

Microbiological Quality Evaluation of Raw Milk Consumed in and Around Rawalakot, Azad Jammu and Kashmir

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Abstract: For the microbiological examination of milk, total bacterial count, Antibiotic sensitivity test, White side test and Surf test were performed. The result of total bacterial count showed the variation 4.5×10^5 , 8.3×10^7 and 1.7×10^9 among the milk samples. The contamination rate was increased in all three sources i.e; house hold animal, milkman and restaurant milk. The results of antibiotic test showed that Amoxicillin given good result among all other antibiotic discs which were used. Whereas, Ofloxacin and Kinanycin showed the poor results. The result of Whiteside test show 14% positive in house rholed milk, 29% and 26% in milkman and restaurant milk respectively. The contamination of pathogenic organisms was become double in milkman milk and restaurant milk, whereas the result of surf test was much similar to white side test having 11%, 23% and 20% in house holed milk, milkman milk and restaurant milk respectively.

Key words: Microbiology • Quality • Raw Milk

INTRODUCTION

Milk plays a vital role in building a healthy society and can be used as vehicle for rural development. Fresh or raw milk as diet contributes to infants and adults in all over the world [1].

Pakistan is at 3rd position in milk production with annual contribution of 43.29 million tons (Ministry of Livestock and Dairy Development Pakistan, 2009-10). Buffalo and cow account for 96% of the total milk production in Pakistan, of which of which buffalo is at fist and cattle at 2nd position [2]. Livestock sector contributes 11% of total GDP and providing main work force for 30-35 million people of rural population [3]. There are several factors which decreases the quality of raw milk. These include poor animal health, delivery services, poor house hygiene, unhygienic milking practices, udder infection and zoonotic infection. Other factors such as presence of antibiotic residues and animal diseases

(mastitis) also affect the milk quality. On the other hand, environmental changes such as seasonal changes and nutritional change also affect the milk composition. Absence of cooling rooms and hygiene facilities may affect the quality standards badly of milk [4-6].

Milk is naturally a good medium for microbial growth. Due to specific nature of milk, there is no way to prevent the microbial contamination, that's why the microbial contamination is major factor in determining its quality. Milk-borne outbreaks are recorded as 2-6% that is due to bacterial food-borne [7].

Microbial contamination can originate from different sources including air, milking equipments, feed, soil, faeces and milking place [8]. Moreover, the utensils, animal skin, environment, water used for adulteration may also cause great contamination [9]. Transportation of milk at ambient temperature results in enhanced and multiple number of microbes that deteriorate the overall milk

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quality [10]. So examination of fresh milk samples, final products and equipment sites for microbes is an important part of quality standards or quality assurance plan.

Quality and safety improves the shelf life, also help to introduce the new variety of different products and convenience for the consumer [11]. The storage life of a dairy product has the better time during which the product can remain wholesome and having no physical or sensory defects. Quality milk is required to fulfill the consumer's demand, i.e. free from pathogenic bacteria, harmful toxins, sediment and extraneous substances, flavor, normal composition, good keeping quality and with low bacterial load. The milk quality is still evaluated throughout the world on the basis of physical and chemical tests such as milk density, fat content, hygienic and microbial analysis [12].

The general way to assess the milk quality is microbial assessment such as standard plate counts (SPC) and antibiotic tests (detection of drug residues) in milk. Besides microbial testing, other quality parameters such as fat percentage, protein percentage and assessment of antibiotics are very important. Hence the milk quality with reference to composition, microbes as well as hygiene, is required for the security of human health so also need to maintain the high biological value of raw milk and ensuring the technological process throughout the milking processes [13,14].

In Rawalakot (AJK) majority of people use raw milk but no proper guidelines are available for milking, handling and storage at house hold level restaurants and shops. In AJ&K very limited work has been undertaken to assess the quality standards of milk. The objective of this study was to evaluate the hygienic quality and level of microbial contamination in raw milk which was collected from house hold animals, milk man and restaurants. The present study was therefore planned to investigate the quality and safety standards.

MATERIALS AND METHODS

The experimental study was carried out in the Food Technology Research Laboratory of Department of Food Sciences and Technology Faculty of Agriculture Rawalakot University of Poonch, Rawalakot.

Collection of Samples: Milk samples were collected from house hold animal milk, milkman and restaurants in bottle from Rawalakot Azad Jammu & Kashmir and its surroundings. Milk samples were labeled and placed the each specimen in ice packing and then transported to the

laboratory for further analysis. These samples were transferred in to the refrigerator immediately at (4°C) and culturing was conducted within 24 hours. The sample was subjected to following quality tests.

Methylene Blue Test (MBRT): Methylene blue test for the assessment of mastitis was performed according to procedure described by method followed by [15]. The test is used to diagnose mastitis, the ability of bacteria to reduce the color of methylene blue dye from the milk sample. Dye reduction time is inversely proportion to the presence of total number of bacteria in sample, hence greater the bacterial population shorter the dye reduction time.

Total Bacterial Count (TVC): Total bacterial count in different milk samples was determined by method followed by Esron *et al.* [16]. Count the microbial population in sample as number of microscopic fields present in 1 cm square prescribed area of microscope glass slides.

Antibiotic Sensitivity Tests: Antibiotic sensitivity test for the assessment of treatments was performed according to procedure described by method followed by Narang [17]. Antibiotic test is used to determine the sensitive of organism against the diseases (mastitis) treatment. The test is carried out on pure culture of raw milk.

White Side Test: It is an indirect method used to assess the number of cell (mastitis) present in milk. Viscosity of the milk increase due to release of nucleic acid by the cell when treated with alkali. It was determined according to method used by Narang [17].

Surf test (SFMT): The surf field mastitis test (SFMT) was used to evaluate the mastitis by house hold detergent; (Surf Excel). This test is used on the basis of closely similar to diagnostic frequencies of SFMT and CMT in term of sensitivity examination. SFMT used for the detection of animals suffering from sub-clinical mastitis and used as good alternative animal sub clinical mastitis diagnostic test in poor Country. According to method followed by Ghulam *et al.* [18].

RESULTS AND DISCUSSION

The present investigation was carried out to assess the microbiological quality of raw milk from Rawalakot and its surroundings. 102 milk samples were collected from different sources mainly, the house hold (HH), milkman

Table 1: Values for Methylene blue test of different milk samples

| Sources | A+ (%) | B+ (%) | C+ (%) | D+ (%) |
|------------|--------|--------|--------|--------|
| House hold | 29e | 44b | 17f | 11h |
| Milkman | 14g | 62a | 11h | 14g |
| Restaurant | 14g | 33c | 33d | 11h |

A+ 8hr, B+ 6hr, C+ 4hr, D+2hr.

(MM) and restaurant milk (RM). These samples were analyzed for different parameters like, MBRT (methylene blue reduction test) and microbiological (total bacterial count, antibiotic test, white side test, surf test).

Methylene Blue Reduction Test (MBRT): The data regarding to methylene blue reduction test (MBRT) has been given in Table 1. The results obtained from three different sources found highly significant. The mean values of A+, B+, C+ and D+ for HH was 29%, 44%, 17% and 11%, 14%, 62%, 11% and 14% was recorded for MM and 14%, 33%, 33% and 11%, in RM respectively. In all three sources of milk grading was different; milkman milk contains high percentage (14% D+) of poor quality milk. However, the restaurants milk containing the high percentage (33% C+) of fair milk quality, whereas milk quality of HH milk was comparatively better than the other two sources. The poor quality of milk for restaurants and milkman may be due to the following reasons;

The milkman collected the milk from the infected animals and the casual organism of mastitis may easily transferred from one animal to another animal, even transferred by milking practices that is also reason of poor milk quality. In case of mastitis positive test animal need timely curing to recover but the common people of the area were not well aware about the mastitis and its curing. The quality of milk dependant on the degree of diseases invasion and the rate of contamination, as the number of organisms (*Staphylococcus aureus*) was more in milk, the quality of milk was lower. If the microbial load is low milk is considered of good quality. Further more increase in microbes decrease the quality of milk graded as B,C,D or poor quality of milk. According to the results, the high quality of milk was observed in house hold animal whereas milkman milk and in restaurant milk this percentage decreased. The result of the test was agreed with Chatterjee *et al.* [19].

Grading of Samples on the Basis of (Mbrt) in Different Milk Sources.

| | |
|-----------------|-------------------------------------|
| Quality of milk | Decolonization time |
| Excellent | More than 8 h |
| Good | Between 6 hours |
| Fair | Between 2 to 6 h Poor Less than 2 h |

Microbiological Tests: Microbiological examination is most commonly used parameter to assess the milk quality. Different parameters such as total bacterial count, antibiotic sensitivity test, surf test and white side test were used to analyze the milk quality

Total Bacterial Count: The data regarding the total bacterial count test (TBC) has been presented in Table 2. The data shows highly significant results. The average value of TBC was observed 4.5×10^5 in HH milk, 8.3×10^7 in MM and 1.7×10^9 in RM respectively. The high (TBC) values in MM milk and RM might be due to the following reasons. Milk sold in market (restaurants) was transported from far distance (200) kilometer. Due to prolong time, bacteria multiplied and became sources of high bacterial count. The high counts may also due to miss handling during milking, animal bedding and by mixing of abnormal milk in good quality milk. Bacterial counts were abundantly found in raw milk samples collected from three different sources of the city and its surroundings. High bacterial count was increased due to insufficient hygienic condition during raw milk collection from house hold animals. Handling and transportation also involved in microbial growth. The addition of impure water in milk may play vital role to enhance the count population. Bacterial growth increased rapidly and finally quality of milk reached at un-acceptable level.

Milk secreted from healthy animals is almost free from bacterial contamination but contamination can be possible from dairy farm and their environment, udder or animal body, utensils, addition of contaminated water. Milk is an ideal medium for bacterial growth which leads to heavy contamination, the results of the present study are in line with that of Muhmmad *et al.* [20].

Antibiotic Sensitivity Test: The data pertaining to anti biotic sensitivity test have been given in Table 5 whereas its statistical evaluation is presented in Table 3. The average values showed that effect of all three sources of milk found to be highly significant. The data also indicate that Amoxicillin given the more effective results among the all antibiotic disc which has been used. Peniciline and Gentamicine also gave the better result than the Ofloxacin and Kinamicin. The presences of antibiotic in milk samples was negligible, because inhibition zoon was recorded less than (2mm) in all samples. The test is sensitive to discriminate the use of drugs (tetracycline) and antibiotic injections (oxytocin) that are dangerous for human health (tooth decay). Tetracyclin is also secreted in freshly drown milk in its original form but it can not secreted in

Table 2: Mean values for total bacterial count (CFU/mL) of different milk samples.

| Source | Mean |
|------------|----------------------------------|
| House hold | 4.5×10 ⁵ _c |
| Milkman | 8.3×10 ⁷ _b |
| Restaurant | 1.7×10 ⁹ _a |

Table 3: Values of different Antibiotic sensitivity test

| Inhibition zone | Penicillin | Gentamicin | Amoxicillin | Ofloxacin | Kinamicin |
|-----------------|------------|------------|-------------|-----------|-----------|
| 0-5mm | 32b | 42b | 40b | 43b | 45b |
| 5-15mm | 54a | 50a | 56a | 49a | 55c |
| 15-25mm | 8c | 6c | 3c | 8c | ---- |
| 25-35mm | 2c | 2c | 3c | ---- | ---- |

Table 4: Values for Whiteside test of different milk samples.

| Source | Positive (+ive) % age | Negative (-ive) % age |
|------------|-----------------------|-----------------------|
| House hold | 14c | 66b |
| Milkman | 29a | 71a |
| Restaurant | 26a | 74a |

Table 5: Vvalues for Surf Test of different milk samples

| Source | Positive (+ive) % age | Negative (-ive) % age |
|------------|-----------------------|-----------------------|
| House hold | 11c | 89a |
| Milkman | 23a | 77c |
| Restaurant | 20b | 80b |

boiled milk, that's why boiled milk is safe from diseases. The milkman also used the injection (oxytocin) for the milk led down which might be the sources of low milk quality.

White Side Test: The result regarding the Whiteside test has been presented in Table 4. The values of positive results 14%, 29%, 26% were obtained from HH animal milk, MM and RM respectively. Household animal milk had lower percentage of positive cases as compared to other two sources of milk.

In small animal's herds at house hold level (HH), easy to manage the, hygienic condition as compared to large herds. Un-hygienic surrounding also played an important role to enhance the chances of diseases outbreak. Mastitis causing organisms are abundantly found in animal fesses and their environment as well and can be sources of diseases spreading in other animals. In case of milkman holding, the number of animals/herds was higher than house hold animals and it is difficult to manage the maintenance of herd. The mastitis causing organism may be transferred from one animal to another easily, so in small holding capacity sheds which containing the large numbers of animals are the main cause of diseases spreading. Hence more positive cases were recorded in MM and RM milk, due to lack of management and un-hygienic conditions. As it is difficult to manage the large herds manually and all these circumstances may favor to the diseases (mastitis) outbreak among the

animals and as a results, milk quality may also be affected. The results of the study are in line with findings of Ghulam *et al.* [18].

Surf Test: The data regarding to surf test has been presented in Table-5. The data of the test show the highly significant result for all three sources of milk. The results of Surf test showed significant differences between the MM and RM while in case of HH milk; the positive cases were showed the lower % age then other two sources of milk. The positive results 11 % in HH animal milk, 23 % in MM milk and 20 % RM respectively.

Because an infected animal shed mastitis causing organism (*Staphylococcus aureus*) which may be the sources of the clinical or apparent mastitis. The milk of such infected animals also the sources of diseases transfer especially when milkman mixed the infected milk with healthy animal milk. In case of MM animal's un-hygiene conditions, an infection, bedding material and presence of dampness in the surrounding environment is also a contributing factor to increase the number of microbial (Coli form) count and hence cause the serious problems. Addition of water by MM which is mostly contaminated with infected animal faces can also be the source of diseases transfer (mastitis) and contaminated water use for cleaning purpose is also objectionable. All these practices may be basis for the outbreak of mastitis. The results are in line with Bachaya *et al.*[21].Who observed the similar prevalence of (SFMT) 15% in Thesil Jand, 10% in Attock, 13.33%, Pindighaib.10% in Fateh Jang.

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