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Hygienic production practices and microbial evaluation of cow milk quality in District Gilgit

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ABSTRACT

Background: Dairy cow's raw milk may get contaminated by pathogens released by diseased animals, bacteria from the environment, or germs from the udder. Insufficient hygiene on the udder and teat surfaces, as well as contaminated milking equipment, can transfer environmental organisms into the milk. **Methods:** To examine the practices of hygienic cow milk production, a study was conducted with 175 respondents from District Gilgit, who were selected to evaluate their understanding and implementation of hygienic milk production methods. The participants were interviewed to determine their level of understanding. In addition, fifty milk samples were collected from the producers to assess the microbiological purity of the milk. **Results:** The findings revealed that a significant proportion of milk producers neglected or were unaware of essential hygiene practices, such as washing hands, udders/teats of cows, and milking utensils before milking. The predominant water sources used were tap and stream, with the barn conditions largely being dusty despite daily cleaning efforts by most respondents. It was observed that milk processing and refrigeration post-milking were common practices, though 14.6% reported using injections and supplements preceding milking activities. The quality of milk was evaluated using the Methylene Blue Reduction Test (MBRT), Total Bacterial Count (TBC) and Total Coli form Count (TCC). Analytical results indicated MBRT durations ranging from 1 to 8.5 hours, with TBC and TCC levels extending from $2.16x10^6$ to $1.008x10^7$ and $3x10^5$ to $6.56x10^6$, respectively. Most of the milk samples were of fair quality having average MBR time and the TBC and TCC have values above the upper limits set to 10^5. **Conclusion:** we concluded that inadequate hygiene, low quality stream and tap water for cleaning, and poor barn conditions contributes to the low milk quality in Gilgit. Therefore, it is essential to enhance the hygienic dairy practices and promote public health.

Key Words: Raw milk, microbial quality, hygiene practices, Total Bacterial Count, Total Coliform Count, Methylene Blue Reduction Test.

Introduction

Milk is a crucial dietary element for a vast number of people worldwide, due to its substantial nutritional benefits. The hygienic quality of milk is of paramount importance from a public health perspective (1).

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Beyond its nutritional value, milk is esteemed as an essential component of human diets, often referred to as 'the most nearly perfect food,' owing to its rich content of calcium and phosphorus—crucial for the health of bones and teeth—alongside significant levels of vitamins B6, A and B1 (2,3).

The composition of milk, while generally consistent in its chemical constituents, exhibits variability based on factors such as the source species (e.g., cows, goats, buffaloes, sheep), breed, lactation stage, milking frequency, age, seasonal variations, diet, health conditions, and the administration of pharmaceuticals and hormones(4).

Concerns regarding the safety of dairy products are pronounced globally, particularly in developing nations where milk and a wide array of dairy products are produced under conditions marred by poor hygiene and substandard production practices (5, 6, 7, 8). The tendency of milk, whether raw or processed, to support microbial growth is well-documented, attributed to its high water content, nearly neutral pH, and the presence of essential nutrients (9, 10). The quality of raw milk can be compromised by bacterial contamination, which, along with the enzymes and toxins associated with certain bacteria, may pose health risks even after pasteurization (11).

Such microbial contamination can arise from various sources, including the air, milking apparatus, feed, soil, fecal matter, and vegetation, potentially affecting the exterior of the udder as well as the surfaces of milk handling and storage equipment (12). It is posited that the microbial quality of milk can be influenced by the dietary and housing conditions provided to cows (13). Handlers of milk and milk products who lack good hygiene suffer the risk of introducing harmful microbes into their products (14). Hygienic measures are essential to minimize microbial contamination and produce safe and high-quality goods for consumption. It also helps to minimize product loss and strengthens the position of smallholder milk producers in the selling of quality milk and milk products (15, 16).

Therefore, detailed investigation of hygienic production practices and microbial quality is very important to identify existing hygiene related problems. This study aims to evaluate the hygiene practices employed during milking and to conduct a microbial analysis of various milk samples, thereby contributing to the broader understanding of factors influencing milk quality and safety.

Methods

The study was conducted in the district Gilgit, Gilgit-Baltistan, Pakistan, focusing on the areas of Jalalabad, Oshikhandass, Danyore, Sultanabad and Nomal, where mixed crop-livestock farming systems predominate.

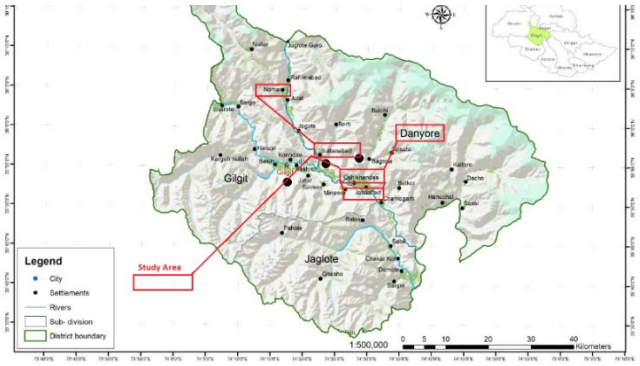

Figure-1: Map of Study Area.

This cross sectional analytical study was conducted in the Food Microbiology Laboratory of the Department of Agriculture and Food Technology, Karakoram International University from June 2021 to June 2022. This study was approved by Institutional review board of Karakoram

International University dated 3rd June 2021. The research comprised of two principal components: A questionnaire survey to examine milking hygiene practices among milk producers and laboratory analysis to assess milk hygiene and bacteriological load. For the laboratory analysis, 50 raw milk samples were randomly collected across the five regions representing 10 samples from every village, ensuring a comprehensive assessment of milk quality. These samples were analyzed at the Food Microbiology Laboratory of the Department of Agriculture and Food Technology, Karakoram International University. Complementarily, the questionnaire survey targeted 150 cattle owners, probing into their hygiene practices, with questions designed to uncover potential risks of contamination and the presence of extraneous materials. The study meticulously adhered to **(**International Commission on Microbiological Specifications for Foods) ICMSF guidelines for sample transportation and handling.

One milliliter (ml) from each sample of raw milk was transferred to 9 ml of sterile 0.1% peptone water and properly mixed to achieve a 1:10 dilution. Serial dilutions were made by transferring 1 ml of the previous dilution into 9 ml of sterile peptone water.

TBC was determined using the nutrient agar plate method (17). A 0.5 ml sample from the final dilution (10^5) was placed into a sterile Petri dish by adding the nutrient agar. The plate was then incubated for 24 hours at 37°C, with the TBC expressed in colony-forming units per milliliter (CFU/ml).The Total Coli form Count was determined using a MacConkey's agar medium, following the method(18). After incubating the plates at 37°C for 24 hours, typical pink colonies were counted.

The hygienic status of raw milk samples was analyzed using the methylene blue reduction test (19). One milliliter of methylene blue solution (1:25000) was added to each labeled and sterilized 20 ml screw-capped test tube containing 10 ml of sample. The tubes were then capped, inverted gently three times to ensure thorough mixing of the dye with the milk and incubated at 37°C. Observations were made every 2 hours over 8 hours, recording the time required for the methylene blue in the milk to decolorize.

Results

The findings reveal a range of practices at the farm level influencing milk quality, including the state of barns, the frequency of barn cleaning, the practice of washing hands and udders/teats before milking, the type of water utilized for cleaning (both for hands and milking equipment), the variety of storage containers employed, and the duration of milk storage at room temperature. Additionally, the study noted the administration of medicines or injections before milking and the intended purposes behind the milking processes.

In this study, 84% of respondents used metal pots for milking, while 11.8% and 4.2% of respondents across the five villages used plastic and wooden pots, respectively, as mentioned in Table 1. Notably, 69.4% of producers reported washing the cow's udder/teats before milking, contrasting with 30.6% who did not adopt this practice. The survey further revealed hand washing practices among participants, although it also noted the use of poor-quality water for cleaning and a lack of proper drying of hands, utensils, and udders before milking, underscoring the need for improved hygiene practices. Regarding milking frequency, 75.6% of respondents milked their cows twice

daily, while 24.4% did so once daily, indicating varied practices within the area.

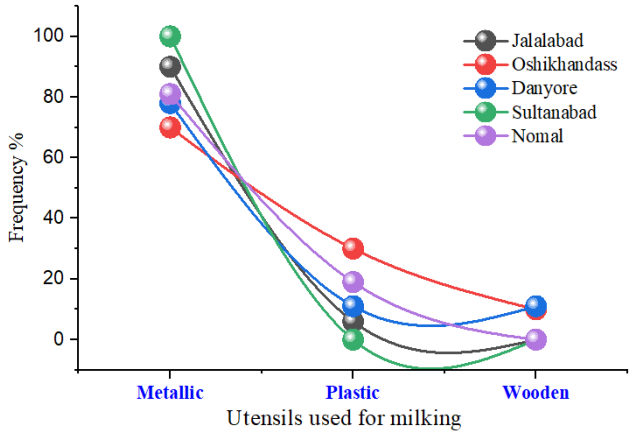

Figure-2: Utensils used for milking purposes.

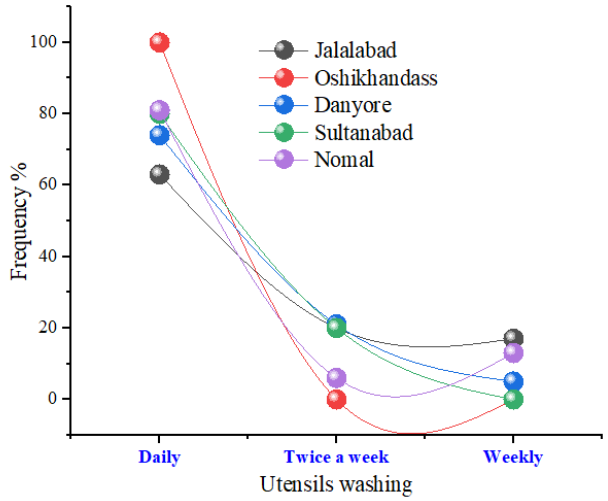

Figure-3: Utensils washing practices during different intervals.

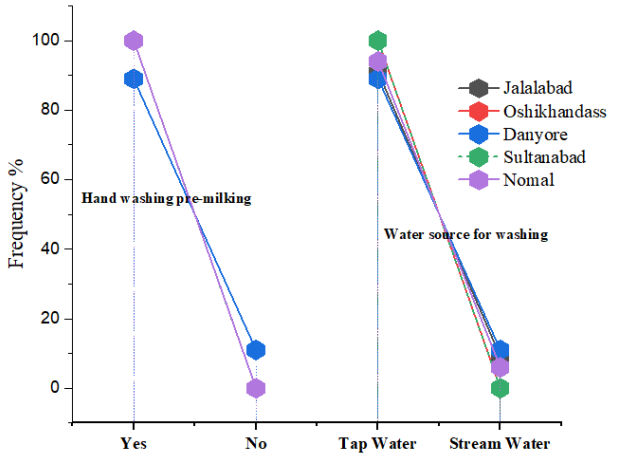

Figure-4. Pre-milking hand washing and using water resources for cleaning utensils.

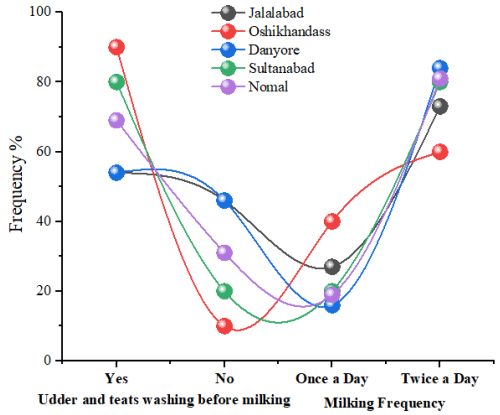

Figure-5. Udder, teat washing and milking frequency of respondents.

Table-1: Barn hygiene and cleaning frequency across the villages of the study area.

Variables	Jalalabad n=35	Oshikhandass n=35	Danyore n=35	Sultanabad n=35	Nomal n=35	Total
Bedding Condition						
Dusty	71% (25)	80% (28)	66% (23)	40% (14)	63% (22)	64% (112)
Muddy	29% (10)	20% (7)	34% (12)	60% (21)	37% (13)	36% (63)
Barn cleaning frequency						
Daily	43% (15)	57% (20)	17% (6)	12% (4)	34% (12)	32.6% (57)
Twice a week	25% (9)	23% (8)	20% (7)	14% (5)	20% (7)	20.4% (36)
Weekly	20% (7)	14% (5)	23% (8)	23% (8)	29% (10)	21.8% (38)
Monthly	12% (4)	6% (2)	40% (14)	51% (18)	17% (6)	25.2% (44)

Table-2: General milk handling practices adopted by producers across the villages.

Variables	Jalalabad n=35	Oshikhandass n=35	Danyore n=35	Sultanabad n=35	Nomal n=35	Total
Storage after milking						
Refrigerator	36% (13)	60% (21)	68% (24)	60% (21)	69% (24)	58.6% (103)
Cool place	64% (22)	40% (14)	21% (7)	20% (7)	31% (11)	35.2% (61)
Room temperature	0% (0)	0% (0)	11% (4)	20% (7)	0% (0)	6.2% (11)
Medication before milking						
Yes	9% (3)	20% (7)	5% (2)	20% (7)	19% (7)	14.6% (26)
No	91% (32)	80% (28)	95% (33)	80% (28)	81% (28)	85.4% (149)
Purpose of Milking						
House hold use	91% (32)	100% (35)	79% (28)	100% (35)	100% (35)	94% (165)
Commercial use	9% (3)	0% (0)	21% (7)	0% (0)	0% (0)	6% (10)
Animal feeding frequency						
Twice a day	27% (10)	50% (18)	0% (0)	20% (7)	6% (2)	20.6% (37)
Thrice a day	45% (16)	40% (14)	37% (13)	60% (21)	81% (28)	52.6% (92)
Four times a day	18% (6)	0% (0)	63% (22)	20% (7)	6% (2)	21.4% (37)
More than above	9% (3)	10% (3)	0% (0)	0% (0)	7% (3)	5.2% (9)
Milk processing						
Yes	91% (32)	100% (35)	47% (16)	100% (35)	88% (31)	85.2% (149)
No	9% (3)	0% (0)	53% (19)	0% (0)	12% (4)	14.8%26

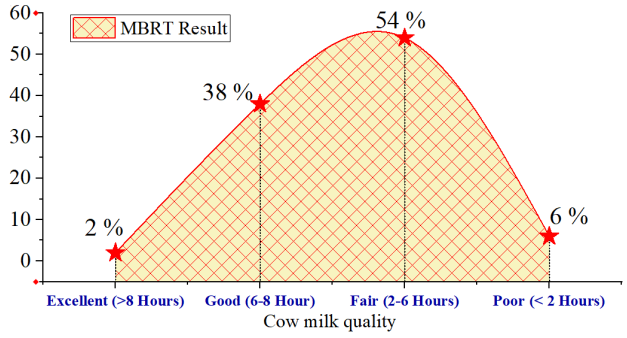

Figure-6: Mean values of MBRT of different milk samples collected from the study area.

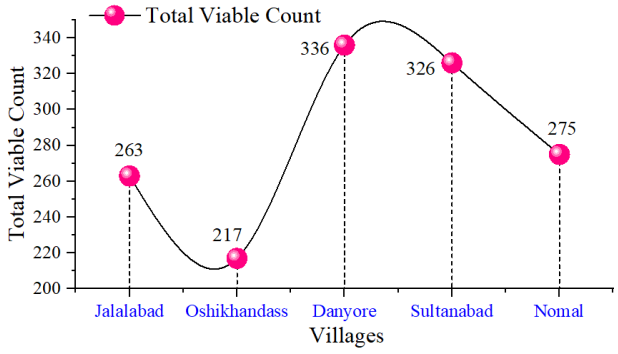

Figure-7: Mean value of Total Bacterial Count (TBC) at 10^5 dilutions in the study area of district Gilgit.

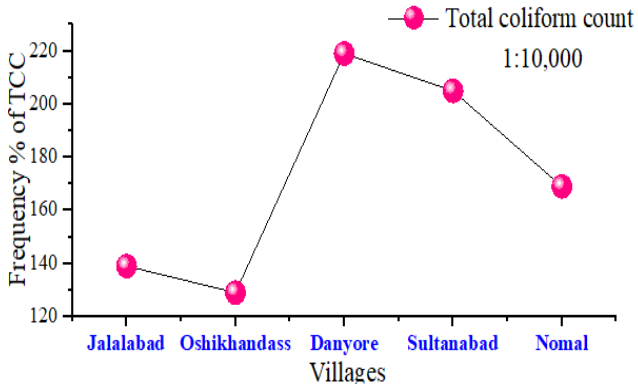

Figure-8: Mean value of Total Coli form Count (TCC) at 100 cell/ml in the study area of district Gilgit.

As indicated in Table 1, there is a significant variation among the villages of the study area, having dusty and muddy conditions used for their cattle. According to the survey, a majority (32.6%) of respondents clean their barns daily, while 20.4% do so twice a week, and 21.8% and 25.2% undertake cleaning weekly and monthly, respectively.

Figure 6 presents the results from the Methylene Blue Reduction Test (MBRT) conducted on 50 raw milk samples, collected

equally across five selected villages (10 milk samples from each village) in the Gilgit district. Of these, only 2% demonstrated excellent quality, while 38% were classified as good, 54% as fair and 6% as poor in quality.

Table-3. Mean value of Methylene Blue Reduction Test (MBRT) in district Gilgit.

Quality	Parameters	Study Area				
		Jalalabad	Oshikhandass	Danyore	Sultanabad	Nomal
Excellent	>8 Hours	0	1	0	0	0
Good	6-8 Hours	4	4	2	3	6
Fair	2-6 Hours	6	5	6	7	3
Poor	< 2 Hours	0	0	2	0	1

The analysis of raw milk samples from five distinct villages in the Gilgit district revealed significant variations in quality. The distribution of samples categorized as Excellent, Good, Fair, and Poor quality in Jalalabad was 0, 4, 6, and 0, respectively. For Oshikhandass, the breakdown was 1 (Excellent), 4 (Good), 5 (Fair), and 0 (Poor), while Danyore reported 0, 2, 6, and 2 in these categories, respectively. Sultanabad showed results of 0, 3, 7, and 0, and Nomal had 0, 6, 3, and 1, respectively. Specifically, Danyore's samples included two of good quality, six of fair, and two of poor quality. Sultanabad followed, with three samples of good quality. Conversely, Nomal and Jalalabad samples were predominantly of good quality, with counts of 6 and 4, respectively, alongside fair quality samples of 3 and 6. Notably, Oshikhandass stood out with one sample of excellent quality, alongside 4 good and 5 fair, indicating the highest overall milk quality among the villages surveyed.

Figure 7 displays the mean Total Bacterial Count (TBC) for raw milk samples from five villages in the Gilgit district, with means of 263, 217, 336, 326, and 275 colonies for Jalalabad, Oshikhandass, Danyore, Sultanabad and Nomal, respectively. The results indicate significant variations in TBC across the villages.

The Total Coli form Count (TCC) was analyzed in the laboratory, with results presented in Figure 8. Comparable to the Total Bacterial Count previously mentioned, the TCC revealed significant differences among the villages. Specifically, samples from Danyore exhibited the highest coli form numbers, in contrast to those from Oshikhandass which demonstrated the lowest. The data in the table represent the mean values derived from 10 samples collected from each village within the Gilgit district.

Discussion

The study highlights a range of farm-level practices influencing milk quality and these practices are crucial as they directly impact the microbial quality of milk. According to the study (20), emphasizes the crucial impact of the pre-milking udder and teat cleanliness on reducing milk's microbial load, preventing mastitis, and minimizing environmental contamination. The observation that 69.4% of producers washed the cow's teats/udder before milking, whereas 30.6% did not, consistent with the findings(21), which found that 82.5% of smaller farms in Hawassa city practiced udder washing before milking, highlighting its effectiveness in removing bacteria and visible dirt from the surface of the udder.

Similarly, study(22) investigated that moisture on teats could facilitate the entry of skin and environmental bacteria into the mammary gland, further emphasizing the importance of thorough cleaning practices. Therefore, it is crucial to ensure that the teats are thoroughly cleaned to prevent any bacterial contamination. Achieving hygienic milk production for consumer safety necessitates stringent hygiene practices, including the cleanliness of milking utensils, hand washing by milkers, and udder washing before milking and handling (23).

In the surveyed region, although most respondents reported cleaning their milking utensils, the water used for this purpose was often unhygienic, rendering the cleaning practices insufficient for eliminating milk residues and microbial contamination. The study highlights how contamination can occur from surfaces in contact with milk, such as hands and milking utensils. Water from rivers and taps, used for various cleaning tasks, was noted to be of substandard quality. Another study suggests that when relying on non-tap water for cleaning, it should be treated through heating or filtration to mitigate bacterial contamination risks (24).

The results also revealed varied milking practices within the area, with 24.4% of respondents milking their cows once daily, while 75.6% did so twice daily. The presence of dusty and muddy conditions, as observed in some villages, aligns with the findings that such conditions expose the teat end to sources of organic bedding, enhancing the risk of contamination and mastitis (25). Furthermore, maintaining clean and dry bedding is essential to minimize the proliferation of pathogenic microbes, as supported by the study (26). Moreover, another study stated that producing milk of high quality depends on keeping the barn clean (27). Another study finding, with 32.6% of respondents cleaning their barns daily, reflects the importance of a well-maintained housing environment for acceptable milk quality levels (28).

These findings highlight significant variations in milk quality within the Gilgit district. Notably, Oshikhandass emerged with a unique instance of excellent quality, indicating superior milk quality metrics overall. In contrast, Danyore was distinguished by the presence of poor-quality milk, signaling critical areas for enhancement. Both Nomal and Jalalabad demonstrated a notable prevalence of good quality milk, suggesting more uniform, milk handling practices that yield favorable results. Such disparities in milk quality across different areas emphasize the need for focused interventions to improve dairy hygiene practices throughout the district to provide consistent milk quality for consumer safety.

Conclusion

The investigation highlights varying microbial qualities of raw milk across the Gilgit district, pinpointing hygiene practices as a pivotal factor in ensuring milk safety. Oshikhandass demonstrated comparatively better outcomes, while Danyore identified critical needs for enhancement. These insights call for dedicated efforts to boost hygiene practices in milk production, achievable through targeted hygiene training, secure access to clean water, routine quality checks, heightened health awareness and infrastructure upgrades in dairy farming.

Conflict of Interest: The authors have no conflict of interest

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B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion

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