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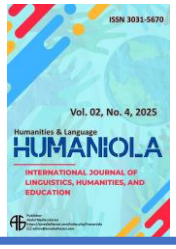

Determination of Hygiene Status of Milk Sold in Lahore, Pakistan

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ABSTRACT

Although milk is a vital source of nutrients and energy, its safety depends heavily on sanitary handling, storage, and marketing procedures. This study used proximate analysis, microbial load estimation, and field-based hygiene assessments to examine the cleanliness state of raw milk sold in Lahore, Pakistan. In accordance with AOAC protocols, samples were gathered from two disparate urban areas (Iqbal Town and Gulberg) and examined for fat, protein, moisture, ash, acidity, total solids, and solids-not-fat content. The Methylene Blue Reduction Test (MBRT) and the Total Bacterial Count (TBC) were used to evaluate the microbiological quality. The majority of the samples did not meet Punjab Pure Food and Codex requirements, according to the data. According to MBRT results, just 13.3% of samples were good, 23.3% were fair, and 63.6% were of poor quality. In almost every instance, TBC levels were higher above allowable limits, suggesting a substantial danger of microbiological contamination. These findings were supported by field surveys, which showed that 80% of vendors sold milk in thin plastic bags that encourage bacterial growth and chemical contamination, 35% used containers that were not properly cleaned, and 65% of vendors had poor personal hygiene. Pathogens including Salmonella, Listeria, and E. coli pose major health concerns to the population, and the lack of cold chain management further degraded milk quality. In order to guarantee milk safety and the welfare of consumers, the study emphasizes the critical need for more hygienic enforcement, better transportation and storage procedures, and awareness campaigns.

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1. Introduction

Pakistan's economy depends heavily on the dairy industry; livestock adds almost 49% to the value of the country's agricultural sector and accounts for 11.4% of its GDP (Nadeem & Ahmad, 2024). More than any other significant crop, milk is the most significant commodity in terms of GDP contribution. Notwithstanding these financial benefits, Pakistan's dairy industry still confronts significant obstacles, notably in the

areas of cold chain management, transportation, and cleanliness upkeep (Rehman et al, 2017). Despite being one of the world's top milk-producing nations, Pakistan frequently falls short of international and national criteria for raw milk safety and quality.

Raw milk is essential for human health since it is a nutrient-rich food that includes proteins, lipids, lactose, vitamins, and minerals (Lucey, 2015). However, when handled under unsanitary settings, its neutral pH and high water content make it a perfect habitat for microbial development (White & McCarthy, 1982). The dangers of consuming raw milk in urban settings have been highlighted by studies conducted in Lahore, which have shown that it frequently contains significant microbiological loads, chemical adulterants, and even heavy metals. Raw milk was found to be inferior in terms of composition and hygiene in other parts of Pakistan, including Rawalpindi, Islamabad, and Swat. It was frequently tainted with water and tainted with pathogens. These problems are not specific to Pakistan; research from Morocco and Ethiopia shows that, as a result of poor handling, storage, and sanitation procedures, raw milk frequently has bacteria levels over permissible limits in underdeveloped nations (Arif et al, 2022; Muhammad et al, 2009).

This circumstance highlights a crucial knowledge gap: although Pakistan produces excellent milk, insufficient attention has been paid to maintaining consumer safety and hygiene standards in urban milk markets (Garcia et al, 2003). In addition to improving public health, closing these gaps is essential for bolstering customer confidence and promoting long-term growth in the dairy industry. Therefore, the current study examines the hygienic condition of raw milk sold in Lahore, paying special attention to its microbiological safety and nutritional value (Wasim, 2005).

Dairy sector is very important for Pakistan’s economy livestock contributes about 49% value addition in agriculture sector and about 11.4% to Pakistan’s GDP. Milk is single most important commodity that contributes more to GDP than any other major crop (Shahzad, 2022). Pakistan is a developing country and dairy sector faces major problems regarding hygiene maintenance, transportation and cold chain. Despite all this Pakistan is one of the largest milk producing country in the world (Nadeem & Ahmad, 2024). Milk is a whitish liquid and it contains milk proteins, fats, lactose, vitamins and minerals. It is produced by mammary glands of female mammals. Milk is the product obtained after milking of female animal. It should be in good health and it should not contain colostrum. Milk is nutrient rich food and variation of amount of nutrients is based upon type of specie, breed lactation time and diet of animal. Whole milk contains about 87.4% water, 3.22% protein, 3.25%fat, 0.69%ash, and carbohydrate 4.52% (Smith et al, 2022). Cow milk is the staple diet, wholesome food and it is nutrient rich food that provides energy and high quality proteins. Milk possesses all the nutrients that are needed for maintenance and growth of human health. It is a source of quick energy, easily digestible and absorbed in intestine (Lucey, 2015).

In year 2013-2014 survey, Pakistan was one of the fourth larger producers of the milk. Pakistan is a developing country and rules and regulations are not followed by farmers, milkmen etc. On farm milk handling and practices should be improved (Nadeem & Ahmad, 2024). In 2019, an extensive research was done in Lahore about the raw milk chemical composition, microbial load, adulteration and heavy metals in dairy farms and urban areas of Lahore. Total three hundred and sixty raw milk samples were collected. Total lactose, fat, total solids, pH, total ash content was determined by standard procedures. Results of lactose presence in all samples had mean value from 2.758+-0.288.For fat overall mean value was 2.629+- 0.180.Proteins had mean value of 1.270+-0.077.pH of the raw milk from dairy farms and urban areas had mean value 6.456 +-

0.264.Ash values were 0.472+-0.022.TPC for raw milk were 4.674+-0.273 and 5.206+-0.823 CFU/ml respectively. Microbial load was not significantly different in both areas. Transportation facilities and seasonal changes also effect the microbial growth. In humid and hot weather microbes grow rapidly (Arif et al., 2024; Haider et al, 2025).

A study is conducted in Rawalpindi and Islamabad regions in which total forty samples of fresh milk of sheep, cow, buffalo and goat were collected in sterile bottles and all the experiments are performed in NARC Islamabad. Lactometer reading, specific gravity, pH determination, titratable acidity, fat, solid not fat, total solids protein content ash content and lactose content is measured by official methods of analysis (AOAC 10th edition). In collected samples milk of buffalo shows 5.25% and 14.04% percent fat and total solids content and in cow milk 4.56% and 13.73% fat and total solid contents (Arjuna, 2017). According to US public health service milk ordinance lactose content should be 4.8%.Collected samples have values near to standard values. Collected milk sample had shown ash content values ranging from 0.4% to 0.58%. Protein content of cow is 5.23% and buffalo 3.87% from collected samples (Kanwal et al., 2004).

A study had been done in Sawat district of Pakistan about milk adulteration; composition and microbial load. Total of 150 samples were collected from 3 tehsils of Sawat. Results for minimum fat value from all the samples were 2.40% and maximum was 6%.Solid not fat mean value was 6.11% and range was 3.91%-7.32%. Protein minimum value was 1.77% and maximum 3.35%.Average lactose value was 2.69% and minimum value was 1.71% and maximum 3.22%. Milk pH was 5.75-6.20. Adulteration of water had average of 36.22% with maximum value of 47.88 % (Khan et al., 2018).

Microbiological analysis to determine the quality of raw milk was done in the north-east of Morocco. Total 80 samples were collected and research was done in three years. Total plate count, coliform count, and fecal coliform analysis were done. Average of total plate count was 1.4x106 CFU/ml. Averages of total coliform and total fecal coliform were 2.6x103 CFU/ml and 1.9x102 CFU/ml respectively (Belbachir et al., 2015).

2. Method

2.1. Study Area and Sample Collection

The study was carried out in the labs of the University of the Punjab's Faculty of Agricultural Sciences in Lahore in 2024–2025. Samples of raw milk were gathered from the Iqbal and Gulberg urban areas of Lahore. These areas were chosen because they reflect various commercial and socioeconomic traits. Iqbal Zone is a mixed-income neighborhood with more conventional milk markets, whereas Gulberg is a comparatively affluent business district with contemporary dairy stores. Comparing hygienic practices under various retail and customer settings was made possible by this contrast. The milk's temperature was noted at the time of collection, and the cleanliness of the stores, milkmen, and storage containers was evaluated using a standardized questionnaire.

2.2. Proximate Analysis

- Titratable Acidity: As per AOAC Official Method 947.05 (AOAC, 1990), titration with standardised 0.1 M NaOH was performed until a persistent pink endpoint was seen.
- In accordance with AOAC Official Method 990.20, the oven-drying method is used to measure total solids and solids-not-fat (SNF). Fat % was subtracted from total solids to determine SNF (Getachew, 2003).

- The Gerber method (AOAC 2000.18) with an acid butyrometer was used to determine the fat content (Richardson, 1985).
- The Kjeldahl method (AOAC Official Method 991.20) was used to estimate the protein content (Lynch & Barbano, 1999).
- • Ash Content: As per AOAC Official Method 945.46, ash was incinerated at 525 °C in a muffle furnace until a consistent weight was reached.
- O'Mahony (1988) states that a lactometer is used to measure specific gravity.

2.3. Microbiological Analysis

- Total Bacterial Count (TBC): Per ISO 4833-1:2013 (Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony count at 30 °C), the spread plate method is used. The criteria used to assess the samples were modified from the International Commission on Microbiological Specifications for Foods (ICMSF, 2006) and Flottorp et al, 2018).
- Methylene Blue Reduction Test (MBRT): Used in accordance with FAO/WHO (2009) and Gashaw & Gebrehiwot (2018) standards to evaluate the hygienic quality of milk. According to the decolonization time (≥8 h, 6–8 h, 2–6 h, and <2 h, respectively), milk quality was categorized as Excellent, Good, Fair, or Poor overall.

2.4. Data Interpretation

The proximate composition criteria were contrasted with the Codex Alimentarius guidelines (Codex Stan 206-1999) and the Punjab Pure Food Rules (2018). Interpretations of microbial quality adhered to the ICMSF (2006) raw milk safety standards.

3. Results and Discussion

The study's findings raise significant questions about the safety and hygienic conditions of raw milk that is sold in Lahore. The MBRT test rated 63.6% of the samples as "Poor" quality, with the majority displaying microbial burdens beyond allowable limits. These results are in line with those of Akinjogunla et al. (2022), who found that raw milk samples taken from Lahore's urban markets and dairy farms had high total microbial counts. In a similar vein, Hussain et al. discovered extensive microbiological contamination and adulteration in Swat milk samples. Our investigation, in contrast to these other research, integrated laboratory analysis with on-site hygiene assessments, offering a more thorough knowledge of the direct relationship between poor handling procedures and decreased milk quality (Nawaz et al, 2022; Khan et al, 2014). Geographical variables, the type of markets examined, and variations in sample collection dates (seasonal temperature effects) can all contribute to discrepancies in microbial counts across research (Zafar et al, 2025).

These discoveries have important implications for public health. Elevated Total Bacterial Counts (TBC) in raw milk indicate possible contamination with foodborne illness-causing organisms as Salmonella spp., Escherichia coli, and Listeria monocytogenes (Kilango, 2011). Typhoid fever, gastrointestinal disorders, and even potentially fatal illnesses can result from consuming such tainted milk, especially in young children, the elderly, and people with weakened immune systems (Arvanitoyannis, 2010). The risks are further increased by the presence of adulterants

and improper cold-chain storage, which lowers the nutritional content of milk and exposes consumers to harmful risks.

Crucially, the results of the cleanliness questionnaire (Table 4) closely match the results of the laboratory. For example, the high percentage (63.6%) of milk samples that scored as "Poor" quality in the MBRT test is congruent with the fact that 65% of merchants demonstrated poor personal hygiene. Similarly, the majority of samples showed high bacterial growth, which was probably caused by inadequate container cleaning (35% rated "poor"). These associations highlight the fact that inadequate hygienic standards in milk handling and marketing are a direct cause of milk contamination, in addition to being a laboratory observation.

This study has many drawbacks in spite of its contributions. The sample size could not be entirely typical of all Lahore milk markets, even though it is enough to show distinct tendencies. Only two zones were sampled, which might not have well represented the city's variety. Furthermore, despite the fact that microbial growth in milk is known to increase during hot and humid months, seasonal fluctuation was not systematically investigated (Fox, 2015; Barbano et al, 2006). To better understand temporal differences in milk quality, future research should conduct sampling throughout multiple seasons and extend the sample coverage to include additional parts of Lahore.

Overall, this investigation supports the significant hygienic and safety issues associated with milk sold in Lahore's urban markets. Strong evidence for the urgent need for enhanced hygiene training, more stringent regulatory enforcement, and public awareness campaigns to protect consumer health is provided by the findings, which link bad laboratory results with observed handling methods.

This study highlights the food safety and hygiene conditions in the Lahore city. It also evaluates the low nutritional values and microbial load presence in raw milk samples. Below mentioned tables describe the representative samples percentages and standard values for proximate analysis and microbial load of raw milk samples.

Table 1. Shows standard values and representative samples% in both zones

Proximate analysis	**Standard Values**	**Representative samples for each test in Iqbal zone**	**Representative samples % for eachtest in Gulberg Zone**
Acidity	0.14-0.16%	13.3 %	6.6%
Total solids	12-14%	56.6 %	46.6%
Solid not fat	8.5-9%	20%	33.3%
Fat	3.5-3.8%	50%	66.6%
Protein	3.1-3.5%	63.3 %	46.6%
Moisture	86.2-87.5%	30%	33.3%
Ash	0.7-0.8%	26.6 %	10%
Specific Gravity	1.026-1.032g/ml	40%	43.3%

Source: Field Survey, 2025

Table 1's "Representative samples %" column could be confusing because it's not immediately apparent what it means. The header should be changed to "Percentage of samples meeting standard values" for clarity if the goal is to display the percentage of milk samples that satisfy the corresponding standard values for acidity, fat, protein, ash, and other criteria. However, this distinction needs to be made clear in the text if "representative samples" refers to the percentage of samples that fall within a wider observed range rather than exactly satisfying the criterion. Readers will be better able to determine if the percentages represent broad distribution trends in the dataset or compliance with quality criteria if the labels are clear.

Table 2. Represents Microbial load in raw and pasteurized milk samples

Good quality milk	**1.3×10^{6} CFU/ml**
Poor quality milk	5×10^{6} CFU/ml
Prema milk	1.1×10^{6} CFU/ml
Adam‟s milk	1.5×10^{6} CFU/ml
All raw milk samples	>300 CFU/ml TNT (TOO NUMEROUS TO COUNT)

Source: Field Survey, 2025

Readers who are not familiar with the local market might not recognize the terms "Prema milk" and "Adam's milk," thus they should be defined in Table 2. These are brands of pasteurized milk that are sold commercially in Pakistan; their inclusion offers a helpful comparison point between the microbiological load of raw milk and that of regulated, processed products. Readers will be assured that these are branded pasteurized samples rather than raw milk if a brief explanation is included in the text or as a table footnote. This will also draw attention to the difference between the severely contaminated raw milk offered in local markets and the comparatively safer commercial products.

Table 3. Represents samples %for methylene blue reduction test

Quality	**De colorization Time**	**Samples%**
Good	> 6 Hours	13.3%
Fair	< 6 Hours	23.3%
Poor	< 2 Hours	63.6%
Excellent	8 Hours	None

Source: Field Survey, 2025

3.1. Visual Hygiene Assessment during Sample Collection

A questionnaire survey was filled during collection of samples from different shops and milk markets in Lahore. It reflects light on basic hygiene conditions of market from which we purchase milk for our consumption

Table 4. Gives visual hygiene assessment of milk collection areas

Hygiene of Containers	25% in good condition	40% in fair condition	35% in poor condition
Personal Hygiene of milkmen and shopkeeper	10% in good personal hygiene	25% in fair personal hygiene	65% in poor personal hygiene
Milk Selling	20% milk in plastic bottles	80% samples in plastic bags	

Source: Field Survey 2025

There are significant ramifications for milk safety from the practice of selling milk in plastic bags, which is seen in 80% of cases as opposed to only 20% in bottles (Table 4). Local markets frequently utilize thin, non-food-grade plastic bags, which increases the risk of contamination during handling and transportation. These bags are rarely sterilized, in contrast to sealed bottles, and they can readily transmit bacteria from the milkman's hands or the surroundings.

Furthermore, milk cannot be kept at the recommended 4 °C temperature because plastic bags lack insulation (Kontominas, 2010). This speeds up bacterial growth in Lahore's hot environment, which is consistent with the high microbial loads and subpar MBRT results observed in this investigation. Chemical safety issues are also brought up by the usage of inferior plastic since dangerous substances could seep into the milk. As a result, marketing milk in plastic bags increases the hazards to customers from microbes and chemicals while also compromising hygiene.

4. Discussion

Results of all the research work were astonishing because of food safety and hygiene point of view. Most of the results were not according to legal standards. According to Punjab pure food regulations 2018 milk should have at least 3.5% fat, it should not have less than 12% total solids. Minimum SNF content in cow milk should be 8.5%. All the samples had the microbial load beyond acceptable limit and hygiene quality was not satisfactory. This is an eye opening situation for dairy industry and respective departments. In Lahore, skimming of milk, addition of water and adulterants is becoming a popular trend. It changes milk composition and increase the profitability for marketers. Moreover, hygiene issues were alarming and milk storage temperature was not maintained at 4℃. Personal hygiene was seen to be breached by workers so strict rules should be implemented regarding hygiene and cleanliness practices. Furthermore, milk having microbial load above acceptable limit can cause serious health hazard to human health. All the legislative and administrative authorities should take proper measures to control these problems. Food safety and personal hygiene awareness should be given to workers and to consumers. This will create a better understanding of food safety and consumer's well-being.

5. Conclusion and Recommendations

Milk temperature should be maintained at 4℃ so that microbial activity could be minimized. Proper awareness programs should be given to workers and consumers. Consumers should be aware of food safety problems and hazards related to unhygienic conditions. Transportation facilities should be improved so that milk transportation became easy and quality would be maintained. Legislative and administrative authorities should accelerate their actions tocontrol these problems.

5.1 Acknowledgement

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5.2 Novelty Statement

Most of the people in Pakistan prefer to buy raw milk for their consumption. This research work will play an important role in highlighting the food safety and hygiene issues in Lahore City. Moreover, this work will emphasize on improving the hygiene standards and regularity measures in shops and retail markets.

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