.174±0.11	9.76±0.47	1.41±0.05	1.41±0.05
7	4.5±0.41	0.67±0.02	0.22±0.04	176.9±0.47	1.068±0.08	12.81±1.48	1.58±0.24	1.58±0.24
8	3.7±0.08	0.4±0.04	0.51±0.03	158.6±1.70	1.157±0.12	12.2±0.47	1.13±0.05	1.13±0.05
9	4.28±0.25	0.33±0.02	0.43±0.03	122±0.47	1.246±0.50	11.59±1.30	0.52±0.18	0.52±0.18
10	5.14±0.17	0.75±0.04	0.52±0.03	115.9±0.47	1.281±0.12	10.98±1.30	1.14±0.11	1.14±0.11
11	4.69±0.41	0.32±0.04	0.52±0.03	103.7±4.08	1.157±0.08	9.15±0.94	0.49±0.05	0.49±0.05
12	4.81±0.08	0.6±0.02	0.52±0.04	54.9±0.47	1.192±0.08	6.1±0.47	0.69±0.16	0.69±0.16
13	3.38±0.41	0.34±0.04	0.51±0.07	176.9±4.08	0.854.±0.29	8.54±0.83	0.53±0.19	0.53±0.19
14	2.84±0.12	0.79±0.04	0.52±0.03	183±0.82	1.584±0.08	10.98±1.21	1.17±0.05	1.17±0.05
15	3.4±0.74	0.56±0.03	0.52±0.04	244±1.63	1.299±0.32	12.2±0.47	1.02±0.18	1.02±0.18
16	4.18±0.08	0.37±0.04	0.46±0.02	67.1±0.82	1.068±0.22	5.49±1.22	0.96±0.30	0.96±0.30
17	5.09±0.	0.21±0.0	0.31±0.0	85.4±2.45	1.192±0.24	6.1±0.47	0.76±0.05	0.76±0.0

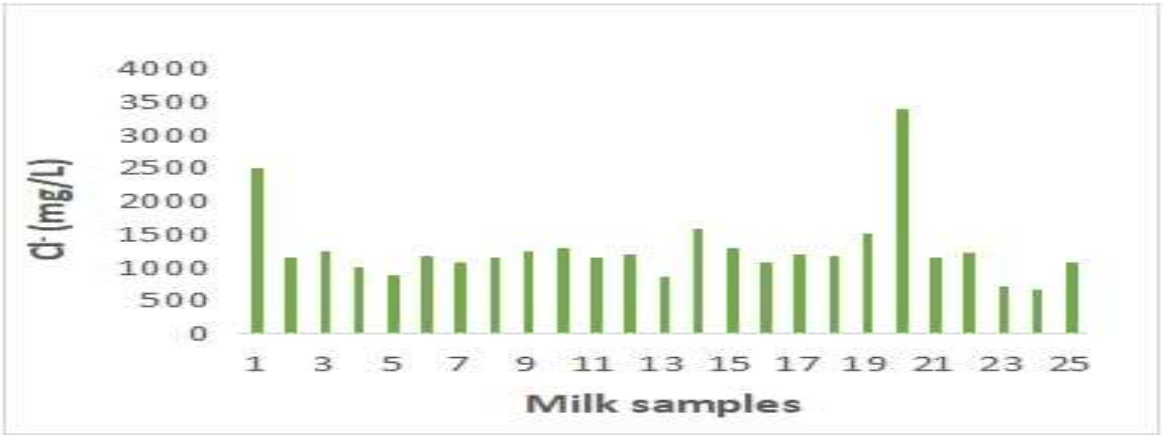
	41	1	4					5
18	5.49±0.49	0.83±0.04	0.53±0.03	85.4±0.82	1.174±0.22	9.76±1.85	0.73±0.19	0.73±0.19
19	5.63±0.16	0.56±0.02	0.48±0.03	292.8±1.63	1.513±0.42	12.81±0.47	1.84±0.05	1.84±0.05
20	3.85±0.41	0.7±0.04	0.51±0.04	24.4±1.63	3.382±0.72	9.76±1.03	2.7±0.05	2.7±0.05
21	5.13±0.16	0.72±0.04	0.48±0.03	115.9±2.45	1.157±0.29	6.71±0.47	1.11±0.17	1.11±0.17
22	4.06±0.41	0.58±0.02	0.48±0.03	292.8±0.82	1.21±0.08	7.93±1.17	1.41±0.17	1.41±0.17
23	4.55±0.12	0.83±0.02	0.5±0.04	48.8±2.45	0.721±0.18	10.37±0.47	1.28±0.05	1.28±0.05
24	5.52±0.41	0.69±0.04	0.48±0.08	274.5±0.82	0.658±0.08	7.93±0.47	1.53±0.27	1.53±0.27
25	4.85±0.12	0.7±0.02	0.5±0.04	48.8±4.08	1.068±0.30	6.1±0.90	0.97±0.14	0.97±0.14

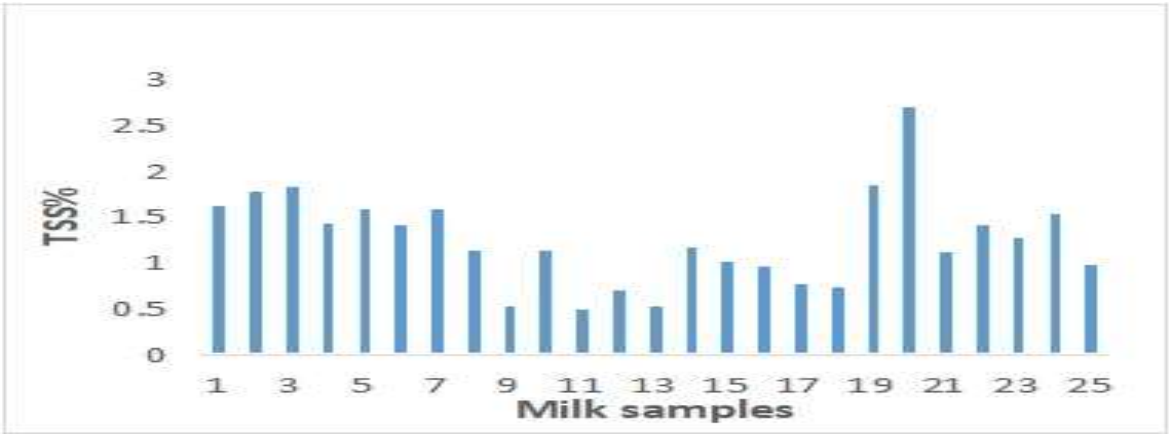


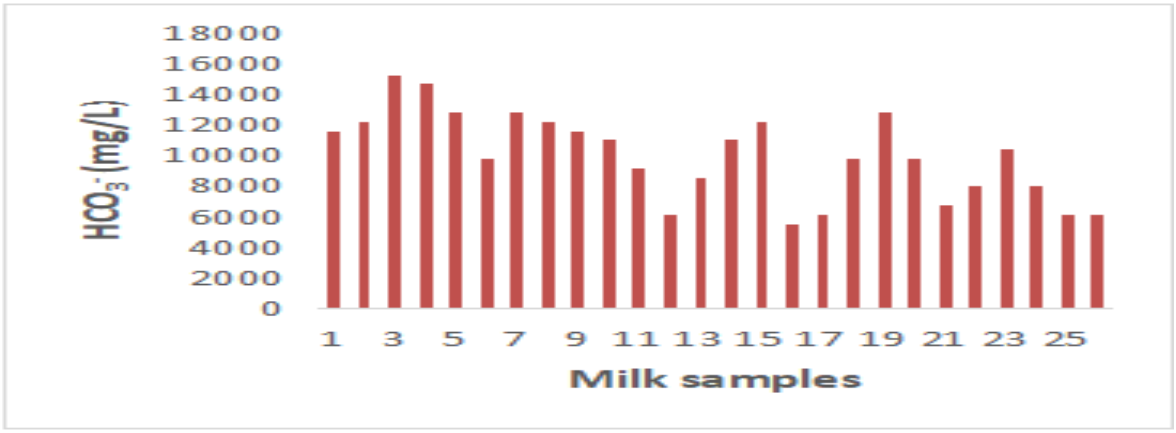


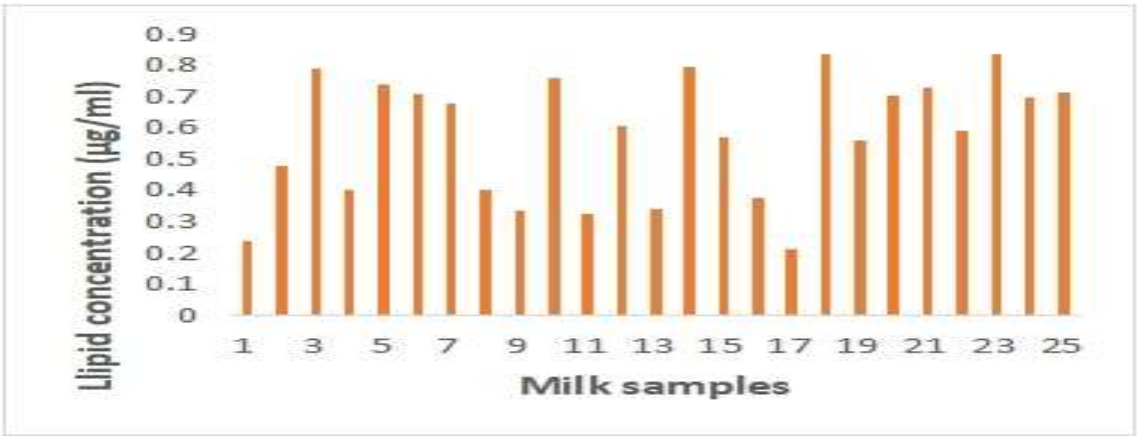

700
600
500
400
300
200
100
0
Na⁺ (mg/L)
1 3 5 7 9 11 13 15 17 19 21 23 25
Milk samples


6
5
4
3
2
1
0
K⁺ (mg/L)
1 3 5 7 9 11 13 15 17 19 21 23 25
Milk samples


4000
3500
3000
2500
2000
1500
1000
500
0
Cl⁻ (mg/L)
1 3 5 7 9 11 13 15 17 19 21 23 25
Milk samples


TSS%
3
2.5
2
1.5
1
0.5
0
1 3 5 7 9 11 13 15 17 19 21 23 25
Milk samples


HCO_3^- (mg/L)
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
1 3 5 7 9 11 13 15 17 19 21 23 25
Milk samples


Lipid concentration (µg/ml)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21 23 25
Milk samples

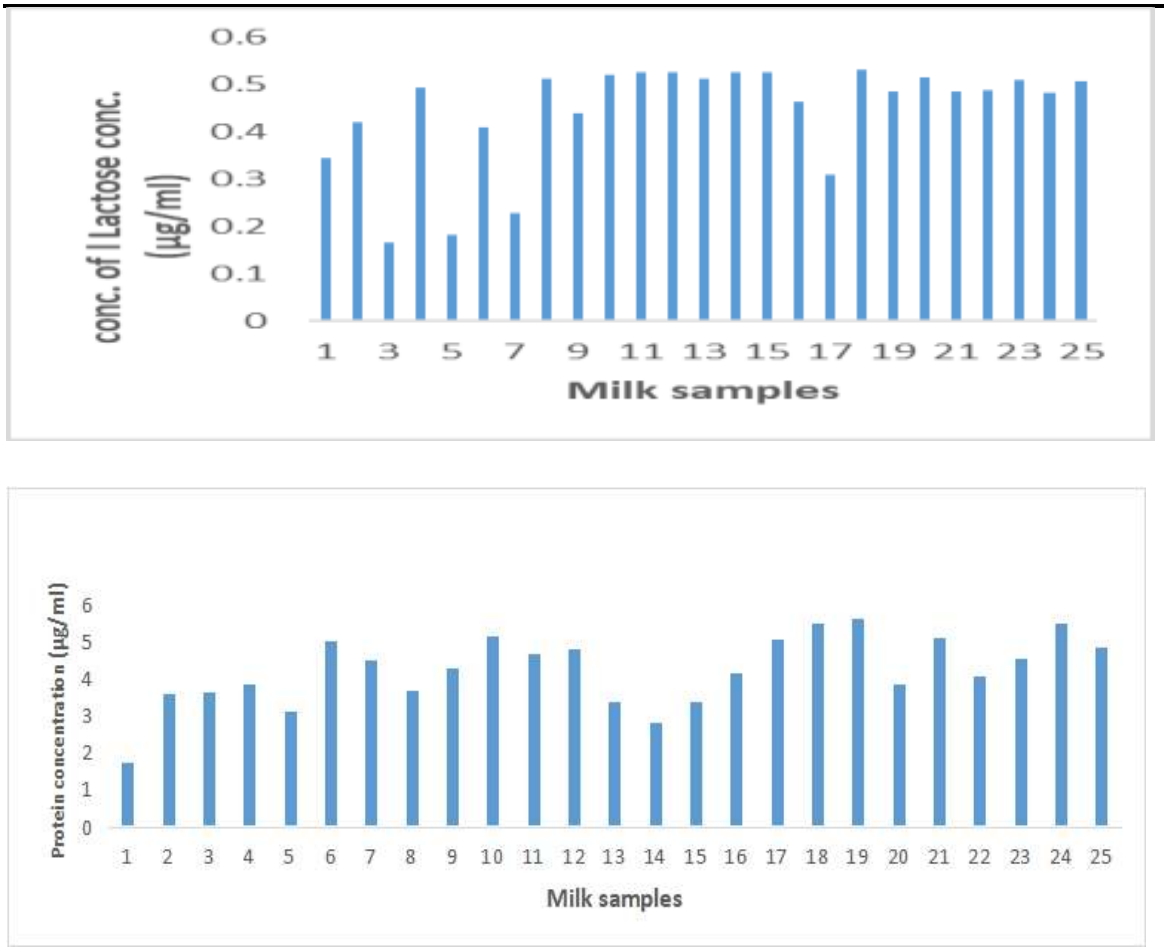


Figure 1: Physiochemical and biological properties of collected milk samples.

2- Identification of *Staphylococcus aureus*

In this study, 25 cow raw milk samples that were taken from different markets in Assiut almost 23 milk samples (92%) were provided the culture properties, biochemical assays that are specific to *S. aureus*. The bacterial count of *S. aureus* isolates in milk samples was presented in table (2), which pointes to the prevalence of *S. aureus* in milk samples.

3- Morphological Identification

The rounded and golden colonies was identified on Mannitol Salt Agar medium (MSA) as well as alter the color of media from light red to yellow (Figure 2).

A B

Figure 2: A: control medium, B: Growing of *S. aureus* on Mannitol Salt Agar medium after 24 hours of incubation at 37℃.

4- Biochemical properties of the isolated *Staphylococcus aureus.*

All *Staphylococcus aureus* isolates collected in this study fit *S. aureus* categorization as Gram (+) and cocci, as well as being able to ferment carbohydrate (lactose, sucrose and glucose) and all strains grow at 37 ^{o}C. The formation of bubbles upon adding hydrogen peroxide reagent to the bacterial isolates confirmed a positive catalase reaction, demonstrating the breakdown of H_2O_2 into oxygen and water. The catalase enzyme functions primarily to prevent the accumulation of toxic levels of hydrogen peroxide, and this reaction is used to distinguish the genus *Staphylococcus* from *Streptococcu*s species [46]. Additionally, a positive coagulase reaction was observed.

5. Prevalence of *Staphylococcus aureus.*

This study revealed high prevalence of *S. aureus* contamination in raw cow milk in Assiut, with an overall rate of 92%.

6- Antibiotic Susceptibility of *Staphylococcus aureus*

The disk diffusion method was employed to assess the susceptibility of seventeen *S. aureus* isolates to seven different antibiotics. The diameters of the inhibition zones were

measured and interpreted according to the standards set by the Clinical and Laboratory Standards Institute (CLSI) [34] (Table 3).

Table: 3 Antibacterial activities of some drugs (expressed by inhibition zone diameters, in mm) against *Staphylococcus aureus* isolates collected from cow milk.

Bacterial isolates	CD2(mm)	E15(mm)	AMC30(mm)	FOX30 (mm)	AK30(mm)	KZ30(mm)	CN10(mm)
1	20	12	22	10	30	-	30
2	8	12	-	12	-	-	7
3	22	15	26	12	22	-	40
4	13	12	20	-	25	-	30
5	15	15	20	7	22	-	28
6	15	10	20	8	20	-	20
7	18	8	15	-	25	-	30
8	-	10	18	-	22	-	30
9	12	8	12	6	25	-	30
10	15	-	16	15	22	-	21
11	ND						
12	12	8	16	8		-	30
13	Negative						
14	8	7	25	15	32	-	35
15	10	-	20	12	25	-	35
16	ND						
17	ND						
18	-	-	18	8	20	-	22
19	ND						
20	Negative						
21	-	15	13	-	26	-	30
22	15	12	18	7	21	-	30
23	18	10	16	-	25	-	30
24	ND						
25	ND						

ND= Not Determined

The antimicrobial discs used include clindamycin CD (2µg), cephazolin KZ (30 µg), amikacin AK (30 µg), amoxicillin/Clavulanic acid AMC (30µg), gentamycin CN (10 µg), cefoxitin FOX (30 µg), erythromycin E (15µg).

Multidrug Resistance

In this study, the antibiotic susceptibility of *Staphylococcus aureus* isolates was tested against several antibiotics., all *S. aureus* isolates (100%, 17/17) exhibited resistance to at least one antimicrobial class, specifically cephazolin (30µg). Additionally, 47% (8/17) of the isolates demonstrated multidrug resistance. The most commonly observed antimicrobial class associated with multidrug resistance was cephazolin. Resistance to other antibiotics, including erythromycin, cefoxitin, amikacin, clindamycin, and amoxicillin/clavulanic acid, was also recorded the following results were observed

regarding the resistance patterns of the isolates: 1. Cephazolin (30 µg): 100% of the isolates were resistant to cephazolin, making it the most widely resisted antibiotic among the tested strains. 2. Cefoxitin (30 µg): 21% of the isolates showed resistance to cefoxitin. 3. Erythromycin (15 µg): 13% of the isolates were resistant to erythromycin. 4. Clindamycin (2 µg): 13% of the isolates exhibited resistance to clindamycin. 5. Amoxicillin/Clavulanic acid (30 µg): A percentage of isolates (approximately 5%) were resistant to amoxicillin/clavulanic acid. 6. Amikacin (30 µg): approximately ٨.6% of the isolates were resistant to Amikacin. 7. Gentamicin (10 µg): Gentamicin was found to be the most effective antibiotic against the *S. aureus* isolates, with the lowest resistance observed

Table 4: Antibiotic resistance patterns of *S. aureus* isolates and the resistance rates for each drug.

Antibiotics	NO. R	R%
Clindamycin (2µg)	3	13
Erythromycin (15µg)	3	13
AmoxycillinL (30µg)	1	4.34
Cefoxitin (30µg)	5	21.73
Amikacin (30µg)	2	8.69
Cephazolin (30µg)	23	100
Gentamicin (10µg)	0	0
R = Resistance NO. R= Number of Resistance		

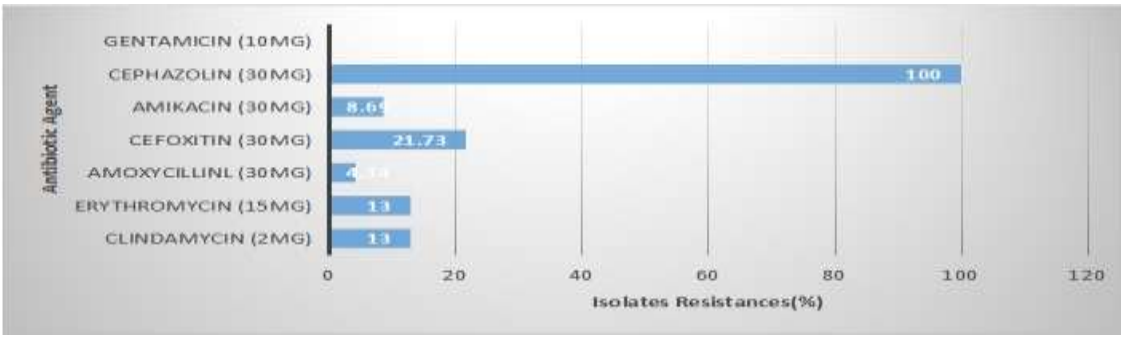


Figure 3: Antibiotic resistance of *S. aureus* isolates to various drugs.

DISCUSSION

The results of this study revealed that, pH values of milk samples were slightly acidic to neutral, ranging from 4.54 - 7. Total Soluble Salt (TSS%) and mineral (Na, K, Ca, Mg, Cl, HCO_3), components of milk samples numbers (15, 16, 21) were found to be nearly similar to the permissible standard levels [36, 37]. Although, in other samples, they were either lower in samples 1, 8, 16, and 23 and higher samples 7,6 ,19.

The result of the analysis of 25 cow raw milk samples collected from dairy markets in Assiut, Egypt show major variability in both physicochemical and microbiological properties. These differences can reflect various factors, including milk quality, handling practices and microbial contamination. These findings integrate that obtained in other studies to highlight common trends and discrepancies in milk quality assessments [38, 39]. The pH values of the cow raw milk samples ranged from 4.54 to 7, indicating that some of the milk was slightly acidic, while others were closer to neutral. Fresh milk typically has a pH ranged from 6.6 to 6.8. A lower pH values in some samples, may indicate that microbial fermentation or spoilage and it could be a direct result of inadequate storage conditions, as microbial activity increases over time. Deviation from neutral may be because milk, being non-fresh and stored at an improper temperature, undergoes lactose fermentation, leading to the production of acid. The results in table (2) showed that pH were 6.39, 6.32 and 6.195, respectively, and this is in consistent with the results of pH in previous literatures [38, 39], that reported similar values in milk obtained from markets, in Ethiopian.

On the other hand, a pH closer to neutral (7) is suggested to be due to good quality milk, which is in agreement with the findings of Jones [40], who reported that milk with a pH near 7 is likely to be fresh and free from excessive bacterial growth. Also, these results underline the significance of pH as an indicator of milk quality and safety. TSS values in the milk samples ranged from 0.49% to 2.70%. Electrical conductivity (EC) is often correlated with the amount of dissolved minerals and salts in milk. High EC and TSS are frequently associated with adulteration, particularly with the addition of salt or water. Furthermore, the high levels of TSS are an indicator of milk dilution or intentional adulteration, which may be used to increase milk volume or mask spoilage (25). The data

illustrated in table (2) reported that, some milk samples had higher TSS and EC values, and this supports the concerns raised in other studies about potential milk adulteration. Moreover, the high values of EC and TSS could also reflect the addition of non-dairy substances, such as water, to increase milk volume, which compromises its quality and safety. These results emphasize the need for regular monitoring of EC and TSS as a standard practice in quality control within the dairy industry. The concentrations of sodium (367–598 mg/L), potassium (1.06–4.84 mg/L), magnesium (24.4–311.1 mg/L), and calcium (70–430 mg/L) varied among the samples. Several studies have highlighted the role of minerals in determining milk quality and nutrition. For example, sodium and potassium levels in milk can be indicative of contamination or adulteration, such as the addition of salt to mask spoilage or increase milk volume. Other minerals, calcium and magnesium concentrations were highly variable depending on the cow's diet, region, and breed.

The protein content of the milk samples ranged from 1.7 to 5.63 mg/L. This finding is consistent with the results reported by Dehinenet [41], although it is lower than the values observed by Derese [42], Kebede and Meskel [43], and Aysheshim et al [44] in cow milk samples from different regions. The observed variation could be attributed to factors such as genetic differences in the cows, as well as environmental influences like feed, lactation stage, milking intervals, seasonal variations, and geographic location. Nevertheless, the protein content found in this study is within the quality standards set in Ethiopia, where the FDA recommends a minimum milk protein content of 2.73% [45]**.**

The formation of bubbles upon adding hydrogen peroxide reagent to the bacterial isolates confirmed a positive catalase reaction, demonstrating the breakdown of H_2O_2 into oxygen and water. The catalase enzyme functions primarily to prevent the accumulation of toxic levels of hydrogen peroxide, and this reaction is used to distinguish the genus *Staphylococcus* from *Streptococcu*s species [46]. Additionally, a positive coagulase reaction was observed. After incubating the bacterial cultures in plasma for 4 to 18 hours, clot formation was detected, which helps differentiate *Staphylococcus aureus* from other *Staphylococcus* species [47]. The coagulase enzyme facilitates the conversion of fibrinogen to fibrin, leading to clot formation in plasma or blood. This enzyme is believed

to contribute to abscess formation in host tissues and plays a role in the pathogen's ability to induce severe sepsis [48].

This study revealed high prevalence of *S. aureus* contamination in raw cow milk in Assiut, with an overall rate of 92%. A global analysis of *S. aureus* contamination rates in raw cow milk reported a comparable pooled prevalence of 33.5% [49]. Similarly, a systematic review from China showed a 61.7% isolation rate of *S. aureus* in raw cow milk [50]. A study conducted in Iran also reported a high prevalence of *S. aureus* (16%) in cow milk, with a rate of multidrug-resistant strains at 47.1% [51]. Consistent with our findings, previous studies indicate that the rate of bacterial contamination increases as the production chain progresses [52]. The high prevalence of *S. aureus* observed in this study (92%) may vary due to factors such as geographical location, farming practices, sample size, and hygiene standards in farms and milk collection centers [53]. Selective media like Mannitol Salt Agar (MSA) has proven effective in identifying *S. aureus*, thus improving detection chances. To mitigate milk-borne hazards, improving hygiene practices for food handlers, equipment, and implementing cold chain facilities is essential [53]. Effective control of *S. aureus* growth in dairy products is critical for both consumer safety and the profitability of small-scale cow farming, as well as for the dairy industry. In other context, 16.78% of respondents in this study reported washing cow udders before milking, which is lower than the 28.21% to 58.9% observed in other studies [15]. *S. aureus* is typically found on the udder surface of infected cows and can be transmitted between udder quarters during milking. The absence of gloves during milking increases the risk of spreading contagious pathogens between cows. Poor hygiene practices during milking can elevate the likelihood of intra-mammary infections caused by *S. aureus* [54]. Post-milking contamination of the liner is also a significant risk factor, as pathogens are transmitted via milker hands, utensils, towels, and contaminated floors. Workers in dairy farms are an important source of *S. aureus* contamination, highlighting the need for improved infection control measures. In line with our results, the incidence of *S. aureus* in this study was higher than that reported in previous studies [55, 56]. The elevated prevalence in intensively managed cows may be due to the lack of bedding materials, inadequate separation of cows, and the unsanitary conditions in barns [57]. Such conditions increase the risk of *S. aureus* contamination in milk. Moreover, *S. aureus* is

well adapted to survive in the udder and is highly contagious, spreading during milking and posing a risk to other cows. Large herds, often managed intensively, have a higher risk of exposure to infection due to increased stocking density [58]. The total count of *S. aureus* in each microbial-positive cow raw milk sample ranged from $2x10^2$ to $78x10^4$ CFU/ml, which is relatively low compared to other studies [15]. According to ISO 6888 standards [59], such milk is considered unsatisfactory, as concentrations above 10^5 CFU/ml of enterotoxigenic *S. aureus* can pose a significant health risk due to the release of enterotoxins [60].

In this study, the antibiotic susceptibility of *Staphylococcus aureus* isolates was tested against several antibiotics., all *S. aureus* isolates (100%, 17/17) exhibited resistance to at least one antimicrobial class, specifically cephazolin (30µg). Additionally, 47% (8/17) of the isolates demonstrated multidrug resistance. The most commonly observed antimicrobial class associated with multidrug resistance was cephazolin. Resistance to other antibiotics, including erythromycin, cefoxitin, amikacin, clindamycin, and amoxicillin/clavulanic acid, was also recorded the following results were observed regarding the resistance patterns of the isolates: 1. Cephazolin (30 µg): 100% of the isolates were resistant to cephazolin, making it the most widely resisted antibiotic among the tested strains. 2. Cefoxitin (30 µg): 21% of the isolates showed resistance to cefoxitin. 3. Erythromycin (15 µg): 13% of the isolates were resistant to erythromycin. 4. Clindamycin (2 µg): 13% of the isolates exhibited resistance to clindamycin. 5. Amoxicillin/Clavulanic acid (30 µg): A percentage of isolates (approximately 5%) were resistant to amoxicillin/clavulanic acid. 6. Amikacin (30 µg): approximately ٨.6% of the isolates were resistant to Amikacin. 7. Gentamicin (10 µg): Gentamicin was found to be the most effective antibiotic against the *S. aureus* isolates, with the lowest resistance observed. These findings contrast with previous studies, which reported lower resistance to amoxicillin, ranging from 30.8% to 68.29% [61, 62, 63, 64]. These findings highlight the significant antimicrobial resistance present in the *S. aureus* isolates, particularly against common antibiotics used in veterinary medicine. The high resistance to cephazolin, amoxicillin/clavulanic acid, and other drugs calls for increased attention to antimicrobial stewardship and the development of alternative treatment strategies in dairy farming.

A limitation of this study was the lack of sampling from environmental sources and personnel due to resource constraints. Additionally, the sample size may limit the generalizability of the results. Future studies should include environmental samples and further characterize *S. aureus* in both milk and the farm environment for a more comprehensive understanding of the contamination risks.

CONCLUSION

This study revealed that the milk quality in the investigated area was suboptimal, as evidenced by the high total bacterial count (TBC). This suggests that there are significant gaps in sanitation practices at various stages, from production to consumption. To improve milk safety, it is crucial to implement proper sanitary measures, which include ensuring appropriate handling of cows, maintaining personnel hygiene, using sanitized milking and processing equipment, and enhancing the overall environment in which milk is produced and handled. The observed poor bacteriological quality also highlights the need for further investigation into the health status of the animals, particularly in relation to mastitis, and the potential impact of containers used in milk storage on microbial contamination. *Staphylococcus aureus* is a highly pathogenic bacterium known to cause mastitis in dairy animals. One of the major concerns with *S. aureus* is its ability to quickly develop resistance to a wide range of antibiotics, making it a serious issue for dairy farming. Therefore, it is recommended that rigorous sanitation practices be implemented across all stages of milk production and processing. Additionally, regular health monitoring of dairy animals, particularly for mastitis, and appropriate treatment protocols should be adopted. Further research is needed to better understand the role of environmental factors and containers in milk contamination, as well as to monitor the development of antibiotic resistance in *S. aureus* and its implications for dairy farming.

REFERENCES

[1] S.P. Oliver, B.M. Jayarao, R.A. Almeida, Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications, *Foodborne Pathogens and Disease*, 2 (2005).

[2] J. Owusu-Kwarteng, F. Akabanda, D. Agyei, L. Jespersen, Microbial safety of milk production and fermented dairy products in Africa, *Microorganisms*, 8, 1–24 (2020).

[3] Food and Agriculture Organization of the United Nations (FAO), *Milk and dairy products in human nutrition*, Rome, 2013.

[4] A.F. Haag, J.R. Fitzgerald, J.R. Penadés, *Staphylococcus aureus* in animals, *Microbiol. Spectrum*, 7 (2019).

[5] S.Y.C. Tong, J.S. Davis, E. Eichenberger, T.L. Holland, V.G. Fowler, *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management, *Clin Microbiol Rev*, 623 (2015).

[6] A. Rajkovic, J. Jovanovic, S. Monteiro, M. Andjelkovic, B. Sas, Foubert A et al., Detection of toxins involved in foodborne diseases caused by Gram-positive bacteria, *Compr Rev Food Sci Food Saf*, 2020:1605–1657 (2019).

[7] J.Y. Park, K.S. Seo, *Staphylococcus aureus*, in: Doyle MP, Diez-Gonzalez F, Hill C (Eds.), *Food Microbiology: Fundamentals and Frontiers*, 5th edn, ASM Press, Washington, DC, 555–584 (2019).

[8] J.A. Hennekinne, M.L. De Buyser, S. Dragacci, *Staphylococcus aureus* and its food poisoning toxins: Characterization and outbreak investigation, *FEMS Microbiol Rev*, 36:815–836 (2012).

[9] A. Fetsch, M. Contzen, K. Hartelt, A. Kleiser, S. Maassen, J. Rau et al., *Staphylococcus aureus* food-poisoning outbreak associated with the consumption of ice-cream, *Int J Food Microbiol*, 187:1–6 (2014).

[10] Y. Motarjemi, G.G. Moy, P.J. Jooste, L.E. Anelich, Milk and dairy products, in: *Food Safety Management* (2014).

[11] C. Verraes, W. Claeys, S. Cardoen, G. Daube, Z.L. De, H. Imberechts, A review of the microbiological hazards of raw milk from animal species other than cows, *Int Dairy J*, 39:121–130 (2014).

[12] S. Johler, D. Weder, C. Bridy, M. Huguenin, L. Robert, J. Hummerjohann et al., Outbreak of staphylococcal food poisoning among children and staff at a Swiss boarding school due to soft cheese made from raw milk, *J Dairy Sci*, 98:2944–2948 (2015).

[13] K. McMillan, S. C. Moore, C. M. McAuley, N. Fegan, E. M. Fox, Characterization of *Staphylococcus aureus* isolates from raw milk sources in Victoria, Australia, *BMC Microbiology*, 16(1):169 (2016).

[14] H. Jørgensen, T. Mørk, L. M. Rørvik, Occurrence of *Staphylococcus aureus* on a farm with small-scale production of raw milk cheese, *J Dairy Sci*, 88(11):3810–3817 (2005).

[15] E.Z. Gebremedhin, A.B. Ararso, B.M. Borana, K.A. Kelbesa, N.D. Tadese, L.M. Marami, E.J. Sarba, Isolation and identification of *Staphylococcus aureus* from milk and milk products, associated factors for contamination, and their antibiogram in Holeta, Central Ethiopia, *Vet. Med. Int.*, 6544705 (2022).

[16] V. Peton, L.Y. Le, *Staphylococcus aureus* in veterinary medicine, *Infect Genet Evol*, 21:602–615 (2014).

[17] S. Kırmusaoğlu, S. Enany, (Eds.), MRSA and MSSA: The mechanism of methicillin resistance and the influence of methicillin resistance on biofilm phenotype of *Staphylococcus aureus*, in: *Antibiotic Resistance in Staphylococcus aureus*, 25–41 (2017).

[18] M. Vestergaard, D. Frees, H. Ingmer, Antibiotic resistance and the MRSA problem, *Microbiol Spectrum*, 7(2):7.2.18, doi: 10.1128/microbiolspec.GPP3-0057-2018 (2019).

[19] P. Nandhini, et al., Recent developments in Methicillin-Resistant *Staphylococcus aureus* (MRSA) treatment: A review, *Antibiotics*, 11(5):606, doi:10.3390/antibiotics11050606 (2022).

[20] C.H. Lowry, N.K. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, *J Biol Chem*, 193:256–275 (1951).

[21] S.K. Mishra, W.I. Suh, W. Farooq, M. Moon, A. Shrivastav, M.S. Park, J-W Yang, Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method, *Bioresour Technol*, 155:330–333 (2014).

[22] A. Jain, R. Jain, S. Jain, Quantitative analysis of reducing sugars by 3, 5-dinitrosalicylic acid (DNSA method), in: *Basic Techniques in Biochemistry, Microbiology and Molecular Biology*, Springer Protocols Handbooks, Humana, New York, NY, https://doi.org/10.1007/978-1-4939-9861-6_43 (2020).

[23] C.M. Johnson, A. Ulrich, Analytical methods for plant analysis, U.S. Dept. Agric., Calif. Univ. Agric. Inform. Bull., 766 (1959).

[24] C.H. Williams, M.E. Twine, A flame photometric method for sodium, potassium, and calcium, in: *Modern Methods of Plant Analysis*, K. Paech, M.V. Tracey (Eds.), Springer-Verlag, Berlin, Vol. 5, 535 (1960).

[25] M.L. Jackson, *Soil Chemical Analysis*, Prentice-Hall, Englewood Cliffs, New York, 498 (1958).

[26] M.L. Jackson, *Soil Chemical Analysis*, Constable and Co. Ltd., London (1958).

[27] M.L. Jackson, *Soil Chemical Analysis*, Prentice-Hall of India, Private Limited, New Delhi, 498 (1967).

[28] A. Collins, P. Lynes, *Microbiological Methods*, 6th ed., Butler and Tanner, Somerset, Great Britain, 233–241 (1989).

[29] P.J. Quinn, B.K. Markey, M.E. Carter, W.J. Donnelly, F.C. Leonard, *Veterinary Microbiology and Microbial Disease*, Blackwell Science (2002).

[30] E.H. Marth (Ed.), *Standard Methods for the Examination of Dairy Products*, American Public Health Association, Washington, DC, 416 p (1978).

[31] B.R. Narender, P. Ravi, A.S. Sunder, V. Mallikarjun, Isolation and characterization of bacteriocins from fermented foods and probiotics, *Int. J. Pharma Bio Sci.*, 1 (2010) 1–6.

[32] G. Land, M.R. McGinnis, J. Staneck, A. Gaston, Aerobic pathogenic *Actinomycetales*, in: *Manual of Clinical Microbiology*, 5th ed., Am. Soc. Microbiol., Washington, D.C., USA, 340–360 (1991).

[33] A.W. Bauer, W.M.M. Kirby, J.C. Sherris, and M. Turck, Antibiotic susceptibility testing by a standardized single disk method, *Am. J. Clin. Pathol.*, 45(4): 493–496 (1966).

[34] Clinical and Laboratory Standards Institute (CLSI), *M100 Performance Standards for Antimicrobial Susceptibility Testing*, 31st ed., Wayne, PA, 2021.

[35] J. McFarland, The Nephelometer: An instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines, *J. Am. Med. Assoc.*, 49 (1907) 1176–1178.

[36] F. Gaucheron, The minerals of milk, *Reprod. Nutr. Dev.*, 45(4), 473–483 (2005).

[37] C.D. Hunt, F.H. Nielsen, Nutritional aspects of minerals in bovine and human milks, in: P.L.H. McSweeney, P.F. Fox (Eds.), *Advanced Dairy Chemistry Volume 3: Lactose, Water, Salts, and Minor Constituents*, Springer, Heidelberg, 392–398 (2009). doi:10.1007/978-0387-84865-5_10.

[38] G.T. Debela, M. Eshetu, A. Regassa, Physico-chemical qualities of raw cow milk in Ethiopia: The case of Borana zone, Yabello District, *Glob. J. Dairy Farm. Milk Prod.*, 3(2), 086–091 (2015).

[39] T. Gemechu, F. Beyen, M. Eshetu, Physical and chemical qualities of raw cow milk produced and marketed in Shashamane town, Southern Ethiopia, *J. Food Agric. Sci.*, 5(2), 7–13 (2015).

[40] A. Jones, B. Smith, C. Taylor, The relationship between milk pH and freshness indicators, *J. Dairy Sci.*, 105(3), 789–795 (2021).

[41] G. Dehinenet, H. Mekonnen, M. Ashenafi, G. Emmanuelle, Determinants of raw milk quality under a smallholder production system in selected areas of Amhara and Oromia National states, Ethiopia, *Agric Biol. J.*, 4(1), 84–90 (2013).

[42] T. Derese, Present situation of urban and peri-urban milk production and quality of raw milk produced in West Shoa Zone, Oromia Region, Ethiopia, Thesis, Haramaya University, Ethiopia (2008).

[43] H. Kebede, D.H. Meskel, Determination of adulteration and composition of raw milk sold in Hossana town, South Ethiopia, *Dairy Vet Sci J.*, 6(5), 001–007 (2018).

[44] B. Aysheshim, B. Fekadu, E. Mitiku, Chemical composition and microbial quality of cow milk in urban and peri-urban areas of Dangila town, Western Amhara Region, Ethiopia, *Glob. J. Dairy Farm Milk Prod.*, 3(1), 081–085 (2015).

[45] H. Raff, Market implications of changing fat content of milk and dairy products, fat content and composition of animal products, *J. Food Sci. Technol.*, 5(2), 6–17 (2011).

[46] F. Götz, T. Bannerman, K.-H. Schleifer, The genera *Staphylococcus* and *Macrococcus*, in: *The Prokaryotes*, 5th ed., 2006.

[47] C. James, S. Natalie, *Microbiology: A Laboratory Manual*, Pearson Education (2014). Available from: https://lib.hpu.edu.vn/handle/123456789/28998.

[48] M. McAdow, D.M. Missiakas, O. Schneewind, *Staphylococcus aureus* secretes coagulase and von Willebrand factor binding protein to modify the coagulation cascade and establish host infections, *J. Innate Immun.*, 4(2), 141–148 (2012).

[49] Q. Ou, J. Zhou, D. Lin, C. Bai, T. Zhang, J. Lin et al., A large meta-analysis of the global prevalence rates of *S. aureus* and MRSA contamination of milk, *Crit. Rev. Food Sci. Nutr.*, 1–16 (2017).

[50] X. Kou, H. Cai, S. Huang, Y. Ni, B. Luo, H. Qian et al., Prevalence and characteristics of *Staphylococcus aureus* isolated from retail raw milk in Northern China, *Front Microbiol.*, 1–13 (2021).

[51] E. Rahimi, F. Alian, Presence of enterotoxigenic *Staphylococcus aureus* in cow, camel, sheep, goat, and buffalo bulk tank milk, *Vet Arh*, 83:23–30 (2013).

[52] Y. Titouche, A. Hakem, K. Houali, T. Meheut, N. Vingadassalon, D. Salmi et al., Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) ST8 in raw milk and traditional dairy products in the Tizi Ouzou area of Algeria, *J. Dairy Sci.*, 102:6876–6884 (2019).

[53] T. Enquebaher, S. Siv, R. Knut, S. Taran, A.N. Judith, *Staphylococcus aureus* and other *Staphylococcus* species in milk and milk products from Tigray Region, Northern Ethiopia, *Afr. J. Food Sci.*, 9, 567–576 (2015).

[54] C. Azevedo, D. Pacheco, L. Soares et al., Prevalence of contagious and environmental mastitis-causing bacteria in bulk tank milk and its relationships with milking practices of dairy cattle herds in São Miguel Island (Azores), *Trop. Anim. Health Prod.*, 48(2), 451–459 (2016).

[55] B. Seyoum, H. Kefyalew, B. Abera, N. Abdela, Prevalence, risk factors, and antimicrobial susceptibility test of *Staphylococcus aureus* in bovine cross-breed mastitic milk in and around Asella town, Oromia Regional State, Southern Ethiopia, *Acta Trop.*, 177, 32–36 (2018).

[56] S. Regasa, S. Mengistu, A. Abraha, Milk safety assessment, isolation, and antimicrobial susceptibility profile of *Staphylococcus aureus* in selected dairy farms of

Mukaturi and Sululta Town, Oromia Region, Ethiopia, *Veterinary Med. Int.*, 2019, Article ID 3063185, 11 pages (2019).

[57] R. Abebe, H. Hatiya, M. Abera, B. Megersa, K. Asmare, Bovine mastitis: prevalence, risk factors, and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia, *BMC Vet. Res.*, 12(1), 270 (2016).

[58] M. Radostits, C. Gay, K. Hinchcliff, *Veterinary Medicine: A Textbook of Diseases of Cattle, Horse, Sheep, Pig, and Goats*, Elsevier, Amsterdam, Netherlands (2007).

[59] International Organization for Standardization (ISO), ISO 6888: Microbiology of food and animal feeding stuff—horizontal method for the enumeration of coagulase positive staphylococci (*Staphylococcus aureus* and other species)—Part 1: Technique using Baird-Parker agar medium, Geneva, Switzerland (1999).

[60] Public Health England, Enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species), in: *National Infection Service Food Water and Environmental Microbiology Standard Method*, 1–23, Crown, New York, NY, USA (2016).

[61] D.G. Daka, S.G. Silassie, D. Yihdego, Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia, *Ann. Clin. Microbiol. Antimicrob.*, 11(1), 26 (2012).

[62] B. Abera, D. Lemma, I. Iticha, Study of bovine mastitis in Asella government dairy farm of Oromia regional state, Southeastern Ethiopia, *Internat. J. Current Res. Acad. Rev.*, 1, 134–145 (2013).

[63] B.B. Asiimwe, R. Baldan, A. Trovato, D.M. Cirillo, Prevalence and molecular characteristics of *Staphylococcus aureus*, including methicillin-resistant strains, isolated from bulk can milk and raw milk products in pastoral communities of South-West Uganda, *BMC Infect. Dis.*, 17(1), 422 (2017).

[64] R. Hazari, S. Hirpurkar, C. Sannat, Antimicrobial drug resistance of *Staphylococcus aureus* from clinical bovine mastitis in Chhattisgarh state, *e Pharma Innovation Int. J.*, 7(8) (2018).