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Analysis of microbiological quality and adulteration of raw milk samples from different areas of Kathmandu Valley

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Abstract

Milk is a highly nutritious product that is susceptible to degradation due to microbial activity. Maintaining milk quality is crucial and can be achieved by monitoring specific parameters. This helps preserve the nutritive value of milk, which is essential for proper growth and health. Adulteration and improper storage can diminish the nutritional quality of milk. Therefore, this study aimed to assess the microbial load and adulteration of milk samples collected from various regions of the Kathmandu Valley. Sixty raw milk samples were gathered from local dairies (45) and cow farms (15) between April 2019 and July 2019. These samples were evaluated for microbial quality (total plate count, total coliform count, *Salmonella* spp., *Shigella* spp., and *Vibrio* spp.) and adulterants (starch, table sugar, soda, soap, and hydrogen peroxide) following standard guidelines. Out of the total samples, 58.3% (35) exhibited coliform growth, while *Shigella* spp. and *Vibrio* spp. did not grow on any media. Among coliforms, *Enterobacter* spp. was the most prevalent at 33.3%, followed by *Escherichia coli* at 32%. Antibiotic susceptibility testing revealed that the highest proportion of bacteria was sensitive to Ciprofloxacin and Gentamycin, followed by Ceftazidime. Adulteration analysis indicated that 33.3% and 48.3% of samples were adulterated with sugar and soda, respectively. Starch and soap were not detected in any analyzed samples. The highest titratable acidity (0.16%) was observed in cow farms compared to dairy farms. The findings of this study suggest an urgent need for routine quality testing of milk samples available in the market to prevent the spread of milk-borne diseases and preserve the nutritive value of milk.

Keywords: Raw milk, Microbiological quality, Antimicrobial susceptibility test, Adulteration, Acidity.

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Introduction

Milk is a nutritious food that provides essential nutrients for a healthy life. Bacteria can quickly grow in milk due to its nutritional content [1]. Although milk is almost sterile before milking, it can become contaminated with microbes during production, processing, and storage. Other sources of contamination include the environment, feed, soil, and feces [2]. Unhygienic and microbe-contaminated milk can transmit zoonotic diseases such as tuberculosis, brucellosis, shigellosis, and salmonellosis. Consuming poor-quality milk can transmit pathogens such as *E. coli* O157: H7, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Salmonella* spp from animals to humans or among humans [3]. Therefore, proper sanitation and hygiene practices during milk processing are essential. The microbiological quality of milk is crucial for consumer health and maintaining a strong relationship between sellers and consumers in the market [4].

Although milk-borne disease outbreaks have not been reported in Nepal, this may be due to a lack of adequate research. The potential for milk-borne diseases cannot be ignored, and daily milk screening is necessary to protect consumer health. High-quality milk is essential for producing high-quality dairy products. This study aimed to evaluate the bacteriological quality of raw milk samples from local dairies and farms in the Kathmandu Valley and to detect the presence of specific adulterants. This study was designed to evaluate the microbiological quality of unpasteurized milk samples from various local dairies and farms in the Kathmandu Valley and to identify the presence of specific contaminants.

Methodology

Study site and study period

Over the course of four months, from April to July 2019, 60 raw milk samples were collected from various local dairies and cow farms within the Kathmandu Valley. These samples were then subjected to thorough analysis at the Med-micro research laboratory in Babarmahal, Kathmandu

Sample and sampling method

A total of 60 raw milk samples were randomly collected from local dairies in the Kathmandu, Bhaktapur, and Lalitpur districts, as well as from cow farms. Within an

Table 1. Distribution of bacterial growth in VRBA and XLD with respect to sample sites.

Sample sites	Bhaktapur	Kathmandu	Lalitpur	Cow farm	Total
No. of samples	15	15	15	15	60
No. of samples with bacterial growth	10	9	8	8	35
No. of isolated bacteria	18	17	11	20	66
Percentage of isolated bacteria	27%	25.7%	16.7%	30.3%	100%

Table 2. Total bacterial and coliform counts accross sampling sites

S.N	Sample collection sites	Total bacterial counts in PCA plates (TBC) cfu m/L	Log cfu m/L	Total coliforms counts in VRBA plates cfu m/L	log cfu m/L
1.	Bhaktapur area	5.1×10^2	2.70	3.6×10^2	2.55
2.	Kathmandu area	4.2×10^2	2.62	3.1×10^2	1.02
3.	Lalitpur area	4.1×10^2	2.61	2.2×10^2	2.34
4.	Cow farms	8.2×10^2	2.91	5.8×10^2	2.76

hour of collection, all samples were transported to the laboratory in an icebox to maintain their integrity. Throughout the sample collection and processing procedures, stringent measures were implemented to minimize contamination.

Microbiological analysis

Serial dilution

Serial dilutions of all samples were prepared in sterile buffered peptone water (BPW). Plate count agar (PCA) was then employed to assess the total plate count (TPC) of bacteria, while violet red bile agar (VRBA) was utilized to determine the total coliform count (TCC), in accordance with the established guidelines [5].

Isolation and Identification of coliforms, *Salmonella* spp *Shigella* spp, and *Vibrio* spp

The isolation and identification of coliforms, *Salmonella* spp, *Shigella* spp, and *Vibrio* spp were carried out by employing their morphological and biochemical characteristics [5, 6].

Antibiotic susceptibility test

To assess the antibiotic susceptibility of the isolated bacteria, the modified Kirby-Bauer disc diffusion method on Muller-Hinton agar was employed, following the guidelines set forth by the Clinical and Laboratory Standards Institute [7].

Chemical analysis

For the chemical analysis, the presence of starch, table sugar, soda, soap, and hydrogen peroxide were tested for all the milk samples [6, 8].

Acidity

The acidity of milk samples was tested by using the following [6].

Titrable acidity = Vol. of NaOH consumed×0.009×100
Vol. of milk sample

Quality control

During sample processing, all the tests were carried out in aseptic conditions. Quality control during antimicrobial susceptibility testing was ensured by using the ATCC strain of *E. coli* (ATCC 25922).

Statistical analysis

The collected descriptive data was subjected to analysis using SPSS software (version 21). For the assessment of statistical relationships, the chi-square test was applied, with a p-value threshold of less than 0.05 considered statistically significant.

Result

Distribution of bacterial growth

All the raw milk samples showed growth in PCA (100%) while in VRBA and XLD, only 58.3% (35) samples showed different bacterial growth. Higher prevalence of contamination was observed in milk from cow farms (30.3%) and lower prevalence of contamination was observed in milk samples from Lalitpur area (16.7%). Statistical association between sampling sites and growth rate was not significant (p>0.005).

Table 3. Distribution of total organisms according to sampling sites

Sample sites	*E. coli*	*K. pneumoniae*	*C. freundii*	*Enterobacter* Spp	*Salmonella* spp	*Shigella* spp	*Vibrio* spp	Total	p- value
Bhaktapur	7	2	1	7	1	0	0	18	
Kathmandu	10	0	3	3	1	0	0	17	
Lalitpur	1	1	0	8	1	0	0	11	<0.05
Cow farms	3	0	11	4	2	0	0	20	
Total	21	3	15	22	5	0	-	66	
Percentage	32	4.5	22.7	33.3	7.5	-	-	-	

Table 4. Antibiotic susceptibility profile of isolated bacteria

Antibiotics	Antibiotics Susceptibility Test (%)				
	E. coli	*K. pneumoniae*	*C. freundii*	*Enterobacter* spp	*Salmonella* spp
Amoxicillin	57.14	-	0	0	-
Ciprofloxacin	100	100	100	100	100
Ceftazidime	100	100	60	100	-
Chloramphenicol	100	100	100	100	100
Cotrimoxazole	100	100	100	100	100
Gentamicin	100	100	100	100	100
Ampicillin	-	0	-	-	60
Ceftriaxone	-	-	-	-	60
Nalidixic acid	-	-	-	-	60

Differential Distribution of Total Bacterial and Coliform Counts in Different Sampling Sites

Cow farms exhibited significantly higher total bacterial and total coliform counts compared to dairies across the three districts investigated (**Table 2**).

Distribution of total organisms according to sampling sites

Sixty-six isolates of 5 different bacterial species were obtained among which, 92.5% (61/66) were coliforms and 7.5% (5/66) were *Salmonella* spp. Among coliform, *Enterobacter* spp 33.3% (22/66) was predominant followed by *E. coli* 32% (21/66), *C. freundii* 22.7% (15/66) and *K. pneumoniae* 4.5% (3/66). In this study, *Shigella* spp and *Vibrio* spp were not isolated. Isolation of coliforms was found to be statistically significant with *Salmonella* spp isolation (**Table 3**).

Antibiotic susceptibility profile of the isolates

Among the bacterial isolates, all *E. coli* and *K. pneumoniae* strains exhibited susceptibility to the tested antibiotics except amoxicillin and ampicillin, respectively. Additionally, all *C. freundii* isolates were resistant to amoxicillin and ceftazidime, while *Enterobacter* spp. isolates were susceptible to all antibiotics except amoxicillin. Furthermore, all *Salmonella* spp. isolates displayed susceptibility to ciprofloxacin, cotrimoxazole, chloramphenicol, and gentamicin, but exhibited resistance to ampicillin, ceftriaxone, and nalidixic acid (**Table 4**).

Distribution of Adulterants according to sampling sites

Among all milk sample, 68% samples were found to be adulterated. In the Bhaktapur area, 46.7% (7/15) samples were found to be positive with soda and 13.3% (2/15) samples were found to be positive with sugar. In the Kathmandu area, 40% (6/15) samples were found to be positive with soda and 33.3% (5/15) samples were found to be positive with sugar. In the Lalitpur area, 6.7% (1/15) samples were found to be positive for soda and 80% (12/15) samples were found to be positive for sugar. In the cow farms, 40% (6/15) and 66.7% (10/15) samples were found to be positive for soda and sugar respectively. However, hydrogen peroxide, soap, and Starch were not found to be adulterated in total samples

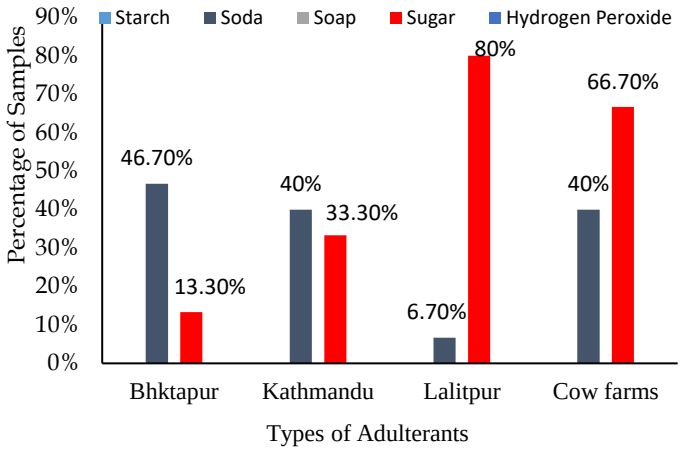

Figure 1. Distribution of adulterants according to sample size

Bacterial growth with respect to adulterated milk samples

Among total samples, 53.65% of bacterial growth was found in adulterated samples while 68.42% of bacterial growth was found in non-adulterated samples (Figure 2).

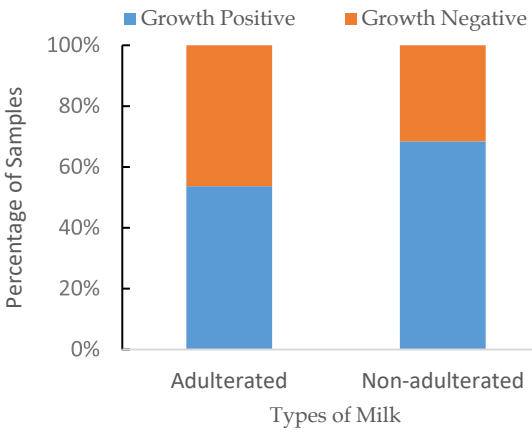

Figure 2. Bacterial growth with respect to adulteration

Titratable acidity of milk according to sampling sites

All milk samples which were collected from different areas showed different titratable acidity values of milk. The highest valve was 0.16% indicating high bacterial activity found in cow farms milk and the lowest valve was 0.13% indicating better quality of milk found from Lalitpur areas (**Table 5**).

Table 5. Titratable acidity of milk according to sample sites.

S.N	Collection area	Titratable Acidity (% lactic acid)
1.	Bhaktapur	0.15%
2.	Kathmandu	0.14%
3.	Lalitpur	0.13%
4.	Cow farms	0.16%

Discussion

Milk is one of the essential foods that provides a vital factor for the growth and development of the body. Since milk is rich in nutrients and acts as a suitable medium for the growth of all selective microorganisms. So, milk can act as a vector to spread infectious agents. These agents can cause mild to severe type of infections in human beings. Therefore good hygienic practice is mandatory [9].

In this study, all milk samples showed bacterial contamination in PCA but only 58.3% of samples showed growth while 41.7% did not show growth in VRBA and XLD. The growth of multi-bacteria in the PCA indicates there might be a chance of contamination with bacteria at the time of sampling. The mean TPC and TCC of raw milk samples were found to be 2.73 log cfu m/L (5.4×10^2 cfu/mL) and 2.55 log cfu m/L (3.6×10^2 cfu/mL) respectively. In the study, the mean of the total plate count of raw milk does not exceed the ranges of FDA Pasteurized milk ordinance i.e. 100000 cfu/mL [10]. The abundant growth of organisms in the TPC clearly indicates that there is a practice of poor milking systems and even dirty equipment surfaces that act as the source of nutrients for the growth and multiplication of bacteria that contaminate the milk [11]. The average total plate count (TPC) value obtained in this study was lower than the findings of previous studies conducted by El-Diasty and El-Kaseh (2009), Tasci (2011), Belbachir et al. (2015), and Acharya et al. (2017). These studies reported mean aerobic bacteria counts in raw milk of 6.1×10^5 cfu/mL, 3.95×10^6 cfu/mL, 1.4×10^6 cfu/mL, and 2.0×10^7 cfu/mL, respectively. However, the TPC value in this study was higher than those reported by Moustafa et al. (1988) and Mohamed and El Zubeir (2007), who found mean values of 1×10^1 cfu/mL and 5.63×10^1 cfu/mL, respectively. The elevated bacteria counts in the milk samples in this study suggest potential issues with hygiene practices or improper pasteurization procedures [18].

The presence of coliform bacteria in food indicates that contamination has occurred, likely from the hands of the milkman, milking equipment, or contaminated water. According to the National Dairy Development Board, a coliform count exceeding 100 cfu/mL suggests poor hygiene practices [11]. In this study, the total coliform count (TCC) of raw milk was 3.6×10^2 cfu/mL, which is lower than the findings of previous studies by Moustafa et al. (1988), Mohamed and El Zubeir (2007), Acharya et al. (2017), and Hassan et al. (2015), who reported TCC values of 1×10^6 cfu/mL, 3.3×10^6 cfu/mL, 1.6×10^5 cfu/mL, and 1.8×10^6 cfu/mL, respectively [15-17, 19]. While the presence of coliform bacteria in raw milk does not always directly indicate fecal contamination, it does signify poor hygiene and sanitation practices during milking and handling [19].

In this study, coliform was obtained in 92.5 % of the total sample which is not in harmony with the aftermath of the Parajuli et al. (2018) study in which they reported 60% coliform from 20 milk samples [20]. The higher coliform count in this study signaled some factors that might be the possible cause; the uncleaned hand of the worker, milk collecting containers, uses of contaminated water, inappropriate storage, and packaging. The study highlights the potential for public health risks associated with milk consumption. This emphasizes the need for improved hygiene practices throughout the milk production chain, including proper milking techniques, sanitation of equipment, and appropriate storage and transportation. According to the California Department of Food and Agriculture, the coliform count in raw milk samples ranges from 4.5×10^3 cfu/mL to 2.03×10^6 cfu/mL [21]. However, the study performed by Srairi and colleagues reported the TCC varied from less than 30 to 2.08×10^7 cfu/mL in raw milk [22].

The presence of bacterial contamination in milk poses significant risks to consumer health because they can cause foodborne illnesses with symptoms ranging from mild gastrointestinal upset to severe complications. Therefore it is necessary for the microbiological analysis of milk in order to enumerate the microorganisms [11]. In this study, 66 isolates of 5 different bacterial species were isolated and identified where *Enterobacter* spp was the predominant isolate with 33.3% (22/66) followed by 32% *E. coli* (21/66), 22.7% *C. freundii* (15/66), 7.5%

Salmonella spp (5/66) and 4.5% K. *pneumoniae* (3/66). The finding from this study is found to be lower than the study reported by Kumar et al. (2010) in the southwestern region where they reported 30 (100%) *E. coli* and 29 (96.66%) *S. aureus* but higher than the study reported by Ali (2010) who reported 2.6% *E. coli* and 1.3% *Enterobacter* spp and Parajuli et al. (2018) who reported 50% *E. coli*, 10% *Citrobacter* spp, 10% *Klebsiella* spp and 20% *Enterobacter* spp [20, 23, 24].

The study also revealed the presence of antibiotic-resistant bacteria in milk samples, raising concerns about the overuse of antibiotics in dairy farming and the potential for the development of drug-resistant pathogens. On antibiotics susceptibility testing, all the *E. coli* was found to be susceptible to the tested antibiotics except amoxicillin. Among 21 *E. coli* isolates, only 57.14% of the *E. coli* isolates were susceptible to amoxicillin. All the *K. pneumoniae* were susceptible to all tested antibiotics except ampicillin. However, 3 *K. pneumoniae* isolates, 100% spp were resistant to ampicillin. All the *C. freundii* were susceptible to the antibiotics used except amoxicillin and ceftazidime. In the case of ceftazidime and amoxicillin, 60% and 0% of 15 *C. freundii* isolates were susceptible respectively. All the *Enterobacter* spp were susceptible to the tested antibiotics except amoxicillin. However, all the isolates of *Enterobacter* spp isolates were resistant to amoxicillin. All *Salmonella* spp were found to be susceptible to ciprofloxacin, cotrimoxazole, chloramphenicol, and gentamicin followed by ampicillin, ceftriaxone, and nalidixic acid. A study performed by Yasmin et al. (2014) reported the susceptibility profile of *Salmonella* spp viz. ampicillin (88.89%), cotrimoxazole (77.78%), chloramphenicol (22.22%), ciprofloxacin (11.11%) and cefixime (11.11%) [25].

Adulteration of milk is a common practice that involves adding poor-quality substances to natural milk to gain economic advantages. However, this practice poses significant risks to human health and compromises the efficacy of milk. In this study, 48.3% of milk samples were found to be adulterated with table sugar, followed by 33.3% with soda. These findings align with previous studies, such as one by Parajuli et al. (2018) which reported 55% of milk samples adulterated with soda and 10% with table sugar. The high prevalence of milk adulteration highlights the need for stricter regulations and enforcement to protect consumer health [20].

Evaluation of acidity in milk is crucial to retain its quality which measures the bacterial load along with their enzymatic activity. In present study, the highest value of titrable acidity was observed in cow farms (0.16%) and the lowest value was observed in the Lalitpur area (0.13%). According to Troy and Sharp, the titrable acidity of fresh milk samples lies between 0.12% and 0.20% [27]. The acidity of the pasteurized milk ranged from 0.14% to 0.16%, whereas the Bangladesh Standards and Testing Institution, BSTI (2002) sets the maximum acidity of pasteurized milk at 0.15% [28].

Conclusion

An analysis of milk samples in current study revealed that the majority were adulterated with sugar and soda, while no adulteration with starch, soap, or hydrogen peroxide was detected. From a microbiological quality standpoint, milk samples from Kathmandu, Lalitpur, and Bhaktapur exhibited higher levels of contamination. The highest bacterial contamination (TBC and coliform) was observed in milk samples collected from cow farms. These findings underscore the need for stringent monitoring and implementation of good hygiene practices during milking and subsequent milk processing steps. The high prevalence of bacterial contamination and adulteration in milk necessitates stricter regulations and enforcement by the government. Developing and implementing national standards for milk quality and safety, including mandatory testing, awarding, and penalties, will be crucial in ensuring adherence to hygiene and sanitation standards. Elevating the investment in research and raising public awareness through targeted campaigns and educational initiatives will foster improvements in milk safety practices. Through collaboration between stakeholders, including government agencies, dairy industry leaders, and consumers, the risk of milk-borne illnesses can be eliminated and a safe and healthy milk supply can be ensured.

Author's Contribution

LP is the principal investigator, carried out laboratory investigation and drafted the manuscript. SP, RK and RM edited the manuscript. AS conceptualized the research and edited the manuscript.

Competing interest

The authors disclosed that there is no competing interest.

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Ethical approval and consent

Not applicable.

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