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Assessment of Bacteriological Quality of Milk Samples Collected in Rural and Urban Areas of Kamrup

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ABSTRACT

Background: Raw milk is a highly nutritious food but also an excellent medium for microbial growth if hygienic handling and cold chain practices are inadequate. In informal dairy supply chains, contamination may occur during milking, storage, transportation and retail sale, posing food safety and public health risks. Standard plate count (SPC) and coliform count are widely used indicators of overall microbial load and sanitary quality of milk. This study assessed the bacteriological quality of raw milk from rural cattle farms and urban milk selling points in Kamrup District, Assam, India (26.22°N, 91.65°E) and compared the microbial status between rural and urban sources.

Methods: A total of 450 raw milk samples were collected, including 225 samples from 75 rural cattle farms (three randomly selected cows per farm) and 225 samples from 75 urban milk selling points (three pooled milk samples per point). Each sample (50 mL) was collected aseptically in sterile, labelled containers and transported to the laboratory under maintained cold chain conditions. Samples were stored at 5°C and processed within 3-4 hours of collection. SPC and coliform counts were determined by pour plate technique using nutrient agar and Violet Red Bile Agar (VRBA), respectively. Colony counts were expressed as log cfu/mL. Rural and urban means were compared using Welch's independent samples t-test in R (version 4.4.2), with $p<0.05$ considered statistically significant.

Result: Both rural and urban milk samples showed very high microbial loads. SPC ranged from 10.62-13.63 log cfu/mL in rural samples and 10.80-13.63 log cfu/mL in urban samples, while coliform counts ranged from 6.49-7.26 log cfu/mL (rural) and 6.49-7.24 log cfu/mL (urban). No statistically significant differences were observed between rural and urban sources for SPC ($p = 0.9987$) or coliform count ($p = 0.9438$). Overall microbial indicators substantially exceeded permissible limits, indicating poor hygienic quality of raw milk in the study area and underscoring the need for strengthened hygiene practices, monitoring and cold chain maintenance across the milk supply chain.

Key words: Coliform, Kamrup, Milk samples, SPC.

INTRODUCTION

Milk is a highly nutritious food and an important component of the human diet; however, it also serves as an excellent medium for microbial growth if produced, handled, or stored under unhygienic conditions. Consequently, the bacteriological quality of raw milk is a critical indicator of its safety and suitability for human consumption. Contamination may occur at multiple stages, including during milking, handling, transportation, storage and marketing, particularly where sanitation and temperature control are inadequate (Chye *et al*., 2004; Saxena and Rai, 2013). Standard Plate Count (SPC) and coliform count are widely used microbiological indicators for assessing the hygienic quality of raw milk. While SPC reflects the overall viable bacterial load and hygiene during production and handling, coliforms indicate sanitary lapses and possible contamination through utensils, water, or handling practices (Houghtby *et al*., 1994; Chye *et al*., 2004). Elevated values of these indicators are commonly associated with poor udder hygiene, contaminated equipment, delayed chilling and breakdown of cold chain, all of which contribute to deterioration of milk quality and increased public health risk (FAO, 2013; Saxena and Rai, 2013). In many parts of India, milk is distributed through informal channels where systematic quality control and cold chain maintenance may be inconsistent. Assam, with substantial dependence on agriculture and allied activities, also faces challenges related to hygiene infrastructure and organized milk handling systems (Bhujel and Gautam, 2021). Previous reports from Assam have highlighted concerns regarding the bacteriological quality of raw milk marketed in and

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around Guwahati, reinforcing the need for location-specific baseline data (Kakati *et al*., 2021).Therefore, the present study was undertaken to assess the bacteriological quality of raw milk samples collected from rural cattle farms and urban milk selling points of Kamrup district, Assam, using Standard Plate Count and coliform count as microbiological indicators (FSSAI, 2015). The study further compares microbial quality between rural and urban sources to generate evidence that may support improved hygienic practices and handling interventions in the milk supply chain.

MATERIALS AND METHODS

The study was conducted following approval from the Institutional Animal Ethics Committee. A total of 450 raw milk samples were collected from dairy farms (n = 225) and urban milk selling points (n = 225) located in different areas of Kamrup District, Assam, India (26.22°N latitude and 91.65°E longitude). From each of the 75 selected rural cattle farms, milk samples were collected randomly from three apparently healthy cows, yielding a total of 225 rural samples. Similarly, from each of the 75 urban milk selling points, three pooled milk samples were collected using standard sampling procedures, resulting in 225 urban samples. All milk samples (50 mL each) were collected aseptically in sterile, labelled containers and immediately transported to the laboratory under maintained cold chain conditions. The samples were stored at 5°C and subjected to bacteriological analysis within 3-4 hours of collection to prevent microbial proliferation. Sampling was carried out during the period from October, 2022 to November, 2023.

The milk samples collected were assessed for bacteriological quality by using standard plate count and coliform count. The milk samples were examined for SPC and coliform count by pour plate technique (FSSAI, 2015). Prior to test, milk samples were thoroughly mixed by shaking the sample bottle for at least 25 times and 10-fold serial dilutions in sterile NSS were prepared up to a final dilution of 10^{-15} for SPC and 10^{-7} for coliform count, respectively. From each dilution, 1 ml was pipetted out on each of the two sterile petri dishes and repeated thrice so as to produce six replicates maintaining all aseptic precautions. Thereafter, 15-20 ml of nutrient agar and Violet Red Bile Agar (VRBA) media previously melted and cooled to 45°C was poured into each pair of petri dishes for SPC and coliform count, respectively. The inoculum and the media were thoroughly mixed by rotating clockwise, anticlockwise and to and fro for 5 times. Then the inoculated media in the petri dishes were allowed to cool down and set by keeping them on a level surface. For coliform count to prevent surface growth and spreading of colonies, the plates were overlaid with 5 ml VRBA and then allowed to solidify. The plates were incubated at 37±0.5 and 35±0.5°C overnight in inverted position for SPC and coliform, respectively. The plates showing 30-300 colony forming units (cfu) were selected for colony count. Coliform organisms produce purple-red colonies that are 0.5 mm or larger in diameter and surrounded by zone of precipitated bile acids. The mean cfu of the six plates multiplied by the specific dilution factor was considered as the number of organism per millilitre (cfu/ml) of milk which was then converted into logcfu/ml.

An independent samples t-test (Welch's t-test) was employed to compare the mean standard plate count and coliform count between milk samples collected from rural farms and urban selling points, as the two groups comprised independent observations. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using R statistical software (version 4.4.2).

RESULTS AND DISCUSSION

Milk is a wholesome food for humans, but it also serves as a good medium for the growth of many micro-organisms, especially bacterial pathogens which include *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Staphylococcus* and *Micrococcus* spp. If milk is kept cool before processing, psychotropic bacteria may predominate in the flora. The presence of coliform bacteria and pathogens in milk indicates potential contamination from the cow's udder, milk equipment, or water source. Normally, fresh milk from a healthy cow has a low microbial count (less than 1000 ml^{-1}), but this count can significantly increase once the milk is stored at room temperature for an extended period (Chye *et al.,* 2004).

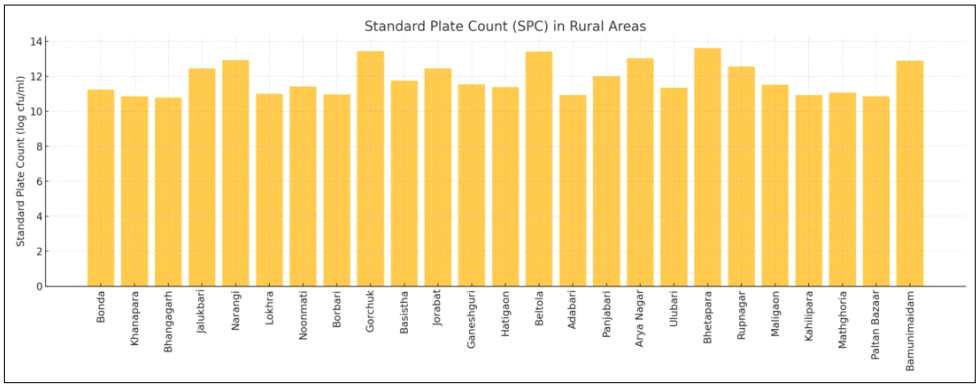

Fig 1: Standard plate count (SPC) in rural areas.

The milk samples collected were assessed for bacteriological quality by using standard plate count and coliform count.

Standard plate count (SPC)

Standard plate count (SPC), also referred to as total aerobic bacterial count, is a widely accepted indicator of the hygienic quality of milk and reflects the overall microbial load arising from production, handling and storage conditions (Houghtby *et al*., 1994). The SPC values of milk samples collected from rural farms and urban milk selling points are presented in Table 1 and Table 2. In rural areas, the SPC ranged from 10.62 to 13.63 log cfu/ml, with the lowest and highest values recorded at Sonapur and Puthimari, respectively. Similarly, urban milk samples showed SPC values ranging from 10.80 to 13.63 log cfu/ml, with the minimum at Bhangagarh and maximum at Bhetapara as depicted in Fig 1, Fig 2 and Fig 5. Statistical analysis using an independent samples t-test revealed no significant difference between rural and urban milk samples with respect to SPC ($p = 0.9987$), indicating comparable levels of microbial contamination in both sources. However, the SPC values observed in the present study were markedly higher than the permissible limit of 4 log cfu/ml prescribed for raw milk by the European Union (Hillerton, 2000), indicating poor hygienic quality of milk in the study area. The SPC values recorded in the present investigation were also higher than those reported by Tekilegiorgis (2018) and Fereja *et al*. (2023). The elevated bacterial counts may be attributed to inadequate cleaning of milking utensils, poor udder hygiene, unhygienic housing conditions and failure to maintain appropriate cold chain practices after milking, as also reported by Saxena and Rai (2013). Similar observations were made by Tekilegiorgis (2018), who reported an average SPC of 6.15 log cfu/ml in raw milk, which was considerably lower than the values observed in the present study.

Coliform count

Milk, being a highly perishable commodity, provides an ideal medium for microbial growth and is therefore highly susceptible to bacterial contamination if proper hygienic practices are not maintained. Consumers increasingly demand milk that is clean, safe and produced under hygienic conditions. However, milk often passes through multiple stages of handling from farm to consumer and failure to maintain adequate sanitation and cold chain conditions during this process significantly compromises its microbiological quality. Poorly processed and improperly handled milk and milk products are well-

Table 1: Standard plate count and coliform count (log cfu/ml) of milk samples collected from milk selling points of different urban locations in Kamrup district.

Location	Standard plate count (logcfu/ ml) of milk samples (Mean± SE)	Coliform count (logcfu/ ml) of milk samples (Mean± SE)
Bonda	11.25±0.14	7.24±0.08
Khanapara	10.87±0.08	6.66±0.05
Bhangagarh	10.80±0.09	6.60±0.09
Jalukbari	12.47±0.30	6.94±0.11
Narangi	12.93±0.27	7.09±0.07
Lokhra	11.01±0.08	6.75±0.08
Noonmati	11.43±0.10	6.92±0.08
Borbari	10.98±0.13	6.72±0.07
Gorchuk	13.44±0.36	7.02±0.12
Basistha	11.77±0.11	6.72±0.11
Jorabat	12.46±0.26	6.74±0.07
Ganeshguri	11.55±0.19	6.72±0.05
Hatigaon	11.39±0.14	6.97±0.10
Beltola	13.43±0.21	6.49±0.05
Adabari	10.94±0.12	6.70±0.05
Panjabari	12.02±0.17	6.52±0.05
Arya nagar	13.04±0.24	6.60±0.10
Ulubari	11.36±0.15	7.20±0.09
Bhetapara	13.63±0.35	7.05±0.08
Rupnagar	12.57±0.19	6.86±0.06
Maligaon	11.54±0.12	6.97±0.09
Kahilipara	10.94±0.10	6.88±0.10
Mathghoria	11.08±0.10	6.98±0.11
Paltan bazaar	10.86±0.06	7.08±0.12
Bamunimaidam	12.90±0.31	7.20±0.01

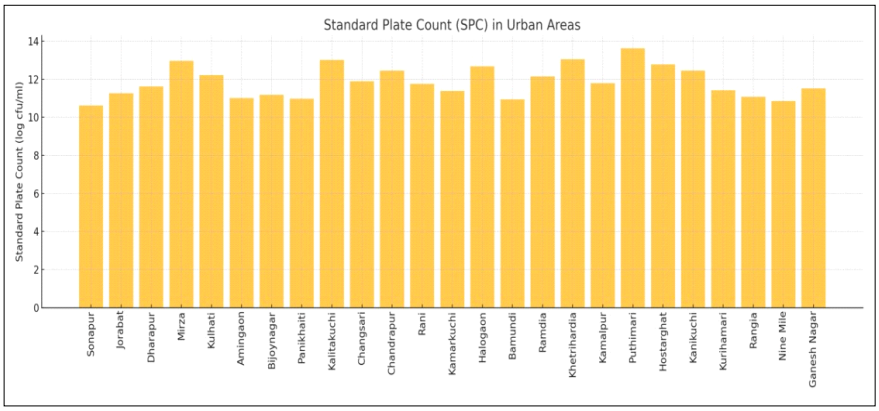

Fig 2: Standard plate count (SPC) in Urban areas.

recognized sources of food-borne illnesses in humans (FAO, 2013). Coliform bacteria are widely regarded as indicators of sanitary quality and post-milking contamination.

Table 2: Standard plate count and coliform count (log cfu/ml) of milk samples collected from cattle farms of different rural locations in Kamrup district.

Location	Standard plate count (logcfu/ml)of milk samples (Mean±SE)	Coliform count (logcfu/ml)of milk samples (Mean±SE)
Sonapur	10.62±0.08	6.60±0.09
Jorabat	11.27±0.11	6.66±0.05
Dharapur	11.62±0.15	7.09±0.09
Mirza	12.97±0.26	6.94±0.11
Kulhati	12.21±0.32	7.26±0.07
Amingaon	11.01±0.08	6.75±0.08
Bijoynagar	11.17±0.07	6.92±0.08
Panikhaiti	10.98±0.13	6.72±0.07
Kalitakuchi	13.02±0.34	7.02±0.12
Changsari	11.90±0.21	6.72±0.11
Chandrapur	12.46±0.26	6.74±0.07
Rani	11.75±0.20	6.72±0.05
Kamarkuchi	11.38±0.16	6.97±0.10
Halogaon	12.67±0.20	6.49±0.05
Bamundi	10.94±0.12	6.70±0.05
Ramdia	12.14±0.17	6.52±0.05
Khetrihardia	13.04±0.24	6.60±0.10
Kamalpur	11.79±0.18	7.24±0.09
Puthimari	13.63±0.35	7.05±0.08
Hostarghat	12.77±0.22	6.86±0.06
Kanikuchi	12.45±0.24	6.97±0.09
Kurihamari	11.41±0.25	6.88±0.10
Rangia	11.08±0.10	6.98±0.11
Nine Mile	10.86±0.06	7.08±0.12
Ganesh nagar	11.51±0.20	7.20±0.10

In the present study, the coliform counts of milk samples collected from different rural and urban locations are presented in Table 1 and Table 2. The highest coliform count in rural milk samples was recorded at Kulhati (7.26 log cfu/ml), while the lowest was observed at Halogaon (6.49 log cfu/ml). Similarly, among urban milk samples, the highest coliform count was observed at Bonda (7.24 log cfu/ml) and the lowest at Beltola (6.49 log cfu/ml) as depicted in Fig 3, Fig 4 and Fig 6. Statistical analysis using an independent samples t-test revealed no significant difference in coliform counts between rural and urban milk samples ($p = 0.9438$).

This indicates that the level of fecal contamination was comparable in both rural and urban settings, suggesting that poor hygienic practices prevail across the entire milk production and distribution chain rather than being restricted to a particular location. Despite the absence of a statistically significant difference between the two groups, the coliform counts recorded in the present study were markedly higher than the maximum permissible limits recommended for raw milk. These findings are in agreement with earlier reports by Tekilegiorgis (2018) and Kakati *et al*. (2021), who also observed elevated coliform levels in raw milk samples. However, the counts observed in the present investigation were considerably higher than those reported by Kalilur *et al*. (2002), Nanu *et al*. (2006), Ali (2010) and Belbachir *et al*. (2015). The elevated coliform load observed in this study may be attributed to several factors, including poor udder hygiene, use of contaminated water, unhygienic milking practices, inadequately cleaned utensils, exposure of milk to dung and lack of proper drainage facilities at the farm level. In addition, the absence of effective cold chain management during storage and transportation likely contributed to bacterial proliferation. These findings highlight systemic deficiencies in milk handling practices and emphasize the urgent need for targeted interventions aimed at improving hygienic practices, infrastructure and awareness among stakeholders involved in the unorganized dairy sector.

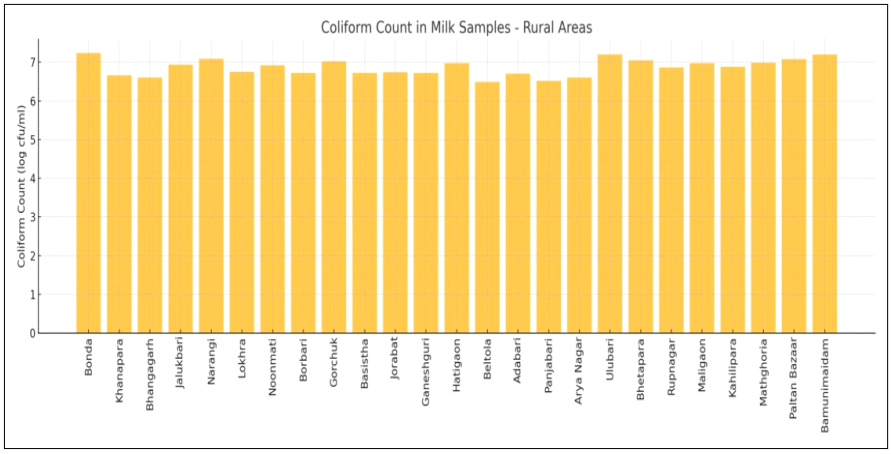

Fig 3: Coliform count in rural areas.

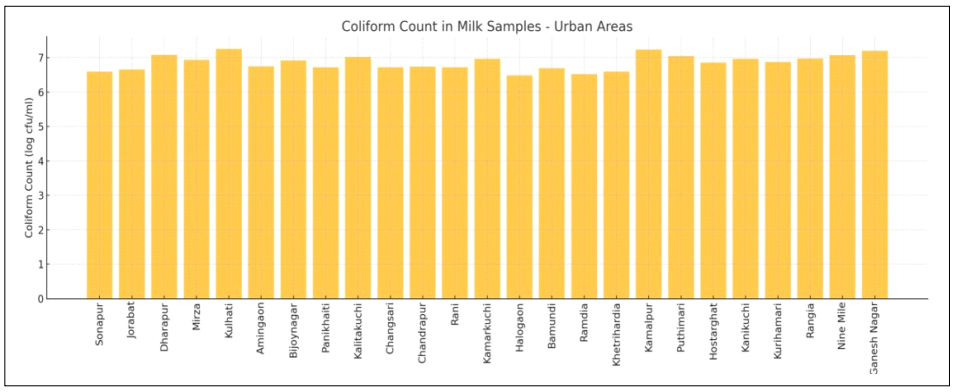

Fig 4: Coliform count in urban areas.

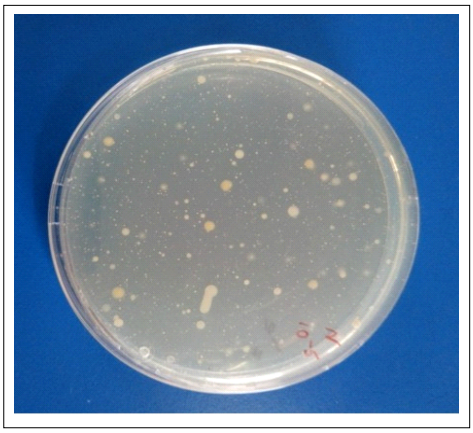

Fig 5: Bacterial colonies in nutrient agar for calculating SPC.

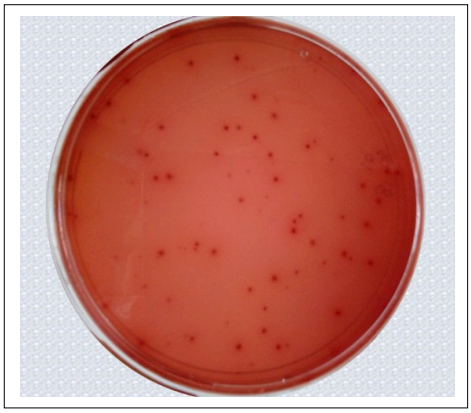

Fig 6: Colonies of coliform organisms in VRBA.

CONCLUSION

The present study evaluated the bacteriological quality of raw milk collected from rural cattle farms and urban milk selling points in Kamrup district, Assam. The findings revealed high standard plate count (SPC) and coliform levels in milk samples from both sources, indicating poor hygienic quality. Statistical analysis showed no significant difference between rural and urban samples for either SPC or coliform count, suggesting that microbial contamination is widespread and not limited to a particular production or marketing system. The observed microbial loads were considerably higher- than the permissible limits recommended for raw milk, reflecting inadequate hygienic practices during milking, handling, storage and transportation. The elevated bacterial counts may be attributed to poor udder hygiene, improper cleaning of utensils, use of contaminated water, lack of cold chain maintenance and unhygienic handling practices. The absence of significant variation between rural and urban samples highlights systemic deficiencies in milk production and distribution systems. These findings emphasize the urgent need for improved hygienic practices, regular monitoring of milk quality, farmer awareness programs and strengthening of cold chain infrastructure to ensure safe milk for consumers and to reduce public health risks associated with raw milk consumption.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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