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Assessment of Microbial and Keeping Quality of Cow Milk Produced under Subsistence Farming Through Better Milking Hygiene in Barisal District

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Abstract: *The present study was conducted with a view to improving the microbial and keeping quality of milk produced by subsistence farmers through better milking hygiene. A survey was conducted to assess the existing hygienic quality of milk. It was found that the present hygienic conditions of the farmers at 9 selected areas were poor. A total of 27 selected farmers were trained to practice better milking hygiene (using a sanitary solution of about 200ppm chlorine). Milk samples (total 135) were collected from the trained farmers to make a comparison. It was found that Standard Plate Count and Coliform Count were reduced by 61 and 69 percent, respectively before and after adopting better milking hygiene practices. Methylene blue reduction time of the milk samples collected from different locations averaged 8.55 hr and 11.55 hr, respectively before and after application of better hygiene. The acidity percentage between two time periods showed no significant difference. A detectable abnormal smell in milk was found after 6.22 hr and 10.83 hr, respectively. The mean values of clot-on-boiling (COB) +ve time (hr) were 8.83 and 12.39, respectively before and after application of better milking hygiene. Time required to develop detectable abnormal smell and to give COB +ve averaged to work out the limit of overall acceptable keeping quality time (KQT). With a significant difference ($p<0.01$), KQT of milk samples were 8.00±0.696 and 11.67±0.395hr, respectively before and after application of better milking hygiene.*

Keywords: *milking hygiene, microbial quality, clot-on-boiling, keeping quality time*

I. Introduction

The nutritive perfection of milk has made it not only a unique food for human being but also a medium most favorable for microbial growth. Organisms that have entered the teat canal through teat orifices are mechanically flushed out during milking (Frazier and Westhoff 1995). During the normal milking operation however, milk is subjected to contamination from the exterior of the udder and adjacent areas, dairy utensils, milk contact surfaces, the hands of milkers or dairy workers, air of the milking parlour, water used and flies. Milk, produced under improper hygienic condition, contain greater microbial load and there have greater chance of being contaminated by human pathogens and such milk is considered as a potential threat for public health. Although, spoilage type of microorganisms present in milk are not considered harmful to human health and even some microorganisms have recognized therapeutic effects, these are harmful to milk itself, as they adversely affect the keeping quality (shelf life) of milk by bringing about a physical and chemical changes in milk. Milk production in our country is characterized by low yield non-descript cows and buffaloes. The rural producers and a few small or medium dairies produce milk without maintaining proper hygienic measures, which results in milk with higher initial microbial load, and the time interval between production and consumption favors a great increase in the microbial number and make the milk bacteriologically of poor quality. The hygienic measures taken during and after milking essentially determine what foreign microorganisms enter the milk, including human pathogens. The count of properly drawn mixed milk from healthy cows is about 100000 $ml^{-1,}$ sometimes even less. If however, the hygienic standards during milking are poor, freshly drawn mixed milk can help a much higher count, upto one million ml^{-1} (Walstra *et al*. 1999). Studies in this regard revealed that proper milking hygiene could significantly reduce microbial load in milk (Islam *et al*. 2009, Petrovic *et al*. 2006) and that lower microbial load is associated with better keeping quality (Lakhani and Singh 1998). It is, therefore, important to train the farmers about proper milking hygiene procedures using cheap and available materials to reduce milk spoilage as well as to get safe and quality milk. The present effort was undertaken to determine the keeping quality of milk under present hygienic condition and to improve the microbial and keeping quality of milk by improving the hygienic condition of milk producers in 3 upazilas of Barisal district in Bangladesh.

II. Materials and Methods

2.1 Study Site and Duration

This study was conducted at Babugonj, Sadar and Wazirpur upazilas of Barisal district and at the Department of Dairy and Poultry Science, Patuakhali Science and Technology University, Bangladesh.

2.2 Baseline Survey and Sample Collection

Baseline data on present milking hygiene were collected using a structured questionnaire. Milk samples (total 135) were collected from different locations at an interval of 3-4 days and analyzed at room temperature during the study period.

2.3 Procedure

The hygienic conditions of the milk producers of the experimental site were assessed from collected data. Milk samples collected from selected farmers initially for the purpose of determination of microbial and keeping quality of so produced milk. The selected producers were trained to practice better milking hygiene using a solution of bleaching powder (around 200 ppm Cl). Milk samples from the same farmers after application of better milking hygiene were collected, and microbial (SPC, CC) and keeping qualities were assessed. Finally, a comparison between the two situations was made.

2.4 Parameters Studied

The milk samples were analyzed at Dairy and Poultry Science Laboratory for initial microbial quality and subsequent keeping quality with the help of Standard Plate Count (SPC), Coliform Count (CC), Methylene Blue Reduction Time (MBRT) test, Acidity test and Clot on Boiling (COB) test. All the parameters were studied as per the procedure described in the Standard Methods for the Examination of Dairy Products (APHA 1998)

2.5 Data Analysis

Data were subjected to paired sample t-test using SPSS computer package. Simple statistical measures like mean and percentage were also used to analyze the data.

III. Results and Discussion

3.1 Existing milking hygiene condition

We have collected information on existing milking hygiene condition of the local farmers in Barisal district. Overall milking hygiene condition is presented in Table 1.

Table 1. Milking hygiene condition of the local farmers (n=60)

Hygienic Aspects	Respondents found as positive	Percent of total
Cleaning and washing of milking cows daily	10	16.67
Washing of udder before milking	5	8.33
Drying of udder with cloth after washing	0	0.00
Washing of milkers hand before milking	5	8.33
Washing of milking pail before milking	3	5.00
Use of disinfectants	0	0.00
Use of soap in washing of milking pail after milking	8	13.33
Use of ash in cleaning of milking pail after milking	23	38.33
Washing of milkers hand in-between two milking	0	0.00

The existing milking hygiene condition at the study area is very poor. Most of the farmers have no knowledge on this aspect. They usually do not wash and clean the cow, cow's udder, milking pails, and hand of the milkers before milking. All washing is mainly done with normal water except using soap (13%) or ash (38%) in some cases in the washing of milking pails after milking.

3.2 Standard Plate Count (SPC)

Results of Standard Plate Count (SPC) as obtained from milks of two hygienic groups (Table 2) indicate that mean values of initial Standard Plate Counts (SPC) were 411±75 and 161±37 thousand cfu/ml, respectively before and after application of milking hygiene practices. The mean SPC values were significantly different (t value of paired t-test was significant at 99% level of confidence). Initial SPC-after were reduced by 61% compared to the count obtained before. Results of initial SPC represent that milking hygiene has significant effect to the initial bacterial number in milk. Proper hygienic measure during milking would help producing milk of highest possible bacteriological quality.

Schoken-Iturrino *et al.* (1982) studied the effect of several sanitary measures during milking on the microbial count of milk. Samples were taken during milking from 16 Friesian cows milked by hand (i) Without

any preparation or with (ii) udder preparation with sodium hypochlorite solution with active Cl at 5ppm (iii) preparation of udder and milkers' hands or (iv) preparation of udders, hands and teat cups. Bacterial count in milk decreased in the order (i)> (ii) = (iii)> (iv) ($p>0.05$). Differences in the mean bacterial count among milking were significant at 1% level.

Table 2. Standard Plate Counts (SPC) of milk collected from different locations before and after application of better milking hygiene practice.

Location	Before ($\times 10^3$ cfu/ml)	After ($\times 10^3$ cfu/ml)	$t_{0.01}$
1	510	115	9.633 (calculated) 2.896 (tabulated)**
2	340	150	
3	285	143	
4	390	165	
5	430	229	
6	470	160	
7	360	157	
8	500	209	
9	410	121	
Mean±SD	**410.5556±75.434**	**161.000±37.286**	

** $p<0.01$

Sakya and Srivastava (1982) reported a 79.7% and 83.6% reduction ($p<0.01$) in mean bacterial count by washing and disinfection (200 ppm Cl) of milkers' hands and cows' udder respectively. Petrova and Petroava (1985) showed that bacterial counts were halved by washing udder with a disinfectant (cleaning agent, Trizonat 0.5%) as opposed to using only a dry cloth for cleaning. Schultze and Smith (1969) showed that chlorohexidine used at 0.2% in an aqueous dip for cows' teats immediately after each milking reduced the resident microflora on the apical teat skin by about 95%. The results of the present study are in agreement with the previous studies throughout the world.

3.3 Coliform Count (CC)

Results of coliform count (CC) are presented in Table 3. The mean values of CC were 359.33±90 and 44.55±20 cfu/ml, respectively before and after application of better milking hygiene. The mean CC showed significant difference (t value of paired t-test was significant at 99% level of confidence). The mean coliform count was reduced by 69 % after practicing suggested milking hygiene practices.

Table 3. Coliform Counts (cfu/ml) of milk collected from different locations before and after application of better milking hygiene practice.

Location	Before	After	$t_{0.01}$
1	470	31	9.608 (calculated) 2.896 (tabulated) **
2	300	45	
3	184	41	
4	410	35	
5	330	38	
6	360	69	
7	390	53	
8	480	27	
9	310	62	
Mean±SD	**359.333±90.00**	**44.555±20.00**	

** $p<0.01$

The coliform count of each location after better hygiene was below the maximum coliform standard (100/ml) for raw milk. Percentage reduction of CC was higher than the reduction of SPC. It may be due to the fact that unsanitary conditions help increased contamination of milk by coliform, and contamination originating from udder, hands, equipment and also water used associated with coliform groups. Henderson (1971) stated that organisms associated with dirt such as manure, bedding, and soil are often of gas forming Escherichia-aerobacter group and may fall from the belly, flanks, tail, and udder into the milk pail and thus carry a considerable number of bacteria into the milk.

From the results it is found that better milking hygiene has significant effect to the reduction of coliform count in freshly drawn milk samples. Some researchers (Hogan *at el.* 1979, Galton *et al*. 1984) reported higher coliform count related to the degree of wetness of udder. Patrova and Patrova (1985) in a study showed that udder disinfection (Cl 5ppm) can half the coliform count in milk, whereas Sakya and Srivastava

(1982) demonstrated a non-significant effect of udder disinfection (200 ppm Cl) on coliform count. Improper equipment sanitation also attribute to the increased coliform counts in fresh milk which was supported by Kantona and Szita (1982). The present experiment design does not support to make such specific distinction but results are in overall agreement with the previous findings and suggest that proper hygienic measure during milking significantly reduce the incidence of coliform counts in milk.

3.4 Methylene Blue Reduction Time (MBRT) test

Methylene blue reduction test shows that microbial quality of milk was improved due to better milking hygiene (Table 4). Though the reduction time was significantly (t value of paired t-test was significant at 99% level of confidence) increased, the initial methylene blue reduction time fails to grade milk properly if the microbial load is low.

Table 4. Methylene Blue Reduction Time of milk collected from different locations before and after application of better milking hygiene practice.

Location	Before (minutes)	After (minutes)	$t_{0.01}$
1	360	720	6.364 (calculated) 2.896 (tabulated) **
2	510	690	
3	570	690	
4	600	720	
5	540	720	
6	510	660	
7	600	690	
8	390	660	
9	540	690	
Mean±SD	**513.333±85.440**	**693.333±23.452**	

** p<0.01

3.5 Acidity test

Samples (before and after) collected from different locations showed no significant (t value of paired t-test was non-significant at 99% level of confidence) difference in the initial acidity percentage (Table 5). Thus initial acidity is not an indication of the hygienic quality of milk.

Table 5. Acidity percentage of milk collected from different locations before and after application of better milking hygiene practice.

Location	Before	After	$t_{0.01}$
1	0.16	0.165	0.525 (calculated) 2.896 (tabulated) **
2	0.17	0.16	
3	0.15	0.15	
4	0.165	0.153	
5	0.153	0.163	
6	0.168	0.155	
7	0.15	0.17	
8	0.17	0.15	
9	0.16	0.16	
Mean±SD	**0.160667±0.008**	**0.158444±0.007**	

** p<0.01

3.6 Organoleptic Evaluation of Milk Smell

On an average normal milk smell was retained for 6.22hr and 10.33hr, respectively before and after better milking hygiene. Normal milk smell gradually decreases after a certain period and the intensity of abnormal smell increases at COB+ve time. Time periods required to develop abnormal smell are presented in Table 6. A detectable abnormal smell was found on an average after 7.17hr and 10.83hr, respectively before and after application of better milking hygiene. Hardling (1995) stated that if the bacterial counts of milk were allowed to increase significantly (e.g. to over 3 million/ml) this could lead to significant degradation of the fat, protein or lactose causing off-flavour. Present study fairly agrees with previous findings.

3.7 Clot-on-Boiling (COB) test

COB tests were performed at an interval of 30 minutes. The results are summarized in Table 6. The mean values of COB +ve time (hr) were 8.833±0.56 and 12.389±0.33, respectively before and after application of better milking hygiene. The COB +ve time was increased by more than 3.5 hr.

Thus milking hygiene can improve the keeping quality of milk by maintaining a lower initial microbial contamination. Lakhani and Singh (1998) showed that average COB +ve for milk obtained by machine and hand milking were 10.6hr and 9.3hr Sharma and Lavania (1988) reported COB +ve time of only 8hr for raw milk from small dairies. Results of the present experiment fairly agree with the previous findings and show the importance of hygienic practices during milking.

3.8 Overall Keeping Quality Time (KQT)

The term keeping quality is generally used to denote the length of time that milk will remain sweet before it commences to sour and become unfit for use, and this period represents the useful life of the liquid (Harvey and Hill 1951). Chalmers (1955) stated that keeping quality of sample of milk is the period in hours elapses from its production until it is considered unsuitable for consumption, either because it curdles on boiling develops an undesirable odour or flavour. Time required in developing detectable abnormal smell and to give COB +ve were averaged to work out the limit of overall acceptable keeping quality time (KQT) in Table 6. There were significant difference (t value of paired t-test was significant at 99% level of confidence) between the average keeping quality times (KQT) of milks obtained before and after application of better milking hygiene practices.

Table 6. Keeping quality time (KQT) based on COB +ve and off-smell.

Location	Before			After			$*t_{0.01}$
	COB +ve (hr)	Off-smell (hr)	KQT (hr)	COB +ve (hr)	Off-smell (hr)	KQT (hr)	
1	8.0	6.0	7.00	13.0	12.0	12.50	11.656 (calculated) ** 2.896 (tabulated)
2	9.5	8.5	9.00	12.0	11.0	11.50	
3	9.5	8.0	8.75	12.5	10.5	11.50	
4	9.0	7.0	8.00	12.5	11.0	11.75	
5	9.0	7.0	8.00	12.5	11.0	11.75	
6	8.5	7.0	7.75	12.0	10.0	11.00	
7	9.0	8.0	8.50	12.5	11.0	11.75	
8	8.0	6.0	7.00	12.5	11.0	11.75	
9	9.0	7.0	8.00	12.0	10.0	11.50	
Mean±SD	**8.83**	**6.22**	**8.0 ± 0.696**	**12.39**	**10.3**	**11.67±0.395**	

* t value was calculated only for keeping quality time; ** $p<0.01$

IV. Conclusion

The study revealed that milking clean cows in dry condition gives better initial microbiological and subsequent keeping quality. It is also readily evident that calcium hypochlorite solution (200ppm Cl) can provide satisfactory reduction in microbial number and can improve keeping quality of milk for a considerable period if used for washing and disinfection of udder and teats, milkers' hands, and rinsing of milking pails just previous to milking.

It may be recommended that calcium hypochlorite solution (200ppm Cl) can be used as an effective and economic sanitizer in milking hygiene operations to improve initial microbial quality and overall keeping quality of milk. The extra cost involved in following such measure appeared to be very negligible compared with the gains obtained through improved milk quality.

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