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Microbiological Quality Assessment of Milk from Local Market in Suburban Regions of Nashik District

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ABSTRACT: High-quality milk and milk products are becoming increasingly popular. As a result, all market participants must ensure clean and high-quality milk production. There are vast opportunities for quality milk and milk products in Indian market. Quality milk production depends on the milking environment, the milker's hygiene, the cleanliness of the milking animals, and the container or packaging of the milk. Contamination of milk is largely due to an unhygienic environment, poor milk handling practices, and milk adulteration. This study was planned to investigate the microbiological quality and safety of milk samples from local market. The samples were assessed for the microbiological quality of milk, including physiochemical parameters, the methylene blue dye reduction test (MBRT), the standard plate count (SPC), the detection of *Salmonella* and *Shigella* species, the presence of coliforms, and molds. After 3 days of storage, a higher SPC of 121 × 10^9 cfu/ml was detected in milk sample. A considerable number of coliform organisms were also found in the sample.

Keywords: Local market milk, Standard plate count, Most probable number, Microbiological quality.

INTRODUCTION

India is the world's greatest producer of milk. India accounts for around 13% of global milk output and consumes nearly all of it (Jadawala and Patel 2017). An immense nature of milk and its nutritional characteristics attract microbes, which contribute predominantly to the perish ability of milk (Argudin *et al*., 2010**;** Islam *et al.*, 2018; Islam *et al.*, 2021; Alice *et al.*, 2021; Agarwal *et al.*, 2012; Tadesse *et al.*, 2020)**.** Milk is a basic food in the human diet with great value as a nutritious, healthy food **(**Agarwal *et al*., 2012)**.** In the first years of human life, milk and dairy products are an important nutritional fact in the diet of the adult population (Amistu *et al*., 2015; Jeppu *et al.,* 2015). Consumers want milk that is safe and of high quality (Tadesse *et al.,* 2020). This creates a necessity to ensure clean milk production by various players in the market. Clean milk production depends on the milking environment, the milker's hygiene, the cleanliness of the animals, processing, and the container or packaging of the milk (Minj and Behera 2012; Kumar *et al.*, 2022). The quality and safety of milk is one of the dairy sector's main issues. However, milk contamination may occur at any point, but the majority of contamination is generally associated with an unhygienic environment and practices (Paraffin *et al*., 2018; Kumar *et al.*, 2022). Contamination of milk after leaving the farm shed and processing unit is largely due to poor milk handling practices which lowers the keeping quality of milk (Azadand Ahmed, 2016; Martin *et al.*, 2018; Collee *et al.*, 2007**;** Ananthanarayan and Paniker 2013).

The microbiological quality of milk plays an important role in ensuring the safety of consumers and producers (Eshetu *et al.*, 2019). According to HACCP, the pasteurization process is one of the important criteria in the production of market milk (Mohamed, 2018). Milk contains all of the nutritional properties required for microbe growth, including spoilage and pathogen populations (Chatli *et al.*, 2014; Melese and Addisu 2015). Milk microbiological quality is of public health concern due to zoonotic diseases that are milk-borne, such as tuberculosis, brucellosis, shigellosis, and salmonellosis (Torkar and Teger 2008; Paraffin *et al.*, 2018; Nur *et al.*, 2021). There are also emerging pathogens of public health importance such as *E. coli, Campylobacter jejuni, Yersinia enterolitica, Listeria monocutogenes and Salmonella* sp. (Senior, 1989; Nur *et al.*, 2021). Due to the fact that milk-borne diseases are of public health importance, there is a serious need to analyze the milk for the sake of consumer health protection (Agarwal *et al.*, 2012). The main objective of this study was to assess the factors influencing the quality of milk. Due to the scarcity of reports pertaining to the microbiological quality assessment of milk, we undertook this study. This study was aimed at checking the physicochemical and microbiological quality of milksamples.

MATERIALS AND METHODS

Sample collection. The milk samples were collected from different local retailers in suburban regions of Nashik District, Maharashtra. Most of the milk and dairy units supply their packaged milk. Samples as available were procured from local market, brought to the microbiology laboratory, and immediately processed for analysis. All samples were analyzed with a 24-hour interval. Milk samples were kept in a refrigerator at 4°C, and analysis of all samples was conducted for three subsequent days.

Physicochemical analysis

— MBRT
— MPN (most probable number) test
— Standard plate count
— Detection of *Salmonella* species
— Detection of the presence of molds

Methylene blue reduction test. In this test, a 9 ml milk sample was taken into the three sterile test tubes, and 1 ml of methylene blue (1:30000) dye was added to it. The tubes were screw-capped and carefully mixed to mix up the dye with the milk sample evenly. All the tubes were incubated in a water bath at 37 °C and examined at intervals of 30 min to 1 h for 6 h (Jeppu *et al.*, 2015). The time taken for the methylene blue dye to decolorize was recorded (Minj and Behera, 2012).

Most Probable Number (MPN) Analysis. The most probable number (MPN) analysis is a statistical method based on the random dispersion of microorganisms per volume in a given sample. In this test, three sets were prepared using single- and double-strength MacConkey's broth (Collee*et al.*, 2007). According to APHA (1992), 10 mL, 1 mL, and 0.1 mL of sample were inoculated in each set.

Standard plate count. The standard plate count method consists of diluting a sample with sterile saline until the bacteria are diluted enough to be counted accurately. One millilitre of the sample was mixed with 99 millilitres of sterile distilled water and kept on an orbital shaker for even mixing of the sample and any microbes present in it, if any. Serial dilutions of samples were carried out, and the 0.1 ml sample (Alice *et al.*, 2021) was taken from desirable dilutions and spread over sterile nutrient agar plates. Plates were incubated at 37 °C overnight. The average count of two plates was taken for the standard plate count as described in APHA (Muhamed *et al.*, 2010). For the molds, milk samples were diluted using the serial dilution method. The 0.1 ml of sample was taken from desirable dilutions and spread over sterile potato dextrose agar plates. Plates were incubated at 37°C for 48 hours (American Public Health Association, 1992).

Confirmation test. EMB agar. Bacteriological Eosin-Methylene Blue Agar, purchased from Hi Media Laboratories Ltd. in India, was used for the confirmed test. The coliform group of bacteria was confirmed using a test.

Salmonella-Shigella (SS) agar. Salmonella-Shigella agar purchased from Hi Media Laboratories Ltd. in India was used for the isolation of *Salmonella* and *Shigella* species. It is a differentially selective medium for the isolation of *Salmonella* and some *Shigella* species.

RESULTS AND DISCUSSION

This study revealed the keeping quality of milk after three days of storage at 4 °C. The physicochemical factors in each milk sample recorded are summarised in Tables 1-3. Milk samples were designated as A, B, C, D, and E. The physical and chemical properties of milk are important factors in attracting consumers. Milk withdrawn from milking animals is typically white or white with a yellow tint. It is opaque in its structure. The colour is influenced by the growth of various microorganisms, which change the colour from white to other unnatural colours (Belitz *et al.*, 2008). All six milk samples were examined and found to have normal colour, smell, pH, and organoleptic properties. Even after being stored for three days, all samples retained their normal consistency.

Analysis of microbial content by MPN Test. MPN tests are commonly used to detect the presence of coliform bacteria in samples. The milking animals' unhygienic conditions may lead to microorganisms in the milk. Other sources of microbes in milk are such as utensils and milk handlers, water, soil, and manure, as well as improper or defective processing of milk (Eshetu, *et al.*, 2019; Zelalem, 2012). Hygienic practices are ways to produce safe and quality products for consumers, thereby reducing microbial contamination and product loss of product (Tadesse *et al.*, 2020; De Silva *et al.*, 2016). MPN is a standard method used determination of the presence of the coliform group of bacteria (Mhone *et al.*, 2011). All six samples were studied for MPN for three consecutive days. Finally, all samples were positive for the presence of coliforms (Tables 4-6**)**. Therefore, it indicates that there might be improper handling and contamination of samples (Alice *et al.,* 2021; Collee *et al.,* 2007; Ananthanarayan and Paniker 2013). There were 180 and 350 discoverable coliform bacteria on 3-day storage in samples B and E, respectively. The presence of coliform organisms in raw and pasteurized milk samples has previously been demonstrated (Islam *et al.*, 2021). The coliform number increased may be due to further storage of the milk sample, even though kept at low temperatures. These results indicated that the quality of the milk was low in few samples.

Demonstration of quality of milk samples by MBRT. This test is commonly used to assess the quality of milk in terms of the microbial load present in it (DeSilva *et al.*, 2016). The microbiological quality of milk samples was assessed by the MBRT test. Results of the test are depicted in Tables 7**-**9**.** The milk samples were graded into different categories using the standard methodology available in literature. As a result, milk samples A, C, and D were found to have excellent, very good, and good qualities in all tests, whereas samples B and E were found to have fair and poor quality, respectively.

Standard Plate Count (SPC) for Bacteria and mold. The aerobic microbial count in milk in terms of colony forming units (CFU) per mL is depicted in Tables 10-12**.** A SPC of 1×10^5 cfu/mL is considered acceptable in raw milk and needs to be treated before its distribution. The European Commission recommends that the standard plate count of milk should be less than 105 cfu/ml of milk. According to the Prevention of Food Adulteration Rule of 1956, the TVC (total viable count) of milk should fall below 100/mL at 20–22 ºC within 3 days. The SPC/TBC was reported in pasteurized milk samples previously (Shojaei and Yadollahi 2008; Agarwal *et al.*, 2012; Mhone *et al.*, 2011). SPC count regarded as important method to determine quality of milk (Shojaei and Yadollahi 2008). The growth of mold was not detected in any sample of milk.

Determination of *Salmonella* and *Shigella* species in milk. According to the Prevention of Food Adulteration Rules of 1956 and Food Safety and Standard Act 2011, none of the milk sample should contain *Salmonella* or *Shigella* species. The milk samples examined for the presence of *Salmonella* and *Shigella* species exhibited growth with striking resemblance to both of the mentioned organisms in one sample, however further identification of these organisms is actually needed. Similar growth was not present in four other samples (Tables 13-15). The presence of pathogens in raw as well as pasteurized milk samples is the matter of concern (Agarwal *et al.*, 2012).

Growth of Coliform group of bacteria on EMB agar. All milk samples were inoculated onto sterile eosin-methylene blue agar and incubated at 35 °C for 24 to 48 hours. Plates were examined for the presence of typical colonies (positive) for organisms like *Escherichia coli*. According to Food Safety and Standards Regulation, 2011 sample should be free from presence of *Escherichia coli* per 0.1 gram of sample. Previously reported that presence of *E. coli* in milk and milk products (Sood *et al.*, 2016). Among the all samples, in E sample was detected positive for the growth of typical colonies which is indicative for contamination and need to employ proper sanitary measures (Islam *et al.*, 2021; Sood *et al.*, 2016).

Table 1: Physicochemical parameters of milk samples on 1st day.

Parameters	**Milk Samples**				
Character	**A**	**B**	**C**	**D**	**E**
Color	White	White	White	White	White
Smell	Milky	Milky	Milky	Milky	Milky
pH	6.7	6.5	6.6	6.8	6.4
Flavor	Semi-sweet	Semi-sweet	Semi-sweet	Semi-sweet	Semi-sweet
Physical state	Liquid	Liquid	Liquid	Liquid	Liquid

Table 2: Physicochemical parameters of milk samples on 2nd day.

Parameters	**Milk Samples**				
Character	**A**	**B**	**C**	**D**	**E**
Color	White	White	White	White	White
Smell	Milky	Milky	Milky	Milky	Milky
pH	6.7	6.5	6.6	6.8	6.4
Flavor	Semi-sweet	Semi-sweet	Semi-sweet	Semi-sweet	Semi-sweet
Physical state	Liquid	Liquid	Liquid	Liquid	Liquid

Table 3: Physicochemical parameters of milk samples on 3rd day.

Parameters	**Milk Samples**				
Character	**A**	**B**	**C**	**D**	**E**
Color	White	White	White	White	White
Smell	Milky	Milky	Milky	Milky	Milky
pH	6.7	6.5	6.6	6.8	6.4
Flavor	Semi-sweet	Semi-sweet	Semi-sweet	Semi-sweet	Semi-sweet
Physical state	Liquid	Liquid	Liquid	Liquid	Liquid

Table 4: Interpretation of MPN test on 1st day.

Amount of Sample inoculated in respective sets	**Milk samples**				
	A	**B**	**C**	**D**	**E**
10ml	5	4	3	3	5
1ml	1	3	2	1	4
0.1ml	1	1	1	1	3
MPN per 100ml of Milk	**46**	**33**	**17**	**14**	**280**

Table 5: Interpretation of MPN test on 2nd day.

Amount of Sample inoculated in respective sets	**Milk samples**				
	A	**B**	**C**	**D**	**E**
10ml	4	5	3	4	5
1ml	2	4	2	1	4
0.1ml	1	0	1	1	3
MPN per 100ml of Milk	**26**	**130**	**17**	**21**	**280**

Table 6: Interpretation of MPN test on 3rd day.

Amount of Sample inoculated in respective sets	**Milk samples**				
	A	**B**	**C**	**D**	**E**
10ml	4	5	4	4	5
1ml	3	3	2	2	4
0.1ml	1	3	0	1	4
MPN per 100ml of Milk	**33**	**180**	**22**	**26**	**350**

Table 7: Demonstration of grading quality of milk samples on 1st day.

Milk samples				
A	**B**	**C**	**D**	**E**
Excellent	Fair	Very good	Good	Poor

Table 8: Demonstration of grading quality of milk samples on 2nd day.

Milk samples				
A	**B**	**C**	**D**	**E**
Excellent	Fair	Very good	Good	Poor

Table 9: Demonstration of grading quality of milk samples on 3rd day.

Milk samples				
A	**B**	**C**	**D**	**E**
Excellent	Fair	Very good	Good	Poor

Table 10: Determination of SPC in milk samples on 1st day.

Sample	CFU per milliliter of sample taken from respective dilution		
A	11×10^7	23×10^8	23×10^9
B	13×10^7	18×10^8	24×10^9
C	31×10^7	22×10^8	12×10^9
D	22×10^7	10×10^8	21×10^9
E	112×10^7	116×10^8	121×10^9

Table 11: Determination of SPC in milk samples on 2nd day.

Sample	CFU per milliliter of sample taken from respective dilution		
	10^7	10^8	10^9
A	5×10^7	32×10^8	12×10^9
B	33×10^7	14×10^8	21×10^9
C	31×10^7	19×10^8	5×10^9
D	27×10^7	3×10^8	9×10^9
E	98×10^7	101×10^8	96×10^9

Table 12: Determination of SPC in milk samples on 3rd day.

Sample	CFU per milliliter of sample taken from respective dilution		
	10^7	10^8	10^9
A	13×10^7	4×10^8	2×10^9
B	23×10^7	20×10^8	13×10^9
C	22×10^7	2×10^8	2×10^9
D	14×10^7	12×10^8	3×10^9
E	87×10^7	80×10^8	78×10^9

Table 13: Presence of *Salmonella* and *Shigella* species growth on day 1 analyzed sample.

Sample	Result
A	Growth was not observed
B	Growth was not observed
C	Growth was not observed
D	Growth was not observed
E	Growth was observed

Table 14: Presence of *Salmonella* and *Shigella* species growth on day 2 analyzed sample.

Sample	Results
A	Growth was not observed
B	Growth was not observed
C	Growth was not observed
D	Growth was not observed
E	Growth was not observed

Table 15: Presence of *Salmonella* and *Shigella* species growth on day 3 analyzed sample.

Sample	Results
A	Growth was not observed
B	Growth was not observed
C	Growth was not observed
D	Growth was not observed
E	Growth was observed

CONCLUSIONS

Total of five samples were collected and assessed subsequently for three days. Principal microbiological quality checking tests were followed, and few samples were detected for the presence of microbes. Considerable bacterial count in samples, suggest improper sanitary measures (Amistu *et al.*, 2015). The samples were found to be positive for the presence of the coliform group of organisms, suggesting the possibility of contamination of samples (Jeppu *et al.*, 2015). Adoption of appropriate sanitary measures and standard operating practices needs to be considerable (Melese and Addisu 2015; Chatli *et al.*, 2014**;** Amistu *et al.*, 2015). These results indicated that the milk samples will meet microbiological quality and that it is essential to follow the prescribed standard operating practices in the interest of consumers (Islam *et al.*, 2021). This study highlights the significance of milk pre- and post-process management in terms of consumer health concerns.

FUTURE SCOPE

Significant measures must be taken when handling, transporting, processing, and controlling the quality of milk. This might ensure public health concerns. More thorough study with controlled analysis is necessary to determine the quality of raw, processed milk, and milk products.

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Conflict of Interest. None.

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