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MICROBIAL ANALYSIS AND QUALITY CONTROL OF MILK COLLECTED FROM VARIOUS DISTRICTS OF KHYBER PAKHTUNKHWA

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Abstract: Milk is a complete diet containing all essential nutritional constituents. The present study has been concede to identify the predominant bacteria, fungi and other contaminants in milk obtained from various sources viz Nestle, Good Milk, Olpe'rs Milk, Tarang, Nurppur Chai Mix and local raw milk (Raw Milk 1, Raw Milk 2 and Raw Milk 3) to evaluate the hygienic quality of milk at storage stages, transportation and during milking. The different bacteria and fungi present in milk are governed by upon the germ-free environment. The results from the given study showed that milking, transportation and storage originated contamination of raw milk. Good quality milk could be obtained following certain steps including refrigeration, radiation and pasteurization etc. Milk contamination can be decreased by proper hygienic packaging of milk. So it is greatly suggested to properly pasteurize the milk for drinking.

Keywords: Milk, Microbe, Chemical analysis and Quality Control

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INTRODUCTION

Milk is the lacteal secretion obtained by the complete milking of mammalian animals. Due to its high nutritional value for human beings, it is a significant food of nutrition of immense population on earth. When temperature is suitable for growth of microorganisms, the milk appears as an excellent medium for their growth. The milk is contaminated very easily if it is handled carelessly and produced un-hygienically results in its early spoilage (Prajapati, 1995; Schmidt, 1982).

Milk serves for the growth of bacterial population. Mostly food-borne diseases are among main public health disquiet throughout the world. Raw and pasteurized milk are daily consumed by millions of people. As a result infected milk either during milk processing or from infected cows results in different zoonotic diseases to many of them. These diseases include brucellosis, typhoid fever and salmonella food poisoning, tuberculosis, gastroenteritis, Q-fever, dysentery, diphtheria and staphylococcal intoxications (Senior et al., 1989).

Present study was carried out to determine the milk, beneficial micro-organisms present in milk, pathogenic microbes in milk and quality control of milk. Microorganisms are important in dairy products. The initial flora of raw milk influenced the microbiological quality of milk products and milk (Ritcher and Vadamuthu, 2001).Milk and its products are generally demanded for nutritional purposes without health risks and hazards, enriched nutritional values and with high biological potential (Khan and Zeb, 2007; Balochet al., 2006)

For the expectant and lactating mothers as well as for growing children milk is an important part of daily diet (Javaid et al., 2009).Mostly microorganisms that include *Staphylococcus, Lactobacillus, coliforms*, *Streptococcus* and *Micrococcus* spp. find milk as a good medium for their growth apart from being an important nutritious food for human beings. Air, soil, feed, milking equipment, grass and feces are the different sources of microbial contamination of raw milk (Torkar and Teger, 2008).

High quality and safe foods with a long shelf life is demanding. Though, milk products and milk deteriorate quickly as they are biochemically unstable. Quality and safety management are the different systems used by food industry. These systems are expensive and complex but are very effective. These systems are ISO 9000, Hazard Analysis and Critical Control Point (HACCP), Total Quality Management (TQM), etc.

METHODOLOGY

Sampling of milk for bacteriological testing:

Milk samples were collected from different regions of Khyber Pakhtunkhwa i.e. Kohat,

Bannu, D.I Khan, Mardan, Malakand, Hazara and Peshawar. The samples were collected by the sanitary inspectors. The tests were carried in Food Testing and Analysis Laboratory Peshawar. Normally $2{\cdot}10^5$ cfu/ml microorganisms are present in raw milk while pasteurized milk has $2{\cdot}10^4$ cfu/ml.

Milk samples for chemical tests:

Potassium dichromate was used to preserved milk samples for butter fat testing. The milk samples must first be warmed in water bath at 40 ºC that have been kept in ice-box, then the temperature was brought to 20ºC, mixed and a sample taken for butter fat determination. Sodium azid at the rate of 0.08% and Bronopol (2-bromo-2-nitro-1, 3-propanediol) used at the rate of 0.02% include the other preservative chemicals.

Standard plate count (SPC):

Through this process we can check only those microorganisms in milk which are alive, those live microorganisms which can grow on normal media. We are unable to check those microorganisms which are dying or not grow on normal media, in this process we did Serial dilution of sample i.e. milk up to require dilution ($2X10^2$ ml). Then we prepare plate and transfer 1-2ml of milk then pour the milk and mix well then incubate at 370C for 24 h. We count visible and scattered colonies multiply with the dilution factor.

Direct microscopy:

It is a simple easy and fast method and giving accurate result. In this process we take the milk and spread on slide of $1cm^2$ then dry and keep on water bath. After this flood Xylol on slide then put Methylene blue on the slide. Then observe on 10X. Count the microorganisms in every field then take the average of the fields. Bacterial cells are of blue color this will equal to cfu/ml.

Test for *Salmonella* and*Shigella*:

Selective medium such as *Salmonella* and*shigella*(SS) agar was autoclaved for 20 min at 121^0Cand it was poured into sterile petri dishes in order to keep it at room temperature for solidification. Afterwards the milk samples (500ul) were transferred to SS agar petri plates and it was spread uniformly. The plates were further incubated for 48 h at 37^0C.

Test for Fecal *E.coli*:

Eyosin Methylene blue agar (EMB) was autoclaved for 20 min at 121^0C and it was poured into sterile petri dishes in order to keep it at room temperature for solidification. Afterwards the milk samples (500ul) were transferred to EMB agar petri plates and it was spread uniformly. The plates were further incubated for 24 h at 37^0C

Test for total viable count:

Serial dilution of milk samples was carried out to obtain the different dilutions. These milk dilutions was further transferred into

sterile nutrient agar petri plates and distributed uniformly. Nutrient agar plates were incubated for 24 h at 37^0C.Bacterial colonies were observed and counted after incubation and it was multiplied by dilution factor. In UHT milk samples, for the total viable count plate count agar media was used (AOAC, 2005).

Test for Fungi:

Milk sample of 500ul was poured intoSabaroud Dextrose Agar and it was distributed uniformly. The total number of colonies were observed and noted after the incubation of SDA plates for 48 h at 20^0C.

Determination of *Coliforms*and *Bacillus* species:

Violet red bile agar was used to determined coli form counts by pour plate method and for isolation and enumeration of *B. cereus* and *B. subtilis,* media such as *B. cereus* selective agar base was used. These plates were incubated for 24 h at 37°C.

Lactose positive tubes from coli form count were further evaluated for *E. coli* count MacConkey' sagar.

Determination of spore formers:

Spore formers were usually enumerated by using plate count agar. Milk sample was first kept in water bath for 10 min at 80°C. With the help of sterile pipette 1 ml of milk sample was inoculated into the petri plate containing plate count agar.The plates were rotated clock wise and anti-clock wise for uniform distribution of milk samples. All petri dishes were placed in inverted position in incubator at 55°C for 72 h.

Methylene blue reductase test:

It is biochemical test. In oxidize form methylene blue have blue color while in reduce form it is colorless. A specific amount of milk was taken in a beaker and 1-2 drops of methylene blue were added, this was incubated at 35°C.Different interval of time is checked. If methylene blue is reducing within 2h then it indicates that the milk is of poor quality because there are large numbers of microbes which reduce the methylene blue soon. If the reduction of methylene blue takes 8h then the milk is of good quality. It is general technique which shows only the presence of microbes. But we can calculate the quality of milk from it.

Phosphatase test:

Buffer substrate solution (5 ml) was taken in a test tube and it was warm in water bath at 37 ºC. Milk sample of 1 ml was added to this test tube and was kept again in water bath. Blank sample from boiled milk was also prepared. Both of these blank samples and test samples were incubated for 2 h at 37ºC.Tubes were removed and mixed properly after incubation. Lovibond comparator" ALL PURPOSES" using A.P.T.W. disc was used in which one sample was used against the blank and disc was rotated till the test sample color matched and further read disc number.

RESULTS AND DISCUSSION

Milk samples were examined for microbial assessment tests and two milk samples (Raw milk 2 and Raw Milk 3) showed high number of colonies out of three samples while one milk sample (Raw Milk 1) showed less colonies. Raw Milk2 and Raw Milk3 samples were the samples which were most contaminated milk samples among all treated samples obtained from different sources. These raw milk samples were collected from Kohat Dairy Farm, QissaKhwani Bazar and from Hayatabad Peshawar.In Nestle, Good Milk, Olper's milk, Nurpur Chai Mixthe number of colonies were less as these were pasteurized milk samples. These milk samples have very good quality as we observed the growth of microbes and none of the contamination appeared in the tests. However, only partial contamination was prominent in theTarang milk sample (Table 1: Figure 1).

The animals are the main source of contamination that probably results in high number of microflora in raw milk. Bacteria can easily enter dairy utensils and milk contact surfaces present in water, soil, manure etc and further transferred to milk. Bacteria might grow in great amounts if the milk contact surfaces are imperfectly cleaned. The present study revealed that raw milk samples (89%) were of poor category while the 95 % of pasteurized milk samples were found to be of good quality as these were properly pasteurized. Similar studies were also carried out by Zineyet *al* (2007) who analyzed samples for numerous microbial quality characteristics including total coliforms, aerobic total plate count (ATPC), faecal coli forms, aerobic mesophilics pore forming bacteria (AMSC), psychrotrophs (PC), *Enterobacteriaceae*, moulds and yeasts.

Donkor*et al* (2007) also conducted the similar studies in milk samples of Accra and Kumasi cities. They cultured and identified different bacterial strains. Due to poor hygiene condition probable faecal contamination of the milk was mostly caused by*Enterobacteria*. They identified most of the microorganisms and prevalence rate were *Mycobacterium*spp. (1%). *Bacillus* spp. (11.5%), *Staphylococcus* spp. (14.6%), *Escherichia coli* (2.1%), *Proteus* spp. (7.3%), *Yersinia* spp. (19.8%), *Enterobacter*spp. (6.3%) and *Klebsiella*spp (16.7%).

The contamination in the milk results from microorganisms during processing and site of production. The air, milk containers, the milk handlers and animals results in entrance of microorganisms in milk. During all aspect of milking, collection and transport it should be protect from any external source of contamination either direct or indirect. The source of bacteria may be from the milking equipment's, udder of the animals or after milking handling procedure.

The two samples (Raw Milk 2 and Raw Milk 3) were poor while the one sample (Raw Milk 1) was fair when raw milk was checked

by methylene blue test. Whereas, out of five pasteurized samples, four samples (Good Milk, Olper's milk, Nurpur Chai Mix and Tarang) showed good quality milk and while excellent milk was of one sample (Nestle) as shown in Table 2, 3.In batch pasteurizer, milk is pasteurized in heat exchanger at 72ºC for 15 seconds or 63ºC for 30 min, pathogenic bacteria present in milk are completely destroyed by continuous flow pasteurizers that results in safe representation of milk safe for human usage. Different enzymes present in milk that affect its flavor are also destroyed simultaneously.

The table 4 shows the percentage of fats in each sample of milk as also shown in table5. The highest fat contents 14% were observed in raw milk sample (Raw Milk3) while least fat contents 3.5% recorded in Nestle milk pack.

Adequate pasteurization of milk can be determined by measuring the phosphatase enzymes present in milk. The pasteurization process may not be done properly if the phosphatase test is positive and it will lead the milk not to safe for human use as well as results in short shelf life. If any pathogenic bacteria and the enzyme have been destroyed during pasteurization it will be negative phosphatase test. The following Table 5 shows the standards of Phosphatase tests for pasteurized milk.

So milk samples were examined for pasteurization and results are given in table 6. By this we concluded that the five sample Nestle, Good Milk, Olper's, Nurpur Chai Mix and Tarang show negative result while Raw Milk (Raw Milk 1, Raw Milk2 and Raw Milk3) show positive result.

CONCLUSIONS

To ensure that only good quality milk is sold, there should be proper quality control system. The processing of milk declares the devastation of pathogens causing diseases in humans, the care of quality of product apart from appearance, nutritive properties and the selection of organisms which may produce unsatisfactory products and the loss of flavor. The milk chain from farm gate to final dairy products should be assured for better quality of milk. Veterinarians, dairy farmers, state regulatory departments, retail distributors, milk and milk product processors, and consumers of dairy products should have a major concern for production of quality milk. We can ensure that good quality milk can be produced by using this simple quality control system. This will improve our reputation as a quality milk supplier, increase the profits and protect the health of consumers.

Table 1.

Enumeration of microorganisms in different milk samples by standard plate count method

SR	samples	Colony forming units (cfu) in Milk Samples	
		1/1000 (cfu/ml)	1/10,0000 (cfu/ml)
1	Nestle	123	31
2	Good Milk	133	43
3	Olper's milk	161	52
4	Nurpur Chai Mix	187	66
5	Tarang	210	82
6	Raw milk 1	287	97
7	Raw Milk 2	451	122
8	Raw Milk 3	551	131

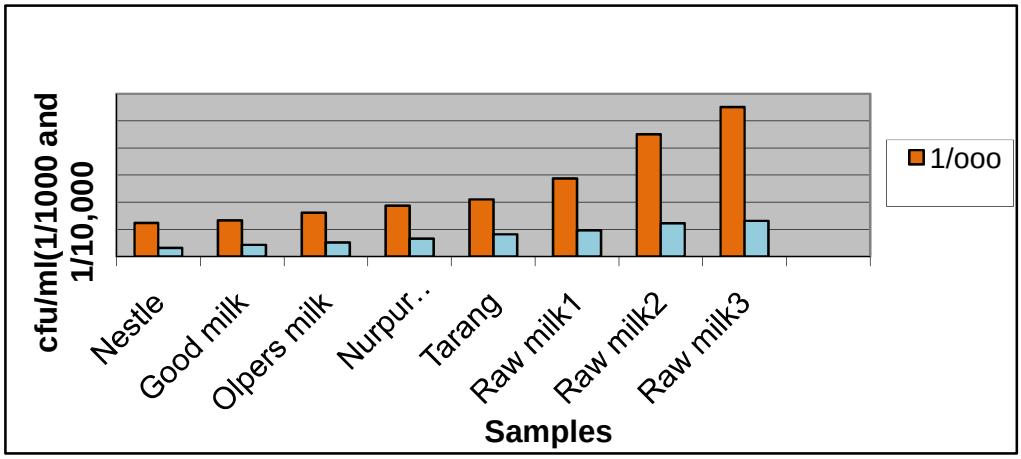

Figure 1: Graphical representation of cfu/ml in (1/1000 and 1/10,000) of milk samples

Table 2

Grading of milk samples on the basis of Methylene-blue reductase test

Quality Of Milk	Decolorization time
Excellent	More than 8 h
Good	Between 6 hours and 8 h
Fair	Between 2 to 6 h
Poor	Less than 2 h

Table 3

Decolorizing time and grading of milk samples

S.R	Samples	Decolorised Time	Grade
1	Nestle	12.56 h	Excellent
2	Good Milk	9.41 h	Excellent
3	Olper's milk	7.30 h	Good
4	Nurpur Chai Mix	6.25 h	Good
5	Tarang	6.55 h	Good
6	Raw milk 1	5.46 h	Fair
7	Raw Milk 2	1.42 h	Poor
8	Raw Milk 3	1.30 h	Poor

Table 4

Percent Fat contents of Milk sample

S.R	Samples	Percentage of Fat
1	Nestle	3.5
2	Good Milk	3.7
3	Olper's milk	3.8
4	Nurpur Chai Mix	7.5
5	Tarang	6
6	Raw milk 1	10
7	Raw Milk 2	13
8	Raw Milk 3	14

Table 5

Standards of milk samples for Phosphatase test

Disc Reading after 2 hours	Remarks
0-10	Pasteurizedproperly
10-18	Pasteurizedslightly
18-42	Below pasteurization
> 42	Below pasteurization

Table6

Represent the pasteurization level of milk samples

S.R	Samples	Disc Reading after 2 h incubation at 37ºC	Remarks
1	Nestle	5	Properly pasteurized
2	Good Milk	6	Properly pasteurized
3	Olper's Milk	7	Properly pasteurized
4	Nurpur Chai Mix	7	Properly pasteurized
5	Tarang	7	Properly pasteurized
6	Raw Milk1	45	Not pasteurized
7	Raw Milk2	47	Not pasteurized
8	Raw Milk3	48	Not pasteurized

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