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COMPARATIVE ANALYSIS OF QUALITY AND MICROBIAL LOAD IN FRESH, RAW AND COMMERCIAL MILK

Original Article

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ABSTRACT

Background: Milk is a staple food globally, recognized for its nutritional value and essential role in human health. However, microbial contamination and adulteration significantly compromise its safety and quality. Microbial contamination can lead to spoilage and the transmission of foodborne illnesses. In developing regions, raw milk is often consumed without proper processing, increasing public health risks. This study aimed to evaluate the microbial quality, safety, and adulteration levels of raw and packaged milk from various sources in Multan, Pakistan.

Objective: To assess the microbial load and the prevalence of adulterants in fresh raw milk from farms, shops, milkmen, and packaged milk available in the local market.

Methods: Milk samples were collected using a random sampling technique from four sources: farms, shops, milkmen, and packaged milk brands. Microbial load was analyzed through Total Plate Count (TPC), Total Coliform Count (TCC), and Escherichia coli detection. Adulteration tests were performed for water, starch, sugar, formalin, urea, detergents, and neutralizers using qualitative detection methods. Data were statistically analyzed using one-way ANOVA and Tukey HSD tests to determine significant differences.

Results: Packaged milk exhibited no microbial growth in TPC, TCC, and E. coli detection, while raw milk from milkmen showed the highest contamination with TPC values of 1.53 (10^5 CFU/mL) and TCC values of 4.63 (10^5 CFU/mL). Farm and shop milk had TPC values of 1.31 (10^5 CFU/mL) and 1.27 (10^5 CFU/mL), respectively. Adulteration was most prevalent in milkman samples, with 100% water addition, 40% urea, and 20% starch and formalin. Packaged milk was free from adulteration except for 80% sugar presence.

Conclusion: Packaged milk demonstrated superior microbial safety and minimal adulteration compared to raw milk. However, milk from milkmen and shops showed significant contamination and adulteration, emphasizing the need for improved hygiene practices and public awareness regarding milk safety.

Keywords: Adulteration, Escherichia coli, Food Safety, Microbial Contamination, Milk Quality, Total Plate Count, Water Adulteration

INTRODUCTION

Milk is a fundamental component of the human diet worldwide, fulfilling vital nutritional requirements across all age groups. With the increasing global demand for milk, farmers are focusing on selective breeding practices to enhance milk production. However, such practices have led to complications in fertility traits, posing challenges to animal health and productivity (1). Moreover, inadequately managed milk, whether due to unhygienic conditions or improper handling, fosters the proliferation of harmful microorganisms, significantly impacting public health by contributing to the transmission of zoonotic and foodborne diseases (2). Various milk sources, including buffalo, bovine, caprine, and camel, have been found to harbor bacteria with high levels of antibiotic resistance to common drugs such as penicillin, gentamicin, erythromycin, and tetracycline. The presence of these antibiotic-resistant bacteria underscores the urgent need for further research to understand their epidemiological patterns and mitigate their impact. Psychrotrophic bacteria, for instance, not only compromise milk quality but also pose direct health risks to consumers. Their contamination with hazardous chemicals highlights the critical need for effective control measures in the dairy industry. Pathogens like Listeria monocytogenes add another layer of complexity due to their resilience in adverse conditions, widespread distribution, and ability to contaminate food at multiple points along the supply chain. This bacterium is not only a major concern for the food industry but also poses significant threats to human health, as its interactions with other microbial strains can influence virulence, proliferation, and the severity of infections (3, 4).

Additional microbial threats such as Escherichia coli and coliform bacteria have a profound impact on the quality and shelf life of milk. Their presence often signifies fecal contamination and poor hygiene during handling, leading to various foodborne illnesses. The consumption of raw milk is particularly concerning as it exposes humans to severe diseases such as brucellosis, which can be transmitted from infected animals. High bacterial loads in raw milk not only diminish its sensory and nutritional qualities but also make it unsuitable for further processing (5). Moreover, pathogens like Campylobacter jejuni and Staphylococcus aureus frequently contaminate milk through environmental sources, including water, bedding, and feeding equipment. These bacteria are known to cause gastrointestinal infections and food poisoning, exacerbating the risks associated with unpasteurized milk consumption (6). Globally, bacterial infections linked to milk, such as salmonellosis, remain a pressing public health challenge, with millions of cases and fatalities reported annually. Salmonella, a rod-shaped, gram-negative bacterium, is a common contaminant in unpasteurized milk and is a leading cause of foodborne illnesses, characterized by gastroenteritis and other severe health complications (7). Contamination pathways often involve the transfer of microorganisms from soil, feed, or unclean equipment to milk during the production process. Modern dairy practices, including refrigeration and sanitation, aim to mitigate these risks; however, lapses in hygiene continue to facilitate the spread of harmful pathogens (8).

In developing countries such as Pakistan, where economic constraints make packaged milk unaffordable for the majority, consumers predominantly rely on fresh, raw milk sourced from local vendors, farms, and shops. Unfortunately, milk adulteration is rampant at the local level, driven by profit motives, and poses significant health risks. This adulteration, coupled with poor microbial quality, underscores the critical need for awareness and regulatory interventions to ensure that milk meets safety standards and does not jeopardize public health. The issue is particularly acute in regions like Multan, where raw milk is a dietary staple, but its microbial quality remains largely unassessed. This study aims to bridge the knowledge gap concerning the microbial quality and associated risks of consuming fresh, raw milk in the Multan region. By evaluating the prevalence of harmful microorganisms and their potential health implications, the research seeks to provide actionable insights to safeguard consumer health and enhance the overall safety of milk consumption.

METHODS

This study was conducted in the Multan area, and all milk sample analyses were carried out in the laboratory of the Department of Food Science and Nutrition, TIMES Institute of Multan. A random sampling strategy was utilized to collect milk samples, ensuring that spoiled or expired samples were excluded from the selection process. The methodology followed a systematic approach to maintain scientific rigor and ensure reliable results. Milk samples were procured from various sources across Multan city, including farms, shops, milkmen, and packaged milk brands available in the local market. To ensure sample integrity, sterilized glass bottles were used for collection, and each bottle was labeled with the date and time. The temperature of the milk samples was recorded at the time of collection, and all samples were transported to the laboratory in iceboxes to maintain optimal conditions for microbiological analysis. A total of 20 milk samples were collected through simple random sampling, with five samples from each source: farms (FA, FB, FC, FD, FE), packaged brands (BA, BB, BC, BD, BE), shops (SA, SB, SC, SD, SE), and milkmen (MA, MB, MC, MD, ME). Microbiological analysis focused on Total Plate Count (TPC), Total Coliform Count (TCC), and the detection of Escherichia coli using established procedures with slight modifications (9).

Source	Samples
Farms	FA, FB, FC, FD, FE
Packaged Brands	BA, BB, BC, BD, BE

Shops	SA, SB, SC, SD, SE
Milkman	MA, MB, MC, MD, ME

Microbiological Analysis

Total Plate Count (TPC):

To assess the microbial load, TPC analysis began with the preparation of saline solution (8.9g NaCl/L). Nutrient agar media was prepared by dissolving 23g of powder in 1 liter of distilled water, followed by heating, boiling for one minute, and autoclaving at 121°C for 15 minutes. The media was poured into sterile glass tubes, set at an angle to create a slope, and allowed to solidify. Serial dilutions of milk samples were prepared in six sterile test tubes (10^{-1} to 10^{-6}) using 9 mL of saline in each tube, with 1 mL of the milk sample transferred sequentially. One milliliter of each dilution was inoculated onto Nutrient Agar plates, evenly spread, and incubated at 37°C for 24–36 hours. Colony counts in the range of 30–300 CFU/mL were recorded using a colony counter, and the microbial load was calculated as follows:

$$\text{Colony Counting (log CFU/mL)} = \frac{\text{Average No. of Colonies} \times \text{Dilution Factor}}{\text{Volume Factor}}$$

Total Coliform Count (TCC):

Coliform analysis was conducted using Eosine Methylene Blue Agar (EMB). Media preparation involved dissolving the agar, sterilizing at 121°C for 15 minutes, and pouring 15–20 mL into Petri plates for solidification. Milk samples were serially diluted (10^{-1} to 10^{-6}) as described in the TPC method, and 1 mL of each dilution was inoculated onto EMB plates. The plates were incubated at 35–37°C for 24 hours. Colonies within the range of 30–300 CFU/mL were counted, and TCC was calculated using the same formula as TPC.

Detection of Escherichia coli:

MacConkey Agar was used to detect E. coli. Media preparation involved dissolving 46.4g of powder in 1 liter of sterile water, followed by autoclaving at 121°C for 15 minutes. The media was poured into Petri plates and allowed to solidify. Ten-fold diluted milk samples were inoculated onto the prepared plates and incubated at 37°C for 48 hours. Colony counts were performed as described for TPC and TCC.

Detection of Adulterants

Several chemical tests were conducted to detect common milk adulterants:

- Starch: A 3 mL milk sample was heated, cooled, and mixed with 2–3 drops of 1% iodine solution. The presence of starch was indicated by a blue color (10).
- Water: Specific gravity was measured using a pycnometer; a lower specific gravity indicated higher water content.
- Sugar: A 3 mL milk sample was mixed with 2 mL hydrochloric acid and 50 mg resorcinol. A red color upon heating confirmed sugar adulteration (10).
- Formalin: A 10 mL milk sample was treated with 5 mL concentrated sulfuric acid and ferric chloride. A violet or blue color at the interface indicated formalin (11).
- Neutralizers: A 5 mL milk sample was mixed with 5 mL alcohol and a few drops of rosalic acid. A pinkish-red color indicated the presence of sodium carbonate or bicarbonate (11).
- Detergents: A 5 mL milk sample was treated with 0.1 mL bromocresol purple solution. A violet color indicated detergent (11).
- Urea: A 5 mL milk sample was mixed with p-dimethylaminobenzaldehyde. A yellow color indicated urea (11).

Data were analyzed using one-way ANOVA and Tukey HSD tests with the software Statistic version 8.1. Results were categorized as highly significant, significant, or non-significant based on the statistical findings (12).

RESULTS

The study analyzed various milk samples from Multan City to evaluate microbial quality and adulteration. Total Plate Count (TPC) indicated significant variation among milk sources. Packaged milk (UHT processed) showed no detectable microbial growth, while raw milk samples exhibited varied microbial loads. The highest mean TPC, 1.53 (10^4 CFU/mL), was observed in milkman samples, followed

by farm samples at 1.31 (10^4 CFU/mL) and shop samples at 1.27 (10^4 CFU/mL). A highly significant difference ($P < 0.01$) in TPC values across sources highlighted contamination in raw milk, with packaged milk demonstrating superior microbial safety. Total Coliform Count (TCC) revealed significant contamination in raw milk, with levels ranging from 0.00 to 1.32 (10^4 CFU/mL). Packaged milk samples showed no detectable coliform presence, while milkman samples exhibited the highest contamination at 4.63 (10^4 CFU/mL). Farm samples averaged 3.82 (10^4 CFU/mL), and shop samples registered 4.50 (10^4 CFU/mL). The absence of coliforms in UHT milk reflected the efficacy of thermal processing.

Escherichia coli contamination was detected in 60% of milkman samples and 40% of farm and shop samples, with no presence in packaged milk. This highlights significant contamination risks in raw milk sources during handling and distribution. Adulteration testing identified starch in 20% of milkman and shop samples, while all packaged milk and farm samples were free from starch adulteration. Formalin adulteration was found in 20% of milkman and shop samples, but not in packaged or farm milk. Urea was detected in 40% of milkman and shop samples, with none in farm or packaged milk. Neutralizer presence was similar, with 40% of milkman and shop samples showing contamination, while packaged milk and farm samples remained free from neutralizers. Water adulteration was the most prevalent, observed in 100% of milkman and shop samples, 60% of farm samples, and absent in packaged milk. Detergents were detected in 20% of milkman and shop samples, with no evidence in farm or packaged milk. Packaged milk demonstrated superior compliance with safety standards, while raw milk sources exhibited significant microbial and chemical contamination.

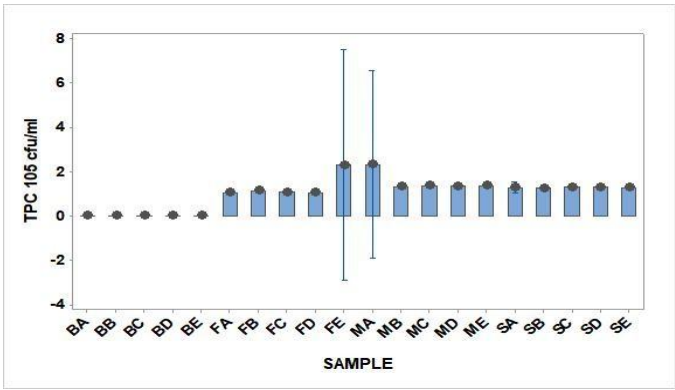

Figure 1: Comparison of TPC of different milk samples

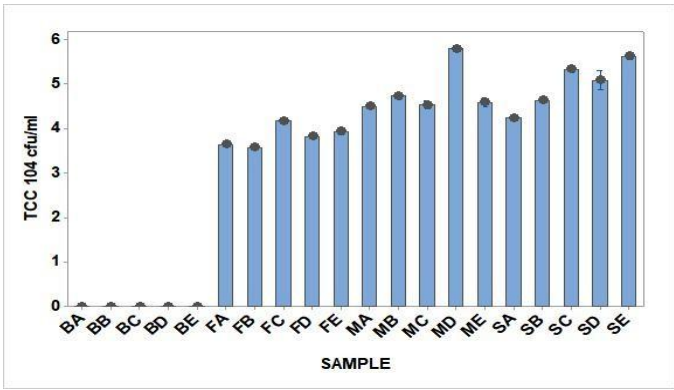

Figure 2: Comparison of TCC in different milk samples tested

Table 1: E. coli detection in different milk samples

Samples	Milk farms					Dairy shops					Milkman					Packaged milk Of different brands				
	FA	FB	FC	FD	FE	SA	SB	SC	SD	SE	MA	MB	MC	MD	ME	BA	BB	BC	BD	BE
E. coli	+	+	-	-	-	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-

Table 2: Percentage of Adulterants Detected in Different Milk Sources"

Source of milk sample	Milk farms	Milkman	Dairy shops	Packaged milk
Starch (P)	0%	20%	20%	0%
Starch (A)	100%	80%	80%	100%
Formalin (P)	0%	20%	20%	0%
Formalin (A)	100%	80%	80%	100%
Urea (P)	0%	40%	40%	0%

Urea (A)	100%	60%	60%	100%
Neutralizer (P)	0%	40%	40%	0%
Neutralizer (A)	100%	60%	100%	100%
Sugar (P)	0%	0%	0%	80%
Sugar (A)	100%	100%	100%	20%
Water (P)	60%	100%	100%	0%
Water (A)	40%	100%	100%	100%
Detergent (P)	0%	20%	20%	0%
Detergent (A)	100%	80%	80%	100%

DISCUSSION

The findings underscored the significant variability in microbial quality and adulteration across different milk sources in Multan City. Packaged milk consistently demonstrated superior microbial safety, as evidenced by undetectable TPC, TCC, and E. coli counts. This highlights the efficacy of UHT processing in ensuring microbial safety and reducing public health risks. In contrast, raw milk from milkmen, farms, and shops exhibited substantial microbial contamination, with milkman samples showing the highest levels of TPC, TCC, and E. coli. Such contamination is attributable to unhygienic handling, poor milking practices, and inadequate equipment sanitation. The results align with prior studies reporting higher contamination rates in raw milk compared to processed milk (13, 14). The study's findings on adulteration reinforce the prevalence of unsafe practices in raw milk handling. Water adulteration was the most frequent, compromising milk's nutritional value and posing risks to consumer health. The detection of neutralizers, starch, urea, and detergents in raw milk further highlights the need for stringent quality control measures. Although packaged milk adhered to safety standards, the presence of sugar adulteration in processed milk raises concerns about product integrity. These observations emphasize the necessity of regular monitoring and strict enforcement of milk quality regulations, particularly for raw milk sources.

A notable strength of this study was the comprehensive analysis of microbial and adulteration parameters across diverse milk sources. The inclusion of both raw and packaged milk provided a holistic view of the milk supply chain's quality and safety. However, a limitation was the relatively small sample size, which may not fully capture variability across all milk vendors in the region. Future studies with larger sample sizes and inclusion of seasonal variability could provide a more nuanced understanding of milk safety trends. The study highlights the dual challenges of microbial contamination and adulteration in raw milk. While packaged milk emerged as a safer alternative, the high contamination levels in raw milk underscore the need for robust interventions to improve hygiene practices and reduce public health risks. Enhanced awareness, coupled with strict regulatory oversight, could significantly improve milk quality and ensure consumer safety. These findings provide critical insights for policymakers and stakeholders in the dairy industry to address prevailing gaps in milk safety and quality assurance.

CONCLUSION

This study aimed to compare the quality of branded milk and raw milk, revealing that while raw milk is more nutrient-dense, it is also significantly more susceptible to microbial contamination due to unsanitary conditions and inadequate hygiene practices. Factors such as poor storage facilities, unstable refrigeration, and improper transportation methods further increase the risk of contamination, particularly in milk sourced from shops and milkmen. The widespread practice of milk adulteration for financial gain, including the addition of water, stabilizers, and other substances, highlights the need for stricter quality control measures. The findings emphasize that improving hygiene standards, maintaining clean environments, and ensuring proper handling and storage methods are essential to minimize contamination, reduce adulteration, and prevent milk wastage. By addressing these issues, safe and high-quality milk can be made accessible to consumers at reasonable costs, ensuring public health and trust in the dairy supply chain.

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