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Original Article

Microbiological Quality and Adulteration of Pasteurized and Raw Milk Marketed in Dharan, Nepal

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Abstract

This study was aimed to evaluate the quality of raw and pasteurized milk marketed in Dharan. Milk may be contaminated with pathogenic microorganisms and a mixture of several adulterants and such milk pose a risk to consumers. The study was carried out from September 2019 to January 2020. Collected samples were tested for adulterants (starch, formalin, neutralizer and table sugar) as well as microbial quality (Total Coliform count, Total Viable Count, Thermoduric Count, *Escherichia. coli* and *Staphylococcus aureus*) as per standard guideline. The adulterants starch, formalin and neutralizer were not detected in both raw and pasteurized milk. However, table sugar was present in 45% (9 out of 20) raw milk and 90% (18 out of 20) pasteurized milk. The average Total Viable Count, Total Coliform Count and Thermoduric Count of raw milk were, 59×10^5 CFU/ml, 14×10^4 CFU/ml and 5×10^3 CFU/ml respectively. Similarly, the average Total Viable Count, Total Coliform Count and Thermoduric Count of pasteurized milk were found to be 15×10^4 CFU/ml, 14×10^3CFU/ml and 4×10^3 CFU/ml respectively. *E. coli* was detected in 30% pasteurized milk whereas *S. aureus* was isolated from only 20%. Likewise, *E. coli* and *S. aureus* were found in 55% and 45% of raw milk respectively. The results of the study indicated that routine monitoring of dairy industries and raw milk vendors, awareness campaign and good hygienic practice should be promoted to upgrade the quality of raw and pasteurized milk.

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1. Introduction

Milk is known as the most complete food found in nature (Marjan et al., 2014). It is an important source of carbohydrate, proteins with all ten amino acids, immunoglobulins, essential fatty acids, and other micronutrients (Hossain et al., 2010). Buffalo milk contains 7.6% fat, 3.8% protein, 4.9% lactose, 0.78% ash and 17% total solids. Cow milk contains 4.5% fat, 3.8% protein, 4.9% lactose, 0.72% ash 13.9% total solids. That is why milk is an important part of the diet of all age group including expectant mothers (Wijesinha-Bettoni & Burlingame, 2013). It is most likely an extremely perishable food (Sebho & Meskel, 2018). Inappropriate handling may cause an outbreak to public health problems and economic losses, thus hygienic vigilance is essential throughout the entire milk chain starting from producer to consumer (Hayes and Boor, 2001).

Various sources are responsible for contamination of the milk that may be through cattle suffered with tuberculosis, brucellosis, and mastitis (Jay et al., 2005). Contamination may also occur from poor hygiene, contaminated utensils, milk-handlers with typhoid fever, diphtheria, dysentery, and scarlet fever (Jay et al., 2005). It is common that dairy cattle and their farm's surroundings may consist of several pathogens such as *Listeria* spp., *Salmonella* and pathogenic *E. coli*. Raw or inadequately pasteurized milk may contain toxin-producing *E. coli*, *Salmonella*, *Listeria monocytogenes* and others (Pal et al., 2016).

An adulteration is an act of purposely debasing the quality of food offered for sale either by mixing or substitution of inferior substances or by the reduction

of some valuable ingredients (FDA, 1995). Adulterants are the substances that are added in the product for making more profit or to extend the shelf life of highly perishable goods like milk (Lateef et al., 2009). Adulterants are mostly harmful to human health and thus should be avoided (FDA, 1995). According to the Department of Food Technology and Quality Control (DFTQC) guideline for milk and milk products, milk should not contain any adulterants (DFTQC, 2011). Adulterated milk has an adverse health effect as it may contain several toxic chemicals and deprive human body of nutrients required for growth and development (Marcus, 1979). Maximization of profit can be done by milk dealers in three ways viz. dilution, extraction of useful components like milk fat which is removed as cream and addition of unwanted substances like starch and table sugar to increase SNF (Lateef et al., 2009). Middlemen are one of the important milk vendors and they may adulterate milk by mixing undesirable substances (Lateef et al., 2009). If good hygiene is not maintained, contamination of milk may come from vegetation, soil, utensils, packaging materials and beddings (Lemma et al., 2018).

Ministry of Finance, Nepal reported that Nepal's total production of milk in fiscal year 2018/19 was 1,475,333 metric tons, in which cow milk occupied 40% and buffalo milk 60% (MOF, 2019). Food and Agriculture Organization (FAO) reported that only about 10% of milk is estimated to be used by the recognized dairy sectors and rest (90%) goes to the informal sector, milk vendor and small dairy cooperatives out of the total milk production in Nepal (FAO, 2010). It was reported that the marketed milk in Kathmandu valley by various dairy industries were contaminated with pathogenic microorganisms (Arjyal et al., 2004).

Presence of *S. aureus* and an intestinal commensal *E. coli* indicates the alarming public health concern. To minimize the risk of milk-borne diseases, an intense study should be done to determine the microbiological quality of milk and other chemical adulterants and their public health impact (Arjyal et al., 2004). Thus, objectives of this study were to determine the bacterial contaminants as well as an admixture of adulterants in both raw and pasteurized milk marketed in Dharan.

2. Materials and Methods

Between September 2019 to January 2020, a total of 20 raw and 20 pasteurized milk samples were collected. We selected this time duration as it represent both summer and winter season. Raw milk samples (500 ml, distributed at atmospheric temperature) were collected in a UV sterilized plastic bottle from different milk vendors of Dharan. Pasteurized milk (a packet of 500 ml, refrigerated) samples marketed by 5 different dairy industries namely Kamadhenu, Gaubarsha, Dudhsagar, DDC and NMC dairy were purchased from different shops. These brands were selected for study as they are being marketed in Dharan. All the samples were collected at morning (7-8), kept in ice box and transported to the laboratory. All samples were processed within 6 hours of receipt.

2.1 Adulteration test

Collected samples were tested for most common chemical adulterants (starch, neutralizer, table sugar and formalin) according to Manual of Methods of Analysis of Foods, Milk and Milk Products published by Ministry of Health and Family Welfare, Government of India, 2016 (FSSAI, 2016). Briefly, the tests were done as follows:

2.2 Starch test

Five ml of milk was boiled and then cooled and few drops of 1% iodine starch were added. The appearance of blue color denoted positive test.

2.3 Neutralizer test

10 ml of sample to be tested and 10 ml of 95% alcohol was taken in test tube. Few drops of 0.1% alcoholic solution (w/v) rosalic acid was added and rossy red color indicate positive result.

2.4 Table sugar test

1 ml of milk and 1 ml of 0.5% resorcinol solution was mixed well and placed in boiling water for 3-5 minutes. The appearance of red colour denoted positive test.

2.5 Formalin test

Two ml of sample to be tested was taken and gently added equal volume of 90% H_2SO_4 containing traces of FeCl3 from top of the test tube. Formation of purple ring at the junction indicated the presence of formalin.

2.6 Microbiological tests

For microbial analysis, collected samples were processed immediately after receipt. Briefly, tests were done as follows:

2.7 Total Viable Count (TVC)

TVC was performed according to Laboratory Handbook for Dairy Industry published by National Dairy Development Board (NDDB), Nepal, 2001 (NDDB, 2001). In which, serial ten-fold dilutions of the milk sample were done and TVC were determined by the pour plate method on nutrient agar and incubated at 37°C for 24 hours.

2.8 Total Coliform Count (TCC)

TCC was performed according to Laboratory Handbook for Dairy Industry published by National Dairy Development Board (NDDB), Nepal, 2001 (NDDB, 2001). Serial ten- fold dilutions of the milk sample were done and TCC were determined by the spread plate method on Mac-Conkey agar and incubated at 37°C for 24 hours.

2.9 Thermoduric bacterial count (TBC)

Thermoduric bacterial count was done following Kimberly et al. (2014).

E. coli

One loopful each of the samples from 10-1 dilution was inoculated on to MacConkey Agar (MA). The plates were incubated at 37° C for 24 hours. Lactose fermenting colonies on MacConkey agar were sub-cultured to obtain pure culture. Pure cultures were tested biochemically (catalase test, oxidase test, Indole test, methyl red test, Voges Proskauer test, citrate utilization test, triple sugar iron agar test, urease test, oxidative fermentative test for confirmation of *E. coli* as described by Isenberg (2007) and Cheesbrough (2006).

S. aureus

Identification of *S. aureus* was done according to Chakraborty, 2011. In which, one loopful of each samples was inoculated into Mannitol salt agar plates and incubated at 37ºC for 24 hours. Identification was done based on colony characteristics, Gram's staining, catalase test, oxidase test and coagulase test.

3. Results and Discussion

The milk samples were tested for adulteration of starch, sugar, neutralizer, and formalin. Chemical analysis of the samples revealed that, most of the pasteurized and raw milk were adulterated with table sugar. However, starch, formalin and neutralizer were absent in both pasteurized and raw milk samples (Table 1).

According to the Department of Food Technology and Quality Control (DFTQC) guideline for milk and milk products, adulterants should be absent in milk (DFTQC, 2011). However, this study found 90% of the pasteurized and 45% of the raw milk samples were adulterated with table sugar. In a similar study of Parajuli et al. (2018) reported that the extent of adulteration in milk of Kathmandu valley with table sugar and soda was 10 and 55% respectively. Findings of this shows that raw and pasteurized milk marketed in Dharan are free from neutralizers. Since the neutralizers are added to neutralize the developed acidity (which in turn is due to increased microbial activity), it can be inferred that the quality of milk sold in Dharan is completely better than that of Kathmandu valley. Table sugar is commonly used as an adulterant to increase Solids-Not-Fat (SNF) level of milk. Starch was not found as an adulterant in the study by Parajuli et al. (2018) and in this study too. The reasons behind not using starch as an adulterant could be the cost of starch.

Among the pasteurized milk (total sample 20), *E. coli*, Total Coliforms, Thermoduric bacteria and *S. aureus* were detected in 30%, 80%, 75%, and 20% samples respectively (Table 2).

The average Total Viable Count (TVC) of pasteurized milk was 15×10^4 CFU/ml. This result was greater than the findings of the previous study of Al-Mazeedi et al. (2013) where, average counts of the aerobic bacteria in the pasteurized milk from three different dairy industries were 3×104 CFU/ml, 9×101 CFU/ml and 5×103 CFU/ml respectively. This finding also did not satisfy with international standard of European Union (EU, 2020) and Mandatory Nepalese Standard (MNS, 2016). Presence of higher load of bacteria in pasteurized milk may be due to inadequate pasteurization and post pasteurization contamination. It also indicated poor hygienic condition during packaging. Similarly, in case of raw milk (total sample 20), 55%, 95%, 45% and 95% samples were contaminated with *E. coli*, Total Coliforms, *S. aureus* and Thermoduric bacteria respectively (Table 3).

Table 1: Adulterants in pasteurized and raw milk

Milk	Table sugar	Formalin	Starch	Neutralizer	Number of samples
Pasteurized	18 (90 %)	ND	ND	ND	20
Raw	9 (45%)	ND	ND	ND	20

**ND= Not detected*

Table 3: Microbiological analysis of raw milk.

Sample	TB	TVC	TCC	*E. coli*	*S. aureus*
R1	5×10^3	9×10^4	16×10^5	P	P
R2	6×10^3	11×10^4	80×10^4	A	A
R3	8×10^3	7×10^4	17×10^5	A	A
R4	6×10^3	8×10^4	27×10^5	P	P
R5	5×10^3	12×10^4	16×10^4	P	P
R6	6×10^3	44×10^4	3×10^4	A	A
R7	5×10^3	55×10^4	20×10^4	P	A
R8	5×10^3	15×10^4	8×10^4	A	A
R9	5×10^3	11×10^5	30×10^4	A	A
R10	7×10^3	20×10^4	5×10^4	A	P
R11	3×10^3	10×10^5	11×10^3	P	A
R12	3×10^3	10×10^4	9×10^5	P	P
R13	4×10^3	18×10^4	22×10^4	A	P
R14	3×10^3	58×10^4	18×10^4	P	A
R15	3×10^3	3×10^4	12×10^4	P	P
R16	4×10^3	12×10^4	11×10^4	A	A
R17	Nil	19×10^4	Nil	A	P
R18	6×10^3	29×10^4	18×10^4	P	A
R19	8×10^3	21×10^4	12×10^4	P	A
R20	6×10^3	11×10^4	13×10^4	P	P
Average/Prevalence	5×10^3	59×10^5	14×10^4	55%	45%

**TB (Thermoduric Bacteria), TVC (Total Viable Count), TCC (Total Coliform Count) measured on CFU/ml, P=Present, A=Absent*

In this study, none of the samples (both pasteurized and raw) were free from bacterial contamination. The average Total Viable Count (TVC) of pasteurized and raw milk was 15×10^4 CFU/ml and 59×10^5 CFU/ml respectively. Likewise, the TVC of raw milk in our study was higher than findings of some former studies in Nepal by Dhungel et al. (2019) and Dahal et al. (2010), in Ethiopia by Tassew & Seifu (2011), in India by Jain & Shreevastav (2014) and in Morocco by Belbachir et al. (2015) who reported average plate count of 15×10^4 CFU/ml, 9.03×10^5 CFU/ml, 3.95×10^6 CFU/ml, 2.34×10^6 CFU/ml and 1.4×10^6 CFU/ml respectively. The reason behind the higher occurrence rate could be related to the difference in time, place and season of research. Additionally, higher prevalence rates might be due to unhygienic processing, improper cleaning, deficient handling, and contamination of utensils. In contrast, our result was lesser than the findings of former studies by Acharya et al. (2017), Dahal et al. (2010), Aaku et al. (2004), Mwangi et al. (2000), Moustafa et al. (1988) and Mohamed & El Zubeir (2007) who found the average value of 104.71×10^5 CFU/ml, 107 CFU/ml, 107×10^6 CFU/ml, 1×10^9 CFU/ml and 5.63×10^9 CFU/ml respectively.

Table 2: Microbiological analysis of pasteurized milk

Sample	TB	TVC	TCC	*E. coli*	*S. aureus*
S1	Nil	11×10^4	16×10^3	P	P
S2	5×10^3	10×10^3	18×10^3	A	A
S3	35×10^2	5×10^3	11×10^2	A	A
S4	5×10^3	10×10^3	3×10^3	A	A
S5	Nil	10×10^2	Nil	A	A
S6	Nil	22×10^2	2×10^3	A	A
S7	2×10^2	11×10^3	Nil	A	A
S8	18×10^2	10×10^2	21×10^3	A	A
S9	3×10^3	19×10^3	14×10^3	P	A
S10	2×10^3	18×10^3	74×10^3	A	A
S11	5×10^3	62×10^3	36×10^3	P	A
S12	5×10^3	13×10^3	2×10^3	A	P
S13	Nil	2×10^2	Nil	A	A
S14	2×10^3	19×10^3	16×10^3	A	A
S15	Nil	2×10^2	Nil	P	A
S16	6×10^2	17×10^3	17×10^3	P	A
S17	9×10^3	15×10^3	9×10^2	A	P
S18	10×10^3	17×10^3	11×10^3	P	A
S19	10×10^3	13×10^3	5×10^3	A	A
S20	8×10^3	14×10^3	32×10^3	A	P
Average/ Prevalence	4×10^3	15×10^4	14×10^3	30%	20%

**TB (Thermoduric Bacteria), TVC (Total Viable Count), TCC (Total Coliform Count) measured on CFU/ml, P=Present, A=Absent*

The differences in findings of these studies can be correlated to difference in time as well as place. Higher bacterial counts indicate poor hygiene practice and ineffective pasteurization of the milk (Harding, 1995). It suggests that proper handling of milk, improvement in sanitation, proper sterilization and disinfestations of contaminated utensils and use of safe water is mandatory for all stakeholders

In this study, 80% of pasteurized and 95% of raw milk samples were tested positive with an average Coliform count of 14×10^3 CFU/ml and 14×10^4 CFU/ml respectively (Table 3). This finding was higher than some previous studies (Silva et al., 2010; El Nahas et al., 2015 and Acharya et al., 2017). The annual report published by DFTQC (2011/2012) reported that out of 65 milk and milk products analyzed, 31 (47%) milk samples were found to be microbiologically unsafe (DFTQC, 2011). Hence, the result of this study complied with the study done by DFTQC showing that most of the milk being sold in Nepal might be microbiologically unsafe for consumption. A similar study was done by Acharya et al. (2017) and reported the Total Coliform count range from 2-52% in raw milk. The higher coliform count detected in this study may be due to poor hygiene of farm, use of contaminated water while milking and use of contaminated utensils by farmers. Since it is not practical to produce a coliform free product, the existence of coliform may not necessarily indicate direct fecal contamination of milk but it is a precise indicator of poor hygiene and sanitation during milking and further handling processes. The presence of coliforms in pasteurized milk sample may be due to defective pasteurization, adulteration of pasteurized milk with raw milk and unsanitary handling (Hassan et al., 2015).

Similarly, current study found that the prevalence of *E. coli*, *S. aureus* and Thermoduric bacteria in pasteurized milk were 30%, 20%, and 75% respectively. Furthermore, the prevalence of *E. coli*, *S. aureus* and Thermoduric bacteria in raw milk were 55%, 45% and 95% respectively (Table 2 and 3). *E. coli* was reported in 18.75% and 20% of pasteurized milk samples by Acharya et al. (2017) and Parajuli et al. (2018) respectively which is just lesser than results of this study.

S. aureus contamination was detected in 12.5%, 15% and 3.9% pasteurized milk (Arjyal et al., 2004; Acharya et al., 2018; and Dai et al., 2019) and this result is a bit lesser than current study (Table 2). The presence of *S. aureus* in raw milk of this study was similar with the study of Schirmer et al. (2006), but did not support the results of other studies (Joshi et al., 2014 and Silvestre et al., 2008). Presence of *S. aureus* in raw milk may be due to poor personal hygiene of milkers, utensils and milk handlers. The higher prevalence of *S. aureus* in pasteurized milk might be due to unhygienic processing, improper cleaning, deficient handling, and post-processing contamination of packaging material from the polluted environment (Sankhar, 2015).

This study showed the average Thermoduric bacterial count of pasteurized and raw milk were 4×10^3 CFU/ml and 5×10^3 CFU/ml respectively. According to Kimberly et al., (2014) an average of 4.07×10^2 CFU/ml and 4.65×10^2 CFU/ ml of thermoduric bacteria were found in raw milk in winter and summer respectively. The average value of thermoduric bacteria in pasteurized milk reported by Delgado et al., (2013) was 3.19×10^2 CFU/ml and was in a range from 2.38×10^4 to 6.82×10^5 CFU/ml in raw milk. The variation on the thermoduric bacteria counts can be related to the difference in time and place. The presence of thermoduric bacteria in milk indicates that the pasteurizer may be defective so that some of the milk unable to reach the up to require pasteurizing temperature; there may be a high amount of foam which is not heated to temperature; or the vats may not be washed between runs (Rogers & Frazier, 1930).

Different reports have suggested that pasteurized milk is contaminated with food-borne pathogens and it indicated that pasteurization alone could not be a stable solution to control the milk-borne diseases (Oliver et al., 2005). Hence, all the quality assurance systems such as Good Manufacturing Practice (GMP), Sanitation Standard Operating Procedure (SSOP), and Hazard Analysis Critical Control Point (HACCP) should be implemented by industries. Our results demand further intense investigation and periodic monitoring of local milk vendors as well as dairy industries.

4. Conclusions

Both raw and pasteurized milks sold in Dharan were found to be free from adulteration with starch, formaldehyde and neutralizers and thus present lesser chemical safety issue, for example, compared to milk sold in Kathmandu valley. The microbiological quality of both raw and pasteurized milk are still very poor. Presence of coliforms in raw milk clearly indicated lack of hygiene and sanitation on the part of milk suppliers. The presence of coliforms, thermoduric bacteria and *S. aureus* in pasteurized milk invites several speculations, ranging from faulty processing, to post-pasteurization contamination. Pasteurization is an effective technique to reduce and eliminate food-borne

pathogens and other bacteria from milk. However, presence of food-borne pathogens in pasteurized milk indicates that pasteurization alone is not a certain solution for controlling milk-borne pathogens. To upgrade the quality of raw and pasteurized milk, legal enforcement on the microbial guideline of marketed milk, routine monitoring of dairy industries and raw milk vendors, awareness campaign and good hygienic practice should be promoted.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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