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Assessment of Microbial Quality and Hygienic Status of Milk, Dairy Products and Dairy-Contact Surfaces in Srinagar, Kashmir

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Abstract
Milk and dairy products are highly susceptible to microbial contamination and may serve as potential vehicles for transmission of foodborne pathogens. The present study was undertaken to assess the microbial quality and hygienic status of milk, dairy products, and dairy-contact surfaces marketed in Srinagar, Kashmir. A total of 210 samples comprising raw milk, pooled milk, pasteurized milk, paneer, ice cream, milking can swabs, and milking pail swabs (n = 30 each) were analyzed for total viable count (TVC), coliform count (CC), and occurrence of coliform bacteria using standard microbiological methods. Significant differences ($P< 0.05$) in TVC and CC were observed among the various sample categories. Paneer recorded the highest mean TVC (4.939±0.039 log10 colony-forming units (CFU) g^{-1}), whereas raw milk exhibited the highest mean CC (4.447±0.105 log10 CFU mL^{-1}). Comparison with Food Safety and Standards Authority of India (FSSAI) criteria revealed that several sample categories exceeded the prescribed microbiological limits. Overall, 24.18% of the samples were positive for coliform bacteria. Presumptive characterization of coliform isolates revealed the occurrence of *Escherichia coli* (*E. coli*), *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp., with *E. coli* (fecal coliform) constituting the predominant coliform isolate. The findings indicate deficiencies in hygienic handling, sanitation, and storage practices within the dairy production chain and underscore the need for implementation of effective hygiene management practices and routine microbiological surveillance to ensure food safety and protect the public health.

Keywords: Total viable count, Coliform bacteria, Food safety and Microbiological surveillance

Introduction
Milk and dairy products constitute an important component of the human diet owing to their high nutritional value and contribution to food security. However, their rich nutrient composition, near-neutral pH, and high water activity make them highly susceptible to microbial contamination during production, processing, storage, transportation, and retail marketing (Quigley *et al*., 2013) [25]. Consequently, ensuring the microbial safety of milk and dairy products remains a major challenge for the dairy industry and public health authorities worldwide (Haug *et al*., 2007) [16]. Microbial contamination of dairy products represents a significant public health concern due to its association with foodborne illnesses and the transmission of zoonotic pathogens (Newell *et al*., 2010) [24]. Contamination may occur at multiple stages of the dairy production chain, including milking, handling, processing, storage, and distribution. Inadequate sanitation of milking equipment, poor personal hygiene, improper storage conditions, and post-processing contamination can substantially increase microbial loads and compromise product safety, thereby posing risks to consumers, particularly children, the elderly, pregnant women, and immunocompromised individuals (Jay *et al*., 2005) [19]. Evaluation of microbial quality is essential for assessing the hygienic status and safety of dairy products. Total viable count (TVC) is widely used as an indicator of the overall microbial burden and sanitary quality of food products, whereas coliform count (CC) serves as an important indicator of fecal contamination and unsatisfactory hygienic practices during production and handling. Elevated TVC and CC may reflect deficiencies in sanitation, inadequate cleaning and disinfection procedures, poor temperature control, or contamination during processing and distribution (APHA, 2015) [3]. Members of the coliform group, including *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp.,

are frequently used as indicator organisms for monitoring the quality of milk and dairy products. Their presence often signifies contamination from environmental, animal, or human sources and may indicate conditions conducive to the survival and transmission of pathogenic microorganisms. Therefore, monitoring coliform contamination constitutes an integral component of food safety surveillance and quality assurance programmes within the dairy sector (Feng *et al*., 2020) [10].

To safeguard consumer health, microbiological criteria and standards have been established by regulatory agencies and international organizations, including the Food Safety and Standards Authority of India (FSSAI) and the International Commission on Microbiological Specifications for Foods (ICMSF). These standards provide benchmarks for evaluating the microbiological quality and acceptability of dairy products. Exceedance of prescribed limits may indicate inadequate hygienic practices and increased risk of foodborne disease transmission, necessitating corrective interventions across the dairy production chain (ICMSF, 2005 [18]; FSSAI, 2011 [11].

Kashmir Valley is characterized by substantial consumption of milk and traditional dairy products; however, comprehensive information regarding their microbiological quality and compliance with established food safety standards remains limited. Furthermore, contamination associated with dairy-contact surfaces such as milking cans and pails has received relatively little attention despite their potential role in microbial dissemination. Generation of region-specific data is therefore essential for evaluating food safety risks, strengthening surveillance programmes, and developing evidence-based interventions aimed at improving dairy hygiene and protecting public health.

In view of the above, the present study was undertaken to assess the microbial quality and hygienic status of milk, dairy products, and dairy-contact surfaces in Srinagar, Kashmir. A total of 210 samples comprising raw milk, pooled milk, pasteurized milk, paneer, ice cream, and swab samples collected from milking cans and pails were examined for TVC and CC. In addition, coliform isolates were characterized using conventional cultural and biochemical techniques to evaluate the microbiological status of the dairy production chain and its potential implications for food safety and public health.

Materials and Methods

Sampling and isolation

A total of 210 samples comprising raw milk (n=30), pooled milk (n=30), pasteurized milk (n=30), paneer (Indian soft cheese) (n=30), ice cream (n=30), milking pail swabs (n=30) and milking can swabs (n=30) were collected randomly from dairy farms, milk vendors, farmers, and retail outlets located in Srinagar, Kashmir. Samples were collected aseptically in sterile containers and transported to the Division of Veterinary Public Health Laboratory, Skuast-K under refrigerated conditions (4±1°C) using insulated ice boxes. All samples were processed immediately upon arrival at the laboratory following standard microbiological procedures (APHA, 2015) [3]. Details of the sampling are given in Table 1.

Table 1: Distribution of milk, dairy products and dairy-contact surface samples examined in the study

S. No.	Nature of Sample	Type of Sample	No. of Samples
1	Milk	Raw milk	30
2		Pooled milk	30
3		Pasteurized milk	30
4	Milk products	Paneer (Indian soft cheese)	30
5		Ice cream	30
6	Swabs	Milking pail	30
7		Milking can	30
Total			210

Enumeration of total viable count (TVC) and coliform count (CC)

TVC was determined using the standard pour plate technique. Briefly, ten-fold serial dilutions of each sample were prepared in sterile diluent and 1ml aliquots were inoculated into molten nutrient agar maintained at 45-50°C. Plates were incubated aerobically at 37°C for 24 h and colonies were enumerated. Results were expressed as $\log_{10}$ CFU mL^{-1} or $\log_{10}$ CFU g^{-1} or $\log_{10}$ CFU cm^{-2} (APHA, 2015) [3]. For CC, serially diluted samples were inoculated onto Violet Red Bile Agar (VRBA; Hi-Media, Mumbai, India) using the spread plate technique. Following incubation at 37°C for 24 h, characteristic lactose-fermenting colonies surrounded by a reddish-purple halo were counted as coliforms. Results were expressed as $\log_{10}$ CFU mL^{-1} or $\log_{10}$ CFU g^{-1} or $\log_{10}$ CFU cm^{-2} (APHA, 2015) [3].

Isolation and characterization of coliform bacteria

Presumptive coliform colonies obtained on VRBA were sub-cultured onto MacConkey agar (Hi-Media Laboratories, Mumbai, India) and incubated aerobically at 37°C for 24 h. Lactose-fermenting colonies were subsequently streaked onto Eosin Methylene Blue (EMB) agar and incubated under similar conditions. Colonies exhibiting characteristic metallic green sheen suggestive of *Escherichia coli*, along with other distinct morphotypes, were purified on nutrient agar slants. Pure isolates were subjected to Gram staining and conventional biochemical characterization, including catalase, oxidase, indole, methyl red, Voges-Proskauer, and citrate utilization tests. Identification of isolates as *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp. was carried out according to standard microbiological procedure described by Quinn *et al*. (2011) [26] and Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994) [17].

Statistical Analysis

Microbial counts were converted to logarithmic values ($\log_{10}$ CFU mL^{-1} or $\log_{10}$ CFU g^{-1} or $\log_{10}$ CFU cm^{-2}) prior to analysis and expressed as mean ± Standard error (SE). Statistical analysis was performed using IBM SPSS Statistics version 26.0. Differences among sample categories were evaluated at a significance level of $P< 0.05$.

Results and Discussion

The analysis of milk and dairy product samples unveiled a diverse microbial population, underscoring potential public health concerns. The TVC differed significantly ($P< 0.05$) among the various sample categories (Table 2; Fig. 1), indicating differences in microbiological quality and hygienic conditions across the dairy production and distribution chain. Elevated bacterial load in milk and dairy products is generally associated with inadequate sanitation during milking, processing, transportation, storage, and retail handling, thereby adversely affecting product quality and shelf-life (Bonfoh *et al*., 2003 [4]; Chye *et al*., 2004 [6]).

Table 2: Total viable count (TVC) of milk, dairy products and dairy-contact surface samples

S. No.	Source	No. of +ve samples (%)	Range*	FSSAI permissible limit	Mean ± SE*
1.	Raw Milk	30(100%)	4.041-5.164	< 2 million /ml	4.737±0.056^{ac}
2.	Pooled Milk	21(70%)	3.301-4.832	<2 million/ml	4.192±0.110^{d}
3.	Pasteurized Milk	14(46.6%)	4.000-5.021	<30,000 /ml	4.594±0.107^{a}
4.	Paneer	26(86.6%)	4.579-5.421	<1,50,000/g	4.939±0.039^{c}
5.	Ice-cream	28(93.3%)	3.301-5.113	<2,50,000/ g	4.564±0.088^{a}
6.	Milking Can	19(63.3%)	0.497-0.752	<27/cm^2	0.621±0.016^{b}
7.	Milking Pail	24(80%)	0.497-0.720	<27/cm^2	0.064±0.012^{b}

Values are expressed as log_{10}CFU mL^{-1}(milk), log_{10}CFU g^{-1}(dairy products), and log_{10}CFU cm^{-2}(swab samples)*

Means bearing different superscripts within a column differ significantly ($P< 0.05$)

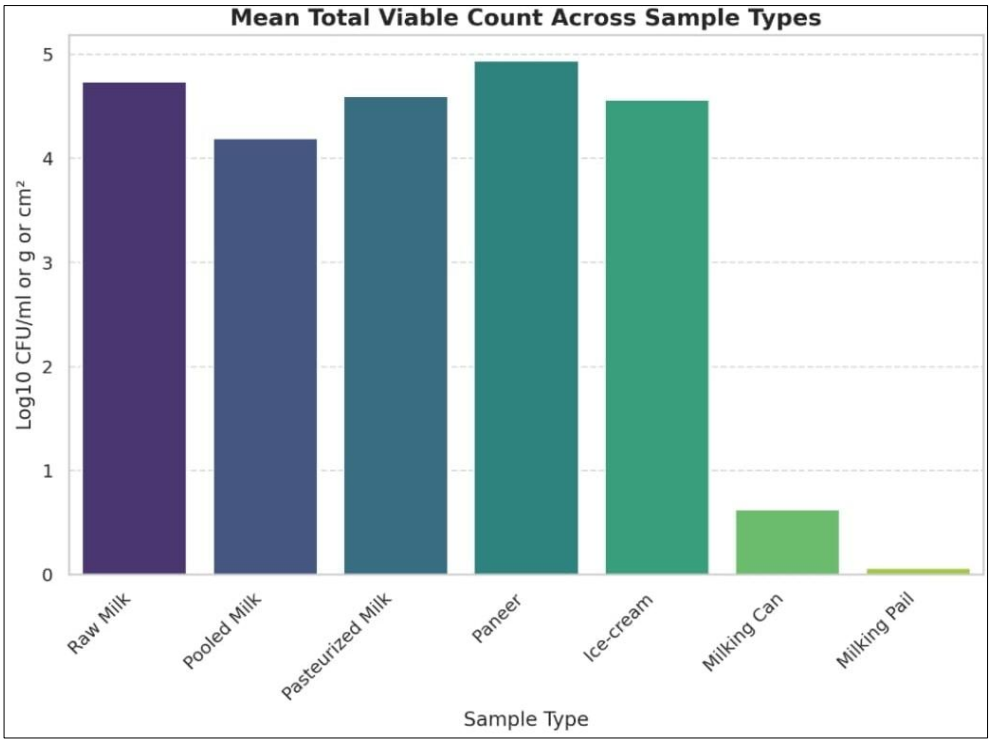

Fig 1: Total viable count across different sample types

The comparatively higher TVC observed in raw milk and paneer in this study are similar to reports observed by Chye *et al*. (2004) [6]; Hasan *et al*. (2015) [14] and may be attributed to increased exposure to environmental contamination, extensive handling during production, and inadequate temperature control during storage and marketing. Similarly, findings have been reported in dairy products where poor hygienic practices and handling conditions contributed to elevated bacterial counts (Soomro *et al*., 2002 [31]; Elmoslemany *et al*., 2010 [8]; Millogo *et al*., 2010 [22]; Shekhar and Joshi, 2010 [30]). The presence of appreciable bacterial loads in pasteurized milk is of particular concern which may arise due to defective pasteurization machinery, survival of pasteurization resistant and endospore producing bacteria, post-pasteurization handling, inadequate storage conditions (Ranieri and Boor, 2009 [27]; Hasan *et al*., 2015 [14]. Coliform bacteria are widely regarded as indicators of sanitary quality and their presence in milk and dairy products generally reflects inadequate hygiene during production, processing, handling, or storage (ICMSF, 2005) [18]. In this study, significant differences ($P< 0.05$) in total coliform counts were observed among the various sample categories (Table 3; Fig. 2), indicating the variations in hygienic quality and the degree of fecal contamination within the dairy production chain.

Table 3: Total coliform count (CC) of milk, dairy products and dairy-contact surface samples

S. No.	Source	No. of +ve samples (%)	Range*	FSSAI permissible limit for CC	Mean ± SE*
1.	Raw Milk	23(76.6%)	3.000-5.062	100/ml	4.447±0.105^{c}
2.	Pooled Milk	14(46.6%)	3.477-4.968	100/ml	4.242±0.134^{c}
3.	Pasteurized Milk	11(36.6%)	3.000-4.973	Absent /0.1ml	4.217±0.232^{c}
4.	Paneer	16(53.3%)	3.301-5.000	Max. 10/g	4.229±0.142^{c}
5.	Ice- cream	21(70%)	3.000-5.113	Max. 10/g	3.669±0.094^{a}
6.	Milking Can	15(50%)	0.428-0.704	Absent	0.593±0.023^{b}
7.	Milking Pail	9(30%)	0.528-0.685	Absent	0.616±0.017^{b}

Values are expressed as log_{10}CFU mL^{-1}(milk), log_{10}CFU g^{-1}(dairy products), and log_{10}CFU cm^{-2}(swab samples)*
Means bearing different superscripts within a column differ significantly ($P< 0.05$)

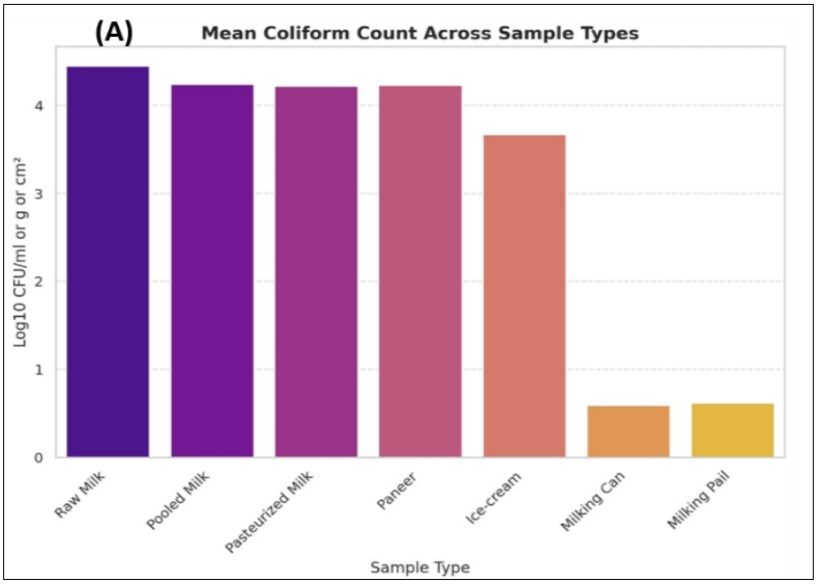

Fig 2: Total coliform count across different sample types

The elevated coliform counts observed in raw milk and ice cream suggest contaminated water sources, environmental exposure, and improper handling practices, similar to the findings reported by Abdalla and El Zubeir (2006) [1]; Chatterjee *et al*., 2006 [5]; El-Diasty *et al*., 2007 [7]; Fakruddin *et al*., 2011 [9]; Vahedi *et al*., 2011 [34]; Salman *et al*., 2013 [29]. The detection of coliforms in pasteurized milk is noteworthy and of serious concern since they are expected to be absent following effective treatment. Their occurrence may therefore indicate post-processing contamination or poor-storage conditions after pasteurization as also reported previously by several workers (Garrote *et al*., 2000 [12]; Gran *et al*., 2003 [13]; Tambekar and Shirsat, 2004 [33]; Abdel All and Dardir, 2009 [2]; Mhone *et al*., 2011 [21]).

Furthermore, comparison of the observed total viable and coliform counts with FSSAI standards revealed non-compliance in several sample categories, indicating deficiencies in hygienic handling and sanitation.

Overall, 24.28% of samples harbored coliforms with predominance of the fecal coliforms, aligning with global contamination rates in dairy products (Hassan and Elmalt, 2008 [15]; Kumar *et al*., 2011 [20]; Mhone *et al*., 2011 [21]; Salman *et al*., 2011 [28]; Mohamed and Ahmed, 2018 [23]). Higher occurrence of *E. coli* (fecal coliforms) observed in this study along with other previous findings indicates *E. coli* species as the most common bacterial enteric pathogen contaminating the food products as the organism is widely excreted through feces contaminating the environment (Zhao *et al*., 1995 [36]; Strachan *et al*., 2001 [32]). These findings (Table 4; Fig. 3) highlight urgent needs for stricter hygiene protocols, improved pasteurization monitoring, and standardized cleaning practices across dairy supply chains to mitigate foodborne risks.

Table 4: Occurrence of presumptive fecal and non-fecal coliforms isolated from different sample types

S. No.	Type of sample	Number of samples	Number of positive fecal coliforms (*E. coli*)	Number of positive non-fecal coliforms			Percentage of positive coliform samples
				Enterobacter spp.	*Klebsiella* spp.	*Citrobacter* spp.	
1.	Raw Milk	30	13	2	1	1	56.6%
2.	Pooled Milk	30	7	1	0	0	26.6%
3.	Pasteurized Milk	30	2	0	0	0	6.6%
4.	Paneer	30	7	1	1	0	30%
5.	Ice cream	30	9	1	2	0	40%
6.	Milking Can	30	2	0	0	0	6.6%
7.	Milking Pail	30	1	0	0	0	3.3%
	Total						24.28%

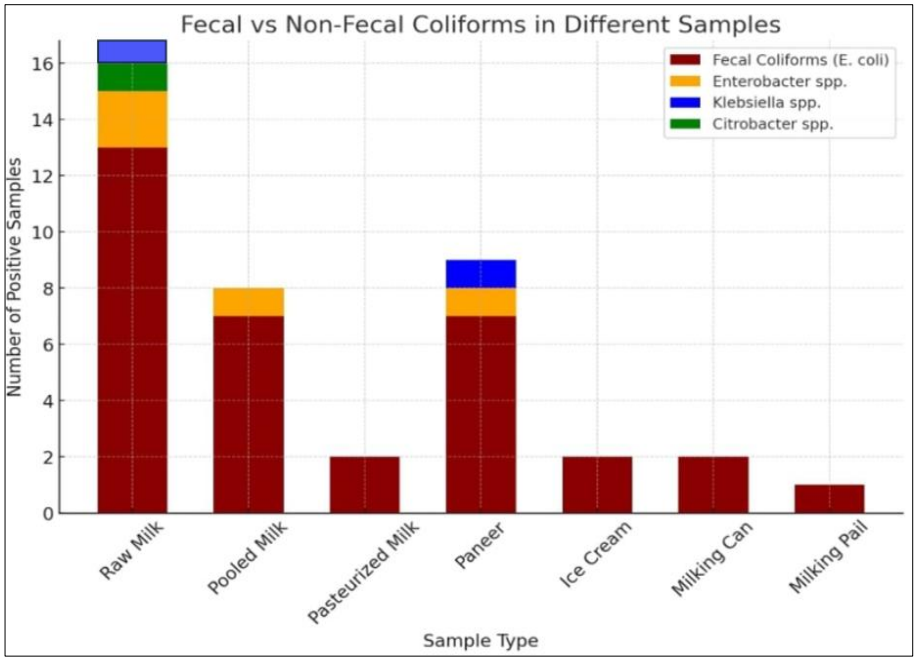

Fig 3: Distribution of presumptive fecal and non-fecal coliform isolates recovered from different sample types.

Conclusion

The present study demonstrated widespread microbial contamination of milk, dairy products and dairy-contact surfaces marketed in Srinagar, Kashmir. Elevated TVC and coliform counts, together with the occurrence of presumptive fecal and non-fecal coliforms, indicate deficiencies in hygienic handling, processing and sanitation practices across the dairy production chain. These findings highlight the need for strict implementation of good hygienic practices, routine microbiological surveillance and adherence to FSSAI standards to improve dairy safety and protect public health.

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Competing interests

There are no competing interests, according to the authors

Author's contributions

The first author completed this work. This study was overseen by the second author, who also provided direction for the research.

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