2026, Scienceline Publication
World's Veterinary Journal
World Vet J, 16(1): 159-170.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2026.wvj15
PII: S232245682600015-16

Microbiological Quality and Adulterants of Cattle Milk in Egypt

Mona Mohamed Hassan Soliman[1]*, Azza Sayed Mohammed Abuelnaga[1], Khaled Abd El-Hamid Abd EL-Razik[2], Mai Mohamed Kandil[1], and Eman Ramadan Hassan[3]

[1]*Department of Microbiology and Immunology, National Research Centre, Giza, Egypt*
[2]*Department of Animal Reproduction, National Research Centre, Giza, Egypt*
[3]*Department of Poultry and Rabbits* Diseases, National Research Centre, Giza, Egypt

*Corresponding author's Email: mona_nrc.micro@yahoo.com

ORIGINAL ARTICLE
Received: January 06, 2026
Revised: February 08, 2026
Accepted: February 29, 2026
Published: March 25, 2026

ABSTRACT

Milk is a rich, highly nutritious food; therefore, it is essential to ensure its purity by eliminating harmful microbes and adulterants that could affect public health. This cross-sectional, laboratory-based analytical study aimed to evaluate the hygienic quality of milk and detect chemical adulterants, with a focus on differentiating buffalo milk from cow milk by utilizing PCR to detect cow and buffalo DNA. A total of 110 milk samples, including raw milk, pasteurized milk, and milk powder from milk shops and dairy companies in Cairo and Giza governorates in Egypt, were collected. The present findings revealed that raw milk exhibited the highest levels of standard plate count ($7 \times 10^5 \pm 1.7 \times 10^6$ CFU/mL), *Staphylococcus aureus* ($1.9 \times 10^3 \pm 2.9 \times 10^3$ CFU/mL), coliforms ($2.8 \times 10^2 \pm 3.1 \times 10^2$ CFU/mL), and *Escherichia coli* (*E. coli*) O157 ($7.4 \times 10 \pm 7.8 \times 10$) and yeast ($1.7 \times 10^2 \pm 1.7 \times 10^2$ CFU/mL), indicating poor hygiene and presenting significant public health concerns. Pasteurized milk and milk powder exhibited superior but variable microbial quality, although mold contamination levels remained consistent across all milk sample types. Sugar, soap, starch, salicylic acid, and formalin were detected at 2%, 6%, 16%,20%, and 6% in raw milk, respectively. Starch was found at 2% and 10% in pasteurized milk and milk powder, and salicylic acid was detected at 10% in milk powder. In the current study, 40% of the raw milk was cattle milk, 50% was buffalo milk, and 10% was mixed milk. In addition, 50% of the pasteurized milk was from cows, 26% from buffalo, and 24% was mixed milk from cows and buffalo. Milk powder was 100% from cows. The current results indicated that although pasteurized milk and milk powder are safer choices, raw milk poses notable chemical and microbiological risks, underscoring the need for stronger regulations to reduce milk contamination.

Keywords: Adulteration, Milk, PCR, Total bacterial count, Total fungal count

INTRODUCTION

Milk is a distinctive component of the human diet, containing numerous micro- and macronutrients and serving as an appropriate medium for the proliferation of various microbes. Furthermore, despite the significant impact of the dairy industry on humans, particularly children and newborns, it also plays an important role in the world economy (Ibrahim et al., 2022; Xue et al., 2023). The safety and quality of milk and dairy products depend on proper hygiene and sanitation protocols, milk-handling practices, farm management, milking procedures, storage and transportation methods, animal health, and the water used to clean milk equipment, udders, teats, and related surfaces. These are the principal risk factors that mitigate microbial contamination of milk and safeguard consumers and the public from milk-borne diseases (Garedew et al., 2012; Deddefo et al., 2023). Milk and milk-derived products have significant potential to cause serious food-borne diseases in underdeveloped countries. Raw milk and its derivatives may contain several pathogenic bacteria, including *Escherichia coli (E. coli)*, *Staphylococcus aureus (S. aureus)*, *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes*, which may pose significant health risks upon consumption (Gebremichael et al., 2024). The relative abundance of yeasts and molds can differ significantly, with yeasts often predominating, although molds are found in different ecosystems (Abuelnaga et al., 2022).

When milk is not properly pasteurized, its microbial count can increase. To make milk safe and extend its shelf life, dairy businesses use heat treatment. Since most Egyptian milk is ultra-high-temperature (UHT) sterilized, many customers consider it expensive and prefer buying raw milk (Ahmed et al., 2022).

Milk adulteration is a worldwide concern, especially in developing countries. Unfortunately, mixing milk with other dangerous chemicals can lead to a wide range of health issues for customers. Quality and safety are typically negatively impacted. Adulteration of milk is a multi-step process that starts with the animals' owners and moves through the

To cite this paper: Soliman MMH, Abuelnaga ASM, Abd EL-Razik KAE-H, Kandil MM, and Hassan ER (2026). Microbiological Quality and Adulterants of Cattle Milk in Egypt. *World Vet. J.,* 16(1): 159-170. DOI: https://dx.doi.org/10.54203/scil.2026.wvj15

milkman, then to rural collection points, and finally to large processing factories. The main reason for adulteration is to maximize profit at the expense of people's health, driven by poverty, a lack of education, and weak law enforcement at all levels (Swar et al., 2021). Milk and its derivatives can be adulterated using different methods to increase volume and viscosity, enhance their physical appearance, and prolong their shelf life (Singh and Gandhi, 2015). Furthermore, common adulterants in milk include the addition of food additives such as vegetable protein, less expensive fats, starch, glucose, whey, salt, and water, which are known as economically motivated adulterants and pose serious health hazards (Tomaszewska-Gras, 2016). In addition, milk adulteration resulting from the addition of harmful chemicals, such as urea, formalin, detergents, boric acid, salicylic acid, hydrogen peroxide, and melamine, has significant adverse effects on consumer health (Salih and Yang, 2017). There are numerous serious health consequences related to consuming adulterated milk and milk products. For instance, milk's peroxides and detergents can cause gastrointestinal implications such as colitis and gastritis. Furthermore, excessive starch in dairy products can lead to severe diarrhea. Urea present in milk may cause renal failure. Additionally, consuming milk contaminated with carbonates and bicarbonates can interfere with the development and reproductive health of humans (Mohammed and Abdel-Aall, 2024).

Detection methods for milk adulteration need to be both rapid and accurate. Two approaches are employed to identify milk adulterants, including qualitative and quantitative techniques. The qualitative method relies on color detection via chemical interactions. Quantitative detection methods are more complicated, including polyacrylamide gel electrophoresis, polymerase chain reaction (PCR), liquid chromatography, and enzyme-linked immunosorbent assay (ELISA; Garcia et al., 2012). Immunological methods are generally preferred (Zachar et al., 2011); however, when assessing milk from closely related animals, they often produce false-negative results. Scientists are therefore looking for more robust and sensitive alternative methods. The most widely used and comprehensive DNA-based assay method is PCR, which can replicate genetic information even when DNA is severely damaged (Pirondini et al., 2010). Recent molecular techniques, such as the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay, can differentiate among milk types. Additionally, PCR-RFLP is more cost-effective than other methods, such as real-time PCR. The cytochrome b gene is said to be highly polymorphic and useful for differentiating between cow and buffalo milk (Abuelnaga et al., 2022).

This study aimed to assess the hygienic quality of milk and identify chemical adulterants, while specifically distinguishing high-cost buffalo milk from low-cost cow milk through the detection of cow and buffalo DNA using PCR.

MATERIALS AND METHODS

Ethical approval

The present study was conducted in accordance with the milk sampling and handling guidelines of the Microbiology and Immunology Department in the National Research Centre (Giza, Egypt) and followed all relevant institutional procedures for sample collection, transportation, and storage.

Sampling and study design

The present study used a cross-sectional, laboratory-based analytical method. A total of 110 milk samples were collected from the governorates of Cairo and Giza in Egypt, comprising 10 milk powder samples, 50 raw milk samples obtained from milk shops, and 50 pasteurized milk samples sourced from ten different dairy companies located in Cairo and Giza governorate from June 2023 to June 2024. The sample size, along with factors such as product availability, variability among different milk types, and logistical considerations, was determined based on similar studies by Alles et al. (2018), Ibrahim et al. (2021), Ndungi et al. (2021), and Chiarlone et al. (2025) to adequately represent the main milk types for microbiological quality assessment. Random sampling was used to obtain representative coverage of the available products in the study area. Raw milk samples (500 mL) were aseptically collected from milk shops into sterile, labeled containers after thorough mixing to ensure homogeneity. Pasteurized milk was obtained from unopened retail packages, and approximately 500 mL from each package was used for further analysis. Milk powder samples (250 g) were obtained from unopened commercial packages and aseptically transferred into sterile airtight containers and stored at 4 °C until analysis. All samples were collected in hygienic conditions and immediately transported to the microbiology and immunology department at the National Research Centre in Giza governorate for analysis.

Microbiological assessment of milk

Bacterial counts of milk

The tested milk samples underwent standard plate counts for total bacteria, coliforms, *E. coli*, *Staphylococcus* spp., and *Salmonella* spp. counts, following established microbiological procedures for milk and dairy products (Chye et al., 2004; Abuelnaga et al., 2022). *Escherichia coli* was identified by the presence of characteristic colonies on eosin

methylene blue (EMB) agar (Oxoid, UK), followed by biochemical analysis according to the procedures in Bergey's Manual of Determinative Bacteriology by Holt et al. (1994). The *Staphylococcus* spp. count was determined using the plate count method outlined in the Bacteriological Analytical Manual and standard dairy microbiology guidelines. The *Staphylococcus aureus* count was assessed using the direct plate count method on Baird-Parker agar, which was supplemented with egg yolk-tellurite emulsion (Oxoid, UK). This method is recommended for isolating and counting coagulase-positive staphylococci from milk and dairy products (Lancette and Bennett, 2001; Gebremedhin et al., 2022).

Fungal count in milk samples

A tenfold serial dilution of milk samples was prepared in sterile 0.1% (weight/volume) peptone solution. Tenfold serial dilutions were prepared by transferring 1 mL of the milk sample into 9 mL of sterile 0.1% (weight/volume) peptone water, then thoroughly mixing. Subsequent dilutions were created by transferring 1 mL from the previous dilution into another 9 mL of diluent. A duplicate Petri dish was filled with 1 mL of each prepared milk dilution. Then, each petri dish was filled with 10-20 ml of molten Sabouraud dextrose agar (SDA) (Oxoid, UK), cooled to 42-45°C. After gently rotating the media and dilutions in both clockwise and counterclockwise directions, the media were allowed to solidify at ambient temperature (Abuelnaga et al., 2022). For 3-5 days, plates were incubated in an incubator at 25°C with lids inverted to prevent contamination. Dull-white, creamy, yellow, pink, regular, and asymmetrical shapes were counted and considered a yeast colony, and yeast count/gram was approximated and noted. Additionally, the plates were stored at 25°C for 5-7 days in the reverse position for mold count. The plates were examined frequently during the incubation period for distinctive star-shaped structures characteristic of mold morphology, and the colonies were assigned numbers and indices (APHA, 1992).

Detection of chemical adulteration of milk

A total of 110 milk samples were analyzed for the presence of chemical adulterants, including sugar, starch, acids, soap, and formalin.

Detection of sugar

Five mL of strong hydrochloric acid was added to ten mL of milk in a glass test tube. After thorough mixing, 0.1 g of powdered resorcinol was added, and the mixture was stirred gently. After that, the test tube was heated for five minutes in a boiling water bath. The presence of sugar (sucrose) in the milk was indicated by the development of a red color during heating (Sharma et al. 2012).

Detection of starch

Three mL of milk was placed in a test tube and kept in a boiling water bath for five minutes. After incubation, the test tube was cooled, and the contents were well mixed with a few drops of 1% iodine solution. The presence of starch in milk was indicated by its blue-black color (Sharma et al., 2012).

Detection of acids

Five mL of milk was poured into a test tube. Concentrated sulphuric acid was added in small amounts. Gently shake the test tube, then add 0.5% ferric chloride solution dropwise with continuous mixing. Ensure the contents are thoroughly combined. The appearance of a buff coloration indicates the presence of benzoic acid, whereas a violet coloration confirms the presence of salicylic acid (Singh and Gandhi, 2015).

Detection of soap

A glass test tube was filled with ten mL of milk, and then an equal volume of hot water was added. Then, one or two drops of phenolphthalein indicator were added, and the contents were gently mixed. The development of a pink color detected the presence of soap in the examined milk sample upon addition of the phenolphthalein indicator (Singh and Gandhi, 2015).

Detection of formalin

Two mL of milk was placed in a glass test tube, and two mL of a 90% sulphuric acid-ferric chloride mixture was added gently. The development of a brownish-pink ring at the boundary of the two layers indicated milk adulteration with formalin (Sharma et al., 2012).

Species adulteration detection using polymerase chain reaction

A total of 110 milk samples (25 mL each) were centrifuged at 2200 g for five minutes to sediment the milk. To prevent casein blockage, 1 mL of the sediment was mixed with 200 µL of Tris-EDTA buffer (containing 1 mM EDTA, 10 mM Tris-HCl at pH 7.6, and 300 µL of 0.5 M EDTA at pH 8) and then centrifuged at 3000 g for 10 minutes (Murphy et al., 2002; Psifidi et al., 2010). Following the manufacturer's instructions, the milk pellet was diluted in 200 µl of phosphate-buffered saline, and DNA was extracted using the GF-1 Tissue DNA extraction kit (Cat. -No. GF-TD-050, Vivantis Co., Malaysia). After being eluted in 50 µL of elution buffer, DNA was stored at -20°C for further analysis (Abuelnaga et al., 2022). Species-specific primers were designed from published mitochondrial cytochrome b sequences

of cattle and buffalo (Table 1).

The PCR reaction was performed in a 25 µl reaction volume containing 12.5 µl of 2 × COSMO PCR RED Master Mix (Cat. W1020300X, Willofort Co., UK.), 1 µl (0.1 mM) of each primer, 1 µl of the purified DNA, and 9.5 µl of double-distilled water. The PCR reaction steps included one cycle at 95°C for two minutes, followed by 35 cycles of 95°C for one minute, 30 seconds of annealing, 45 seconds at 72°C, and a final 10-minute extension at 72°C (GS-96 gradient thermocycler, Hercuvan, Malaysia; Table 1). ViSafe Red Gel Stain (Vivantis Co., Malaysia) was used to stain 1.5% agarose gels for electrophoresis to visualize the amplified PCR products. The InGenius3 gel documentation system (Syngene, UK) was used to visualize amplified products after electrophoresis alongside a 100 bp DNA ladder at 100 V (Abuelnaga et al., 2022).

Table 1. Species-specific PCR primers for the amplification of cattle and buffalo DNA in different milk samples from Egypt (2024)

Species	Sequence	Anneal. Temp.	PCR product	Reference
Cattle	F- GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA-R F-CTAGAAAAGTGTAAGACCCGTAATATAAG - R	59°C	274bp	Matsunaga et al. (1999)
Buffalo	F- TAGGCATCTGCCTAATTCTG -R F- ACTCCGA TGTTTCATGTTTCT -R	61°C	242bp	Rajapaksha et al. (2003)

Statistical analysis

The present results were statistically analyzed using SPSS 14 for ANOVA and descriptive statistics (mean, maximum, minimum, and standard deviation). One-way ANOVA was performed to compare mean values among raw, pasteurized, and milk powder samples, with statistical significance set at p-value less than 5% ($p < 0.05$).

RESULTS

In the current study, raw milk exhibited the highest levels of standard plate count at $7\times10^5 \pm 1.7\times10^6$, *S. aureus* at $1.9\times10^3 \pm 2.9\times10^3$, coliforms at $2.8\times10^2 \pm 3.1\times10^2$, and *E. coli* O157 at $7.4\times10 \pm 7.8\times10$ CFU/mL (Table 2; Graph 1). Pasteurized milk indicated significantly lower microbial loads ($p < 0.05$) compared with raw milk. Milk powder consistently had the lowest microbial counts and exhibited notably greater microbial quality than raw milk. The comparison of the three milk types indicated that the standard plate count revealed significant differences among the groups ($p < 0.05$). Specifically, for *S. aureus*, coliforms, and *E. coli* O157, the differences were highly significant ($p < 0.05$). Raw milk exhibited the highest levels of contamination, posing clear public health risks. Pasteurized milk demonstrated enhanced but variable microbial quality. Milk powder exhibited superior microbiological safety. The current results confirmed that milk processing significantly reduced the microbial loads, making processed milk considerably safer than raw milk ($p < 0.05$). In the present investigation, the yeast count differed significantly among the three milk types (Table 3; Graph 1). Raw milk had significantly higher yeast counts with mean ± SD ($1.7\times10^2 \pm 1.7\times10^2$) than both pasteurized milk ($1.2\times10 \pm 2\times10$) and milk powder ($0.95\times10 \pm 1.3\times10$, $p < 0.05$).

Pasteurized milk and milk powder did not differ significantly from each other, but both had much lower yeast counts than raw milk ($p < 0.05$). The difference in mold counts among the milk types was not statistically significant ($p > 0.05$).

In this study, *Aspergillus* and *Penicillium* were the most common molds isolated, accounting for 40% and 32% in raw milk, and 24% and 20% in pasteurized milk, respectively. Milk powder had the lowest mold counts with 20% and 10% for *Aspergillus* and *Penicillium*, respectively (Table 4).

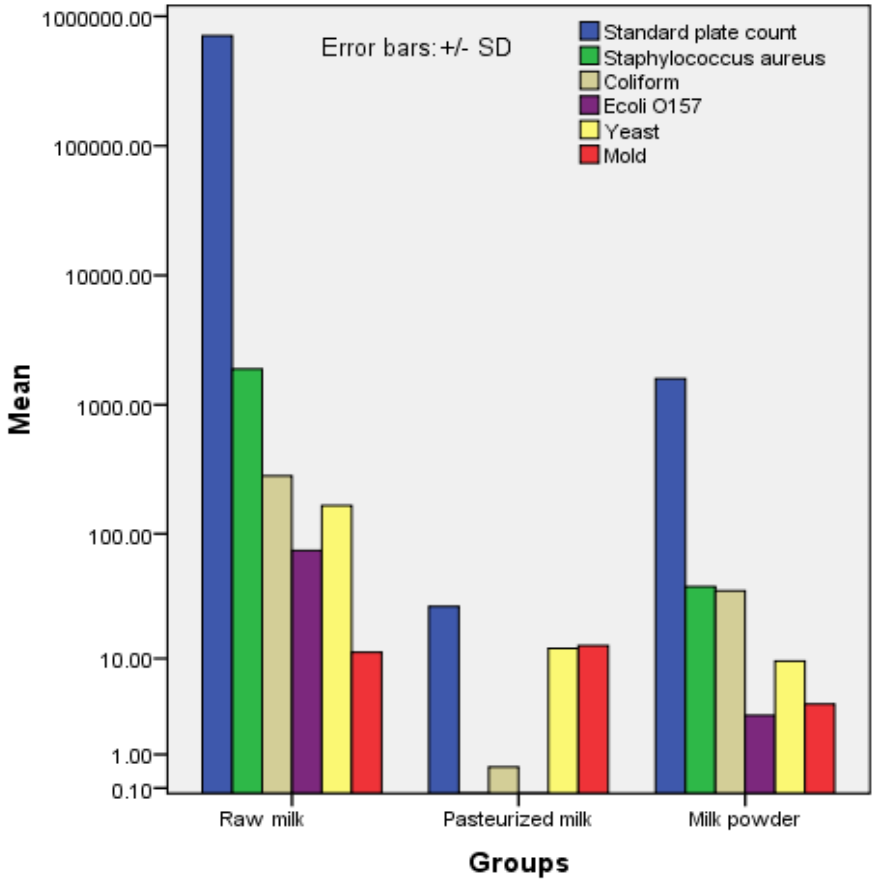
In the current study, sugar and soap were found in 2% and 6% of raw milk samples, respectively, but not in pasteurized or milk powder samples (Table 5; Graph 2). Starch was detected in raw milk, pasteurized milk, and milk powder at 16, 2, and 10%, respectively. Salicylic acid was found in 20% of raw milk samples and in 10% of milk powder samples, but it was not present in pasteurized milk. Formalin was detected only in raw milk at 6% and was not found in pasteurized milk or milk powder.

The primers produced distinct species-specific fragments of 274 bp for cattle milk and 242 bp for buffalo milk (Figures 1 and 2). In the current study, 40% of the raw milk was cattle milk, 50% was buffalo milk, and 10% was mixed milk (Table 6). In pasteurized milk, 50% consisted of cattle milk, 26% comprised buffalo milk, 24% was blended milk, and 100% of the milk powder was cattle milk only.

Table 2. Microbial examination of different cattle and buffalo milk samples in Egypt (2024)

Parameter		Raw milk (N = 50)	Pasteurized milk (N = 50)	Milk powder (N = 10)	P-Value
Standard plate count	Min.	1.7×10^3	0	0	
	Max.	6×10^6	1.8×10^2	5.5×10^3	
	Mean ± SD	$(7\times10^5 \pm 1.7\times10^6)$	$(2.7\times10 \pm 4.7\times10)$	$(1.6\times10^3 \pm 1.9\times10^3)$	0.012
Staphylococcus aureus	Min.	1.8×10^2	0	0	
	Max.	8×10^3	0	1.1×10^2	
	Mean ± SD	$(1.9\times10^3 \pm 2.9\times10^3)$	(0.00 ± 0.00)	$(3.9\times10 \pm 4.4\times10)$	< 0.001
Coliform	Min.	0.3×10	0.1×10	0	
	Max.	8×10^2	0.7×10	10×10	
	Mean ± SD	$(2.8\times10^2 \pm 3.1\times10^2)$	$(0.06\times10 \pm 0.16\times10)$	$(3.6\times10 \pm 3.8\times10)$	< 0.001
E. coli O157	Min.	0.1×10	0	0	
	Max.	2×102	0	0.9×10	< 0.001
	Mean ± SD	$(7.4\times10 \pm 7.8\times10)$	(0.00 ± 0.00)	$(0.3\times10 \pm 0.34\times10)$	

Values are expressed as Mean ± SD (CFU/mL).

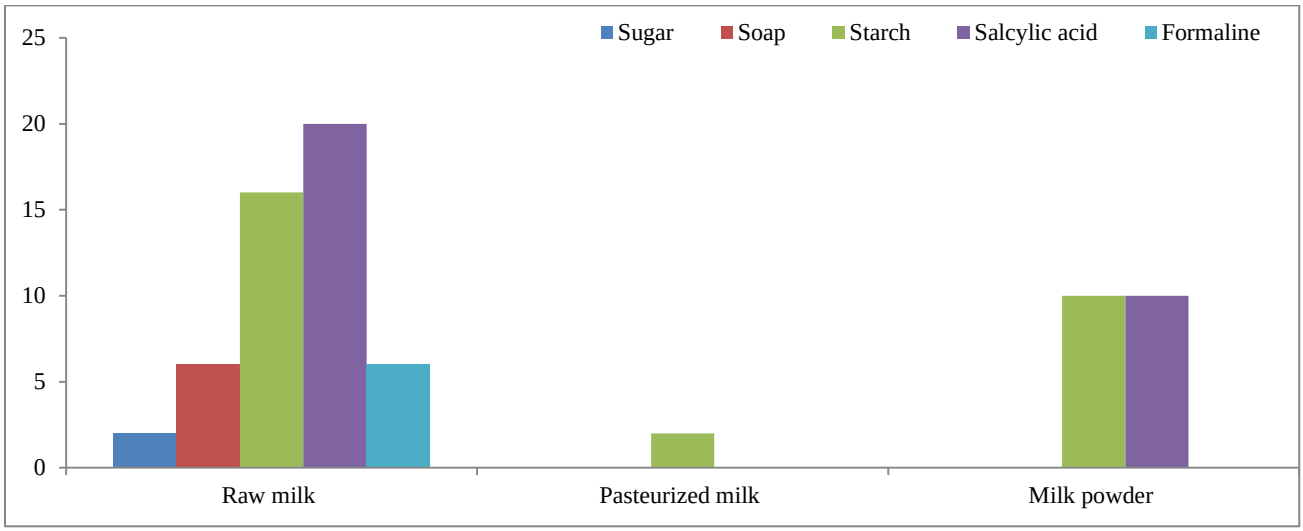


Graph 1. The mean values of bacterial and fungal count in the tested cattle and buffalo milk samples in Egypt (2024)

Table 3. Mycological examination of different cattle and buffalo milk samples in Egypt (2024)

Parameter		Raw milk (N= 50)	Pasteurized milk (N = 50)	Milk powder (N = 10)	P-Value
Yeast	Min.	0.1×10	0	0	
	Max.	5×10^2	8×10	4×10	
	Mean ± SD	$(1.7\times10^2 \pm 1.7\times10^2$	$(1.2\times10 \pm 2\times10)$	$(0.95\times10 \pm 1.3\times10)$	< 0.001
Mold	Min.	0	0	0	
	Max.	5×10^2	7.7×10	1.5×10	
	Mean ± SD	$(0.11\times10 \pm 0.16\times10)$	$(1.3\times10 \pm 1.9\times10)$	$(0.39\times10 \pm 0.5\times10)$	0.322

Values are expressed as Mean ± SD (CFU/mL).



Graph 2. The percentage of chemical adulterants in the examined milk samples in Egypt (2024)

Table 4. Different mold species isolated from the cattle and buffalo milk samples in Egypt (2024)

Mold spp.	Raw milk (N = 50)		Pasteurized milk (N = 50)		Milk powder (N = 10)	
	No.	(%)	No.	(%)	No.	(%)
Aspergillus	20	40	12	24	2	20
Penicillium	16	32	10	20	1	10
Rhizopus	-	-	1	2	-	-
Cladosporium	1	2	1	2	-	-
Alternania	1	2	-	-	-	-
Mucor	1	2	1	2	1	10
Fusarium	-	-	-	-	-	-
Scopulariopsis	2	4	-	-	-	-
Geotrichum spp.	3	6	-	-	1	10

Table 5. Detection of chemical adulteration in different cattle and buffalo milk samples in Egypt (2024)

Items	Raw milk (%)	Pasteurized milk (%)	Milk powder (%)
Sugar	2	-	-
Soap	6	-	-
Starch	16	2	10
Salicylic acid	20	-	10
Formalin	6	-	-

Table 6. The PCR results of different milk samples of cattle and buffalo species in Egypt (2024)

Mold spp.	Raw milk (N = 50)		Pasteurized milk (N = 50)		Milk powder (N = 10)	
	No.	(%)	No.	(%)	No.	(%)
Cattle milk only	20	40	25	50	10	100
Buffalo milk only	25	50	13	26	-	-
Mixed milk	5	10	12	24	-	-

No: Number

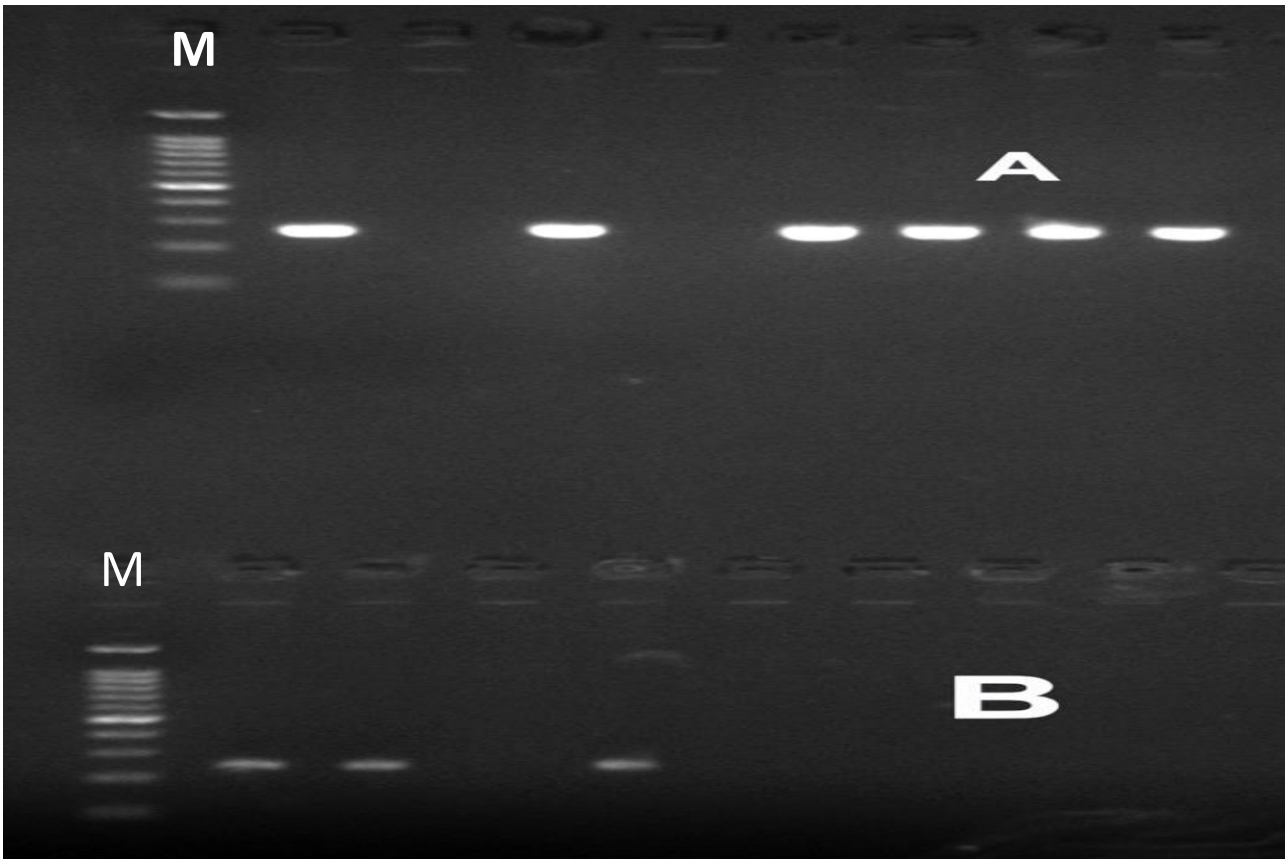


Figure 1. The PCR product of the cattle and buffalo milk samples using species-specific primers on an agarose gel. **A**: Screening of milk samples for cattle DNA (274 bp), **B**: Screening of commercial milk samples for buffalo DNA (242). **M**: Molecular marker (100 bp)

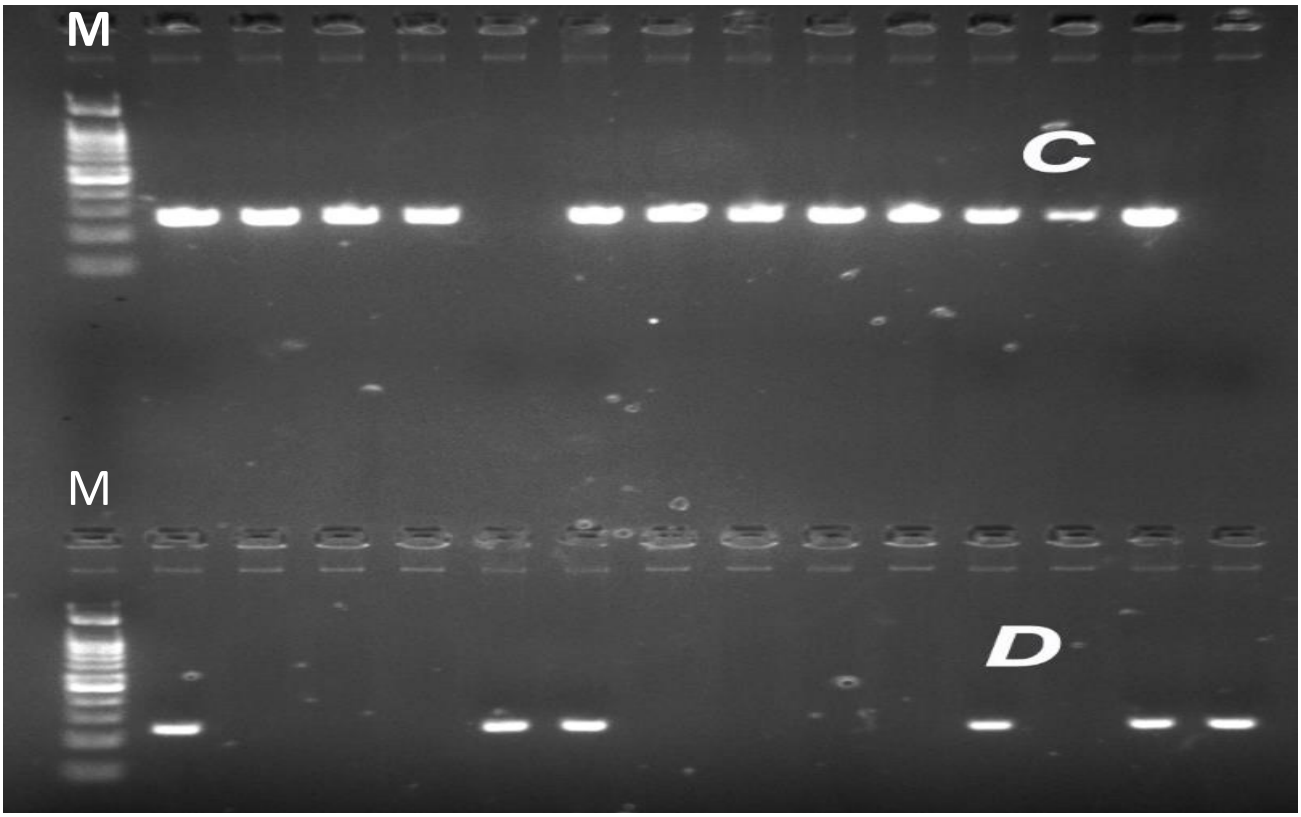


Figure 2. The PCR product of the cattle and buffalo milk samples using species-specific primers on an agarose gel. **C**: Screening of milk samples for cattle DNA (274 bp), **D**: Screening of commercial milk samples for buffaloes DNA (242). **M**: Molecular marker (100 bp)

DISCUSSION

Globally, there is increasing concern about the safety and quality of milk and milk products due to microbial contamination caused by improper handling and hygiene practices. Milk and dairy products are major carriers of milk-borne diseases, including diarrheal illnesses caused by *Salmonella*, *Campylobacter*, STEC, Staph, and *Bacillus* species (Almashhadany et al., 2022). Additionally, milk and dairy products can cause severe invasive infections, including listeriosis, brucellosis, tuberculosis, Q fever, and cryptosporidiosis. Historically, these products have been linked to typhoid, diphtheria, and scarlet fever, all of which have greatly impacted global public health (Almashhadany et al., 2022; Gebremichael et al., 2024).

In the present study, the mean standard plate count was consistent with findings of Mahrous et al. (2023) in Egypt, as they reported a mean count of $2.9 \times 10^5 \pm 0.16 \times 10^5$ CFU/mL, while El-Leboudy et al. (2017) documented an average of $2.6 \times 10^5 \pm 0.2 \times 10^5$ CFU/mL in raw milk. Similarly, Bille et al. (2009) in Namibia reported standard plate count ranging from $7.8 \times 10^4$to 1.3×10^6 CFU/mL of raw cow's milk collected from dairy farms. Abdul Kader et al. (2015) reported an elevated standard plate count from raw cow's milk samples at 38.1×10^6 CFU/mL across different farms in Bangladesh. Additionally, Oladipo et al. (2016) reported aerobic plate counts for raw cow's milk samples in Nigeria ranging from 0.2×10^6 to 4.2×10^6 CFU/mL. Faisal and Ahmed (2018) in Ethiopia reported 5.96×10^8 CFU from milk containers in the distribution centre, while the total aerobic plate count in raw cow's milk samples from the storage area at a dairy farm was 3.4×10^8 CFU/mL. Conversely, Abuelnaga et al. (2022) reported a total aerobic count of 1.6×10^6 CFU/mL from raw cow's milk samples in Egypt.

Staphylococcal food poisoning is one of the most common causes of gastroenteritis worldwide. Initial signs typically include abdominal discomfort, nausea, and vomiting within 2 to 6 hours, and may occasionally be followed by diarrhea. Signs of Staphylococcal food poisoning appear after consuming thermostable staphylococcal enterotoxins at a dose of approximately 0.1-1.0 mg/kg body weight (Ahmed et al., 2022). The present result of *S. aureus* count aligns with the findings of Mahrous et al. (2023) from Egypt, who reported a mean count of $2.7 \times 10^3 \pm 7.8 \times 10^2$ CFU/mL, and Abuelnaga et al. (2022), who recorded a highly comparable mean value of 1.7×10^3 CFU/mL.

The presence of coliforms in raw milk might be attributed to factors such as improper milking practices, use of contaminated water, poor herd hygiene, and inadequate washing and maintenance of equipment (Mahrous et al., 2023). Coliform count in the present study was comparable to those reported by Bille et al. (2009) in Namibia, who documented a range of 2.4×10^2 to 2.3×10^3 CFU/mL, and by Mahrous et al. (2023) in Egypt, who obtained a mean count of $9.29 \times 10^2 \pm 3.8 \times 10^2$ CFU/mL. In contrast, elevated counts have been reported by Banik et al. (2014) with a range of 4.2×10^4 to 1.0×10^5 CFU/mL and also Chowdhury et al. (2022) with a range of 0.6×10^6 to 7.8×10^6 CFU/mL. Conversely, Abuelnaga et al. (2022) reported notably lower levels, with a mean count of $2 \times 10^1 \pm 0.4 \times 10^1$ CFU/mL.

Foodborne fungi produce toxic compounds called mycotoxins, which are extremely harmful to animal and human health. Although food processing may eliminate the microorganisms themselves, any preformed mycotoxins can remain, as these heat-stable compounds are not easily destroyed. Fungi can cause infections or allergic reactions. Yeasts and molds primarily contaminate milk through airborne contaminants, poor storage conditions, or packaging materials, causing different problems in dairy products (Ahmed et al., 2022). Molds and yeasts are undesirable contaminants in milk and dairy products, as their presence, even in low numbers, can negatively affect product quality (Elkot et al., 2025). In the current study, the mean yeast count was similar to that reported by Abuelnaga et al. (2022), who documented a mean count of $2.4 \times 10^2 \pm 7.6 \times 10^2$ CFU/mL in Egypt. Several studies have reported varying microbial counts in Egypt. Ahmed et al. (2022) documented a notably high mean count of $1.29 \times 10^7 \pm 2.48 \times 10^7$ CFU/mL. In contrast, El-shinawy et al. (2018) found a considerably lower mean of $6.22 \times 10^2 \pm 3.62 \times 10^2$ CFU/mL. Regarding mold counts, Abuelnaga et al. (2022) reported similar results with a mean of $4 \times 10 \pm 1.9 \times 10$ CFU/mL, and also El-shinawy et al. (2018) recorded a mean mold count of $2.23 \times 10^2 \pm 9.3 \times 10^1$ CFU/mL, while Ahmed et al. (2022) observed elevated levels, with a mean of $1.04 \times 10^6 \pm 2.52 \times 10^6$ CFU/mL. Additionally, the average mold count across studies was noted as $0.11 \times 10 \pm 0.2 \times 10^1$ CFU/mL.

High total bacterial counts in pasteurized milk may result from high contamination by equipment and personnel, late pasteurization, and inadequate heating procedures, underscoring the importance of stricter post-processing hygiene (Ahmed et al., 2022). In the current study, the standard plate counts for pasteurized milk were in accordance with El-Ziney (2018), who reported a mean count of $2.95 \times 10^1 \pm 0.62$ CFU/mL. However, higher counts were observed by Ahmed et al. (2022), with a mean of $1.55 \times 10^4 \pm 2.25 \times 10^4$ CFU/mL, as well as by Kumala et al. (2021), who recorded mean values of 2.10×10^1 and 2.94×10^4 CFU/mL. *Staphylococcus aureus* was not detected in the current study, as the presence of *Staphylococcus* in pasteurized milk can be attributed to inadequate pasteurization, the persistence of pathogens, or post-pasteurization contamination resulting from poor processing, improper handling, or insufficient worker hygiene (Ahmed et al., 2022). Coliforms are the most important bacteria and serve as indicators of fecal

contamination, implying the presence of other microbes from the digestive tract. Coliforms can be eliminated during pasteurization; therefore, a positive coliform test in pasteurized milk indicates either inadequate pasteurization or post-pasteurization contamination (Ahmed et al., 2022). In the present study, the total coliform count was not detected. Different findings were reported by Ahmed et al. (2022), who recorded a mean count of 9.96×10 ± 9.52×10 CFU/mL. Since most fungi are destroyed by heat treatment, their presence typically indicates post-heat-treatment contamination (Elkot et al., 2025). The mean total yeast and mold count of the present study was lower than the results reported by Ahmed et al. (2022), with a mean yeast and mold count of 1.46×10²±1.64×10² cfu/ml and 1.54×10² ± 3.06×10² CFU/mL, respectively.

The total bacterial count in milk powder was consistent with the findings of El-Etriby (2017), who reported 2.1×10³ ± 0.12×10³ CFU/mL, and also Ibrahim et al. (2021) reported a mean of 4.97×10² ± 1.21×10² CFU/g, while Halim et al. (2022) observed a higher count of 28.15×10⁷ ± 23.4×10⁷ CFU/g. In the current study, *Staphylococci* in milk powder were similar to those obtained by Ibrahim et al. (2021) with a mean of $0.94\times10^2 \pm 0.21\times10^2$ CFU/g. In contrast, Halim et al. (2022) reported higher values, with a mean of $18.38\times10^6 \pm 11.52\times10^6$. The coliform results in the present study were similar to those of Ibrahim et al. (2021), who reported counts less than 3 CFU/g, whereas Halim et al. (2022) reported a mean of $28.74\times10^8 \pm 10.557\times10^8$ for coliforms. Regarding the total yeast count in the present study, Ibrahim et al. (2021) reported lower values, with a mean of $0.65\times10^2 \pm 0.05\times10^2$ CFU/g. Conversely, Halim et al. (2022) found higher counts, with averages reaching $3.09\times10^3 \pm 1.96\times10^3$ CFU/mL. According to the current results, the mean total mold count was 0.39×10 ± 0.5×10. This lack of significance may be attributed to naturally low mold levels, sample variability, or the small sample size in the milk powder group. Comparable findings were reported by Ibrahim et al. (2021), who recorded counts exceeding 10 CFU/g, whereas Halim et al. (2022) observed higher levels, with a mean count of 12.16×10² ± 9.74×10² CFU/mL.

Since most imported dairy products meet international standards, a high bacterial count in milk powder indicated recontamination. No microbial growth was observed during or after manufacturing. Factors such as the heat resistance of spore-forming bacteria, unsanitary storage conditions, post-heat-treatment contamination, poor packaging, mishandling, and poor human hygiene all contribute to recontamination (Halim et al., 2022).

Previous studies have identified different mold species, including *Mucor* spp., *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Absidia* spp., *Penicillium* spp., *Geotrichum candidum*, *Cladosporium* spp., *Phoma* spp., and *Fusarium* spp. Similarly, Elbarbary et al. (2022) observed that the most prevalent mold genera in examined milk samples were *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp., *Mucor* spp., *Rhizopus* spp., *Absidia* spp., and *Fusarium* spp., with *Aspergillus* species being the most dominant.

Chemical adulteration in the current study varied across milk types, with raw milk showing the highest contamination. Sugar and soap were detected in small proportions of raw milk, while starch appeared more frequently, especially in raw samples. Salicylic acid was also common in raw milk and present in milk powder, whereas formalin was found only in a limited number of raw milk samples. Different results were reported by Tubesha in Palestine (2025), who stated that all milk samples tested were free of starch, formalin, and carbonate.

In many countries, including India, Pakistan, Italy, and Nepal, where both cow and buffalo milk are widely produced and sold, milk adulteration is a common problem. Cottenet et al. (2011) proposed a species-specific real-time PCR method targeting the cytochrome b gene to address this issue. This method can detect cow milk content ranging from 0.1% to 2%, combined with buffalo milk.

CONCLUSION

The present findings revealed that raw milk poses significant microbiological and chemical hazards, primarily due to poor hygiene and adulteration. Elevated bacterial and yeast counts, along with the presence of chemical adulterants such as sugar, soap, starch, salicylic acid, and formalin, indicated the improper handling and storage conditions that threaten consumer health. Pasteurized milk demonstrated improved microbial quality but still exhibited variability among samples, whereas milk powder proved the safest product, reflecting effective processing and preservation. The PCR-based method for species identification indicated the presence of mixed and buffalo milk mainly in raw and pasteurized samples, while milk powder was exclusively cattle-derived. The current results highlighted the urgent need for stricter monitoring, improved sanitary practices during milk collection and distribution, and the enforcement of regulatory measures to lower milk adulteration and defilement in the study area. Future studies should involve larger, regionally diverse samples, utilize advanced detection techniques, and assess intervention strategies to further enhance milk safety and quality control.

DECLARATIONS

Funding

The study did not receive any financial support or funding.

Availability of data and materials

Upon reasonable request, the corresponding author will provide the data supporting the study's conclusions.

Authors' contributions

Mona Mohamed Hassan Soliman and Azza Sayed Mohammed Abuelnaga formulated the study, contributed to experimental procedures, data analysis, and manuscript preparation. Khaled Abd El-Hamid Abd El-Razik was involved in the study's conceptualization, experimental execution, and data analysis. Mai Mohamed Kandil participated in experimental procedures and manuscript drafting. Eman Ramadan Hassan contributed to data analysis and manuscript preparation. All authors reviewed and approved the final edition of the manuscript before submission to the journal.

Competing interests

The authors have not declared any conflict of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. The authors confirmed that this study was conducted and prepared without using AI tools.

REFERENCES

Abuelnaga ASM, Ata NS, Abd El-Razik KAEH, Hedia RH, Soliman MMH, Kandil MM, Elgabry EAE, and Arafa AA (2022). Microbiological evaluation and molecular discrimination of milk samples from humans and different animals. World's Veterinary Journal, 12(1): 9-18. DOI: https://www.doi.org/10.54203/scil.2022.wvj2

Abdul Kader D, Abdul Aziz MD, Mehadi MD, Hasan S, and Rahman SR (2015). Evaluation of physico-chemical properties and microbiological quality of milk collected from different dairy farms in Sylhet, Bangladesh. Food Science and Technology, 3(3): 37-41. DOI: https://www.doi.org/10.13189/fst.2015.030301

Ahmed SA, Mostafa AHM, El-Sherbini M, and Abdelkhalek A (2022). Assessment of microbial safety and quality of market raw milk and pasteurized milk sold in Dakahlia Governorate, Egypt. Journal of Advanced Veterinary Research, 12(4): 456-461. Available at: https://advetresearch.com/index.php/AVR/article/view/1068

Alles AA, Wiedmann M, and Martin NH (2018). Rapid detection and characterization of postpasteurization contaminants in pasteurized fluid milk. Journal of Dairy Science, 101(9): 7746-7756. DOI: https://www.doi.org/10.3168/jds.2017-14216

Almashhadany DA, Mohammed HI, Muslat TAM, Hassan RR, Rashid RF, and Hassan AO (2022). Milk-borne diseases. Health risks of food additives-recent developments and trends in food sector, Chapter 3, pp. 45-66. DOI: https://www.doi.org/10.5772/intechopen.108796

American public health association (APHA) (1992). Standard methods for the microbiological examination of dairy products, 16th Edition. American Public Health Association, Washington, D.C., USA. Available at: https://agris.fao.org/agris-search/search.do?recordID=US19950104912

Banik SK, Das KK, and Uddin MA (2014). Microbiological quality analysis of raw, pasteurized, UHT milk samples collected from different locations in Bangladesh. Stamford Journal of Microbiology, 4(1): 5-8. DOI: https://www.doi.org/10.3329/sjm.v4i1.22753

Bille PG, Haradeb BR, and Shigwedha N (2010). Evaluation of chemical and bacteriological quality of raw milk from Neudamm dairy farm in Namibia. African Journal of Food, Agriculture, Nutrition and Development, 9(7): 1511-1523. Available at: https://api.semanticscholar.org/CorpusID:43463261

Bergey DH and Holt JG (1994). Bergey's manual of determinative bacteriology, 9th Edition. Williams and Wilkins., Baltimore, pp. 337-339. Available at: https://www.biodiversitylibrary.org/item/41848#page/182/mode/1up

Chiarlone SA, Gori A, Ravetta S, Armani A, Guardone L, Pedonese F, Bavetta S, Fiannacca C, Pussini N, Maurella C et al. (2025). Microbiological analysis conducted on raw Milk collected during official sampling in Liguria (north-West Italy) over a ten-year period (2014-2023). Animals, 15(2): 286. DOI: https://www.doi.org/10.3390/ani15020286

Chowdhury T, Roky SA, Ashik-uz-Zaman MD, Islam T, and Mohonto B (2022). Coliform bacteria screening and evaluating chemical composition of raw milk from dairy farms of Sylhet Sadar, Bangladesh. Indian Journal of Dairy Science, 75(4): 326-330. DOI: https://www.doi.org/10.33785/IJDS.2022.v75i04.004

Chye FY, Abdullah A, and Ayob MK (2004). Bacteriological quality and safety of raw milk in Malaysia. Food Microbiology, 21(5): 535-541. DOI: https://www.doi.org/10.1016/j.fm.2003.11.007

Cottenet G, Blancpain C, and Golay PA (2011). Simultaneous detection of cow and buffalo species in milk from China, India, and Pakistan using multiplex real-time PCR. Journal of Dairy Science, 94(8): 3787-3793. DOI: https://www.doi.org/10.3168/jds.2011-4195

Deddefo A, Mamo G, Asfaw M, and Amenu K (2023). Factors affecting the microbiological quality and contamination of farm bulk milk by Staphylococcus aureus in dairy farms in Asella, Ethiopia. BMC Microbiology, 23: 65. DOI: https://www.doi.org/10.1186/s12866-022-02746-0

Elbarbary M, Elamin A, Darwish WS, and Tahoun ABMB (2022). Effect of probiotic bacteria on the reduction of aflatoxin risks in milk. Damanhour Journal of Veterinary Sciences, 8(1): 1-5. DOI: https://www.doi.org/10.21608/DJVS.2022.139623.1071

El-Etriby DE (2017). Microbiological study of some cheese and milk powder samples. Egyptian Journal of Medical Microbiology, 26(1): 25-32. DOI: https://www.doi.org/10.12816/0046269

Elkot WF, Mohamed FA, and Ismail HA (2025). Assessment of chemical and microbiological quality of raw cow milk and some dairy products in the local market of Aswan Governorate. Aswan University Journal of Sciences and Technology, 5(1): 189-196. DOI: https://www.doi.org/10.21608/aujst.2025.346562.1158

El-Leboudy A, Amer AA, Abo El-Makarem HS, and Ibrahim EK (2017). Chemical and microbiological status of raw milk sold at local markets. Alexandria Journal of Veterinary Sciences, 55(1): 125-132. DOI: https://www.doi.org/10.5455/ajvs.281717

El-Shinawy SAADIA, El-Kholy ADEL, Meshref A, and Sharkawy S (2018). Mycological evaluation of milk and some milk products in Beni-Suef City. Assiut Veterinary Medical Journal, 64(156): 117-122. DOI: https://www.doi.org/10.21608/avmj.2018.168711

El-Ziney MG (2018). Evaluation of microbiological quality and safety of milk and dairy products with reference to European and Gulf Standards. Food and Public Health, 8(2): 47-56. DOI: https://www.doi.org/10.5923/j.fph.20180802.03

Faisal SD and Ahmed AF (2018). Bacteriological quality assessment of milk in College of Veterinary Medicine (Cvm) dairy farm and Kalamino dairy farm in Mekelle, Tigray, Ethiopia. Dairy and Veterinary Sciences Journal, 8(2): 555734. DOI: https://www.doi.org/10.19080/JDVS.2018.08.555734

Garedew L, Berhanu A, Mengesha D, and Tsegay G (2012). Identification of gram-negative bacteria from critical control points of raw and pasteurized cow milk consumed at Gondar town and its suburbs, Ethiopia. BMC Public Health, 12: 950. DOI: https://www.doi.org/10.1186/1471

Garcia J, Sanvido G, Saraiva S, Zacca J, Cosso R, and Eberlin M (2012). Bovine milk powder adulteration with vegetable oils or fats revealed by MALDI-QTOF MS. Food Chemistry, 131(2): 722-726. DOI: https://www.doi.org/10.1016/j.foodchem.2011.09.062

Gebremedhin E, Ararso AB, Borana BM, Kelbesa KA, Tadese ND, Marami LM, and Sarba EJ (2022). Isolation and identification of *Staphylococcus aureus* from milk and milk products, associated factors for contamination, and their antibiogram in Holeta, Central Ethiopia. Veterinary Medicine International, 2022: 6544705. DOI: https://www.doi.org/10.1155/2022/6544705

Gebremichael D, Tadesse A, Hailemariam F, Hailay B, Hadgu H, and Kalayu G (2024). Investigation of microbial quality of milk and milk products and isolations of some major bacteria in the Central and Northwestern Zones of Tigray, Ethiopia. Veterinary Medicine International, 2024(1): 9989527. DOI: https://www.doi.org/10.1155/vmi/9989527

Ibrahim AS, Saad MF, and Hafiz NM (2021). Safety and quality aspects of whole and skimmed milk powders. Acta Scientiarum Polonorum Technologia Alimentaria, 20(2): 165-177. DOI: https://www.doi.org/10.17306/J.AFS.0874

Ibrahim S, Mohd MT, Khairi MD, Yunus MA, and Sahlan S (2022). An ultrasonic system for detecting foreign contaminants in milk. In: Control, instrumentation and mechatronics: theory and practice. Springer Nature., Singapore, pp. 809-817. DOI: https://www.doi.org/10.1007/978-981-19-3923-5_69

Halim EYA, El-Essawy H, Awad AAN, El-Kutry MS, and Ahmed LI (2022). Estimating the microbial safety and sensory characteristics of some imported dairy products retailed in the Egyptian markets. Advances in Animal and Veterinary Sciences, 10(3): 488-499. DOI: https://www.doi.org/10.17582/journal.aavs/2022/10.3.488.499

Holt JG, Krieg NR, Sneath PHA, Staley JT, and Williams ST (1994). Bergey's manual of determinative bacteriology, 9th Edition. Williams & Wilkins, Baltimore. Available at: https://www.scirp.org/reference/referencespapers?referenceid=42336

Kumala A, Lemma F, and Fitwi BA (2021). Total bacterial count and identification of *Staphylococcus* species from critical control points of raw and processed milk in selected dairy farm in Bishoftu Town, Ethiopia. Journal of Advanced Veterinary Research, 11(4): 208-212. Available at: https://advetresearch.com/index.php/AVR/article/view/782

Lancette GA and Bennett RW (2001). *Staphylococcus aureus* and staphylococcal enterotoxins. In: F. P. Downes and K. Ito (Editors), Compendium of methods for the microbiological examination of foods, 4th Edition. APHA., Washington DC, pp. 387-403. DOI: https://www.doi.org/10.2105/9780875531755ch39

Mahrous E, Abdelgawad MH, Saad SA, and Abdou SE (2023). Chemical and bacteriological assessment of raw milk collected from some dairy farms in El-Behera Governorate, Egypt. Zagazig Veterinary Journal, 51(4): 310-325. DOI: https://www.doi.org/10.21608/zvjz.2023.235070.1215

Matsunaga T, Chikuni K, Tanabe R, Muroya S, Shibata K, Yamada J, and Shinmura Y (1999). A quick and simple method for the identification of meat species and meat products by PCR assay. Meat Science, 51(2): 143-148. DOI: https://www.doi.org/10.1016/s0309-1740(98)00112-0

Mohammed FAF and Abdel-Aall HEDK (2024). A study on detection of adulteration in milk and some milk products. Assiut Veterinary Medical Journal, 70(183): 54-63. DOI: https://www.doi.org/10.21608/avmj.2024.294777.1276

Murphy MA, Shariflou MR, and Moran C (2002). High quality genomic DNA extraction from large milk samples. Journal of Dairy Research, 69(4): 645-649. DOI: https://www.doi.org/10.1017/S0022029902005792

Ndungi F, Muliro P, Faraj A, and Matofari J (2021). Milk handling practices and utilization at dairy farms and collection centers under rural and peri-urban milk value chain systems in Nakuru county, Kenya. African Journal of Food, Agriculture, Nutrition and Development, 21(4): 17815-17833. DOI: https://www.doi.org/10.18697/ajfand.99.20080

Oladipo IC, Tona GO, Akinlabi EE, and Bosede OE (2016). Bacteriological quality of raw cow's milk from different dairy farms in Ogbomoso, Nigeria. International Journal of Advanced Research in Biological Sciences, 3(8): 1-6. Available at: https://ijarbs.com/pdfcopy/aug2016/ijarbs1.pdf

Psifidi A, Dovas CI, and Banos G (2010). A comparison of six methods for genomic DNA extraction suitable for PCR-based genotyping applications using ovine milk samples. Molecular and Cellular Probes, 24(2): 93-98. DOI: https://www.doi.org/10.1016/j.mcp.2009.10.003

Pirondini A, Bonas U, Maestri M, Giovanna V, Marta M, and Nelson M (2010). Yield and amplificability of different DNA extraction procedures for traceability in the dairy food chain. Food Control, 21(5): 663-668. DOI: https://www.doi.org/10.1016/j.foodcont.2009.10.004

Salih M and Yang SY (2017). Common milk adulteration in developing countries. Case study in China and Sudan: A review. Advances in Dairy Research, 5(4): 1000192. DOI: https://www.doi.org/10.4172/2329-888X.1000192

Sharma R, Rajput YS, Barui AK, and Naik LN (2012). Detection of adulterants in milk: A laboratory manual. NDRI, India, pp. 1-110. Available at: https://ndri.res.in/sites/default/files/Manual_Detection_Adulterants_Milk_june21.pdf

Singh P and Gandhi N (2015). Milk preservatives and adulterants: Processing, regulatory and safety issues. Food Reviews International, 31(3): 236-261. DOI: https://www.doi.org/10.1080/87559129.2014.994818

Swar SO, Abbas RZ, Asrar R, Yousuf S, Mehmood A, Shehzad B, Farhan HR, Aleem MT, Marcelino LA, and Mohsin M (2021). Milk adulteration and emerging health issues in humans and animals (a review). Continental Veterinary Journal, 1(1): 1-8. Available at: https://cvetj.com/display.php?page_no=1-8&issue_id=47

Rajapaksha WRAKJS, Thilakaratne IDSIP, Chandrasiri ADN, and Niroshan TD (2003). Development of PCR assay for identification of Buffalo Meat. Asian-Australasian Journal of Animal Sciences, 6(7): 1046-1048. DOI: https://www.doi.org/10.5713/ajas.2003.1046

Tomaszewska-Gras J (2016). Rapid quantitative determination of butter adulteration with palm oil using the DSC technique. Food Control, 60(8): 629-635. DOI: https://www.doi.org/10.1016/j.foodcont.2015.09.001

Tubesha ZA (2025). Adulteration and microbiological quality of raw milk from local markets in Hebron, Palestine. Asian Journal of Dairy and Food Research, 44: 168-174. DOI: http://www.doi.org/10.18805/ajdfr.drf-516

Xue G, Wu M, Liu T, Fang X, Yin J, Lai W, and Peng J (2023). A multiple lateral flow immunoassay based on AuNP for the detection of 5 chemical contaminants in milk. Journal of Dairy Science, 106(6): 3856-3867. DOI: https://www.doi.org/10.3168/jds.2022-23008

Zachar P, Michal S, Radovan K, Jarosla N, Miroslava N, and Dana M (2011). Analytical methods for the species identification of milk and milk products. Mljekarstvo, 61(3): 199-207. Available at: https://hrcak.srce.hr/69397