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Microbial quality analysis of milk from local vendors in Patna

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Abstract
Milk is a complete diet containing all new essential nutritional constituent. The present study was made to identify the predominant bacteria and other contaminants in milk obtained from various sources for *viz* Amul, Sudha, Raj Dairy, Mother Dairy and local Raw milk -1, Raw milk-2, Raw milk-3, Raw milk-4 to evaluate the hygienic quality of milk at storage temperature, transportation and condition during milking time. The present study shown that contamination occur mostly during milking transportation and storage environmental condition of RAW milk. Quality can be obtained by following hygiene steps that includes proper refrigeration pasteurization and applying proper radiation. The contamination can be lower down by proper packaging and handling of milk. Therefore it is suggested to pasteurize the milk followed by immediate cooling for proper maintenance of quality and hygenicity of milk.

Keywords: Microbial quality, bacteriological test, SPC, pasteurization, methylene blue reductase test, phosphatase test

Introduction
Milk is considered as a complete nutrient for infant as it contains all the vital nutrient essential to support human life. It contains an important source of all basic nutrients for mammals *viz*., lactose, fat, protein, mineral and vitamins in balanced ratio which is not in other foods (Khan *et al.*, 2007) [5-6]. The average production of milk in India, as per 2015-16 statistics, was 155.5 million tones which make India the largest producer in the World (Nalwaya *et al.*, 2018) [8]. The adulteration of milk is mainly due to human feature and unhygienic conditions. Typically milk is polluted with diverse types of microorganisms at milk gathering places. The microbial superiority of raw milk is vital for the fabrication of excellence dairy products. There can be deterioration in milk's quality, colour, odour or flavour to a point where it is improper for human consumption (Prajapati 1995, Schmidt, 1992). Raw and pasteurized milk are daily consumed by millions of people. As a result infected milk either during milk processing or from infected cow's 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). Pathogenic micro-organism in milk comprises E. coli, Staphylococcus aureus, Listeria monocytogenes, Clostridium, Microbacterium, Micrococcus and Streptococcus. According to Prevention of food adulteration (PFA) rules, 1956 specifies microbial supplies for pathogens such as E. coli, Staphylococcus aureus, Listeria monocytogenes in foods frequently involved in food-borne diseases. According to standards given by PFA these microorganisms must be absent in one gram of milk. 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; Baloch *et al.*, 2006) [5-6, 2] Hence we checked if the microorganisms were present or absent in one gram of milk representing whether there was contamination or not. This implied the use of selective and differential media. This implied the use of selective and differential media. EMB (Eosine Methylene Blue) agar – for *E. Coli* SS agar for *Salmonella and Shigella* Dabour Dextrose agar for fungi Bacteria proliferate during fabrication and holding of milk, depending on storage time and conditions. The variations take place in the physic-chemical properties of milk is result of the activities of the individual microorganisms during their period of growth and reproduction or of substances produced during such activities. According to the new food safety rules that came into effect, the FSSAI (Food Safety Standards Authority of India) has made it compulsory for milk to be tested for *E. coli*, staphylococcus aureus and Listeria

monocytogenes. E. coli and S. aureus are the most common contaminants and are important from public health point of view as they have been connected with the onset of food poisoning in human beings. Keeping in view the objectives of the investigation was following:

1. To isolate diverse micro-organisms from milk and flavoured milk collected from diverse localities in Bihar
2. Examining the microbial superiority of milk and flavoured milk by detecting the presence or absence in a specific amount.
3. Total plate count of isolated micro-organisms.
4. Five samples of milk were collected from local vendors in Patna area.
5. These samples were carried in ice bag so that the action of the microorganism cease and to reduce the bottle effect.

Bacteriological Count of Milk

The samples of milk were collected from Patna and adjoining area like Mahua Bagh, BVC farm, Local khatals of Jagdeopath. All the samples positive for altered microbial contaminations were established using biochemical examinations. Normally 2×105 cfu/ml microorganisms are present in raw milk while pasteurized milk has 2×104 cfu/ml.

Chemical tests of Milk

The milk samples was first 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. Potassium dichromate was used to preserved milk samples for butter fat testing. Other preservative used for preservation was Sodium azid at the rate of 0.08% and Bronopol (2- bromo-2-nitro-1, 3-propanediol) (0.02%).

Standard plate count (SPC)

One ml of milk sample were taken from each sample and mixed with nine ml of water. All the sample were serially diluted up to 3-4 dilution then 2x102 ml were taken for plating the sample. Nutrient agar media were taken for bacterial analysis. Sample were analyzed in triplicates for each milk sample and incubate at 370C for 24 h. after 24 hours colony were counted and multiplied by dilution factors for actual cfu/ml of bacterial counts. In this procedure we were able to count only live microorganism visible on the plates. Colonies were counted and Colony Forming Unit (Cfu/ml) was calculated by given formula Cfu/ml= (no. of colonies × dilution factor) / volume inocullaed

Direct Microscopic counts

Simple easy and fast method that give accurate result. In this process we take the milk and spread on slide of 1cm2 then dry and keep on water bath. After this sample were flooded with Xylol on slide then Methylene blue was applied on the slide. Colony were observed by viewing under 10X.Microorganisms were Counted in every field then average were taken for each of the fields. All the colored bacterial cell was taken as cfu/ml.

Test for Faecal E. *coli*.

For this Eosin Methylene blue agar (EMB) was autoclaved for 20 min at 1210C and was poured into sterile petri dishes. The plates were kept at room temperature for solidification. One ml of each milk samples were transferred to EMB agar petri plates and it was spread uniformly with the help of spreader. The plates were further incubated for 24 h at 370C

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 370C.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) [1].

Test for Salmonella and Shigella

Selective medium such as Salmonella and shigella (SS) agar was autoclaved for 20 min at 1210Cand it was poured into sterile petri dishes in order to keep it at room temperature for solidification. Afterwards the milk samples 1ml were transferred to SS agar petri plates and it was spread uniformly. The plates were further incubated for 48 h at 370C.Colony were observer after 24-48 hr of incubation.

Test for Fungi

For this Sabaroud Dextrose Agar media were taken. One ml Milk sample was inoculated into media and distributed uniformly. The total number of colonies were observed and noted after the incubation of SDA plates for 48 h at 27^0C.

Enumeration 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.

Biochemical Test (Methylene blue reductase test)

It is quickest and most authentic test for microbiological quality of milk based on biochemical test. The principal lies in the fact that in oxidize form methylene blue have blue color while in reduce form it is color less. The milk sample highly contaminated with microbial load reduces the dye fast and made it colourless while the good milk sample takes longer time to reduce the dye to color less form. 10 ml of each milk were taken in a test tubes and 1 ml of dye was added in each of the milk sample taken in a test tube. This was incubated at 35°C in water bath. The changes in color were observed for different time interval. If time taken for reduction of methylene blue were less than 2-3 hr 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 taken kore than 6-7 h 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

APTW. 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

The analysed sample (Sample-3, 4, and 5) shown two milk samples showed high number of colonies out of total five sample collected from Mahua bagh While the sample obtained from Jagdeopath (Sample-1,and 2) shown less number microorganisms. The Amul milk, Sudha dairy milk, Mother Dairy and Raj dairy have observed as good quality as these milk was properly pasteurized and hence very few microbial contamination was observed. These milk samples have very good quality as we observed the growth of microbes and none of the contamination appeared in the tests. (Table 1: Figure 1).Similar study was done by Mhone *et al.*, 2011; Hossain *et al.*, 2010. Donkor *et al.* (2007) [7, 4, 3] 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 *Enter bacteria*. 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 observed in the Mahua region of milk may be due to poor hygienic condition of the area and the microbes might have enters through handlers and utensils that was not cleaned or sterilized properly. Microorganisms might have arised from the khatals of the Milk man. Other sources may be air or the hide of animal itself which was not washed properly. Like Wise the Methylene blue reductase time (Table-2) was quite long for all the sample that was branded like Amul, Sudha, Raj and Mother dairy. Likewise Jagdeopath araa of milk was also quite good in terms of pasteurization as these area belongs to pause area and literacy rate is high. But in the Mahuabagh the population belongs to poor category adn lavel of hygenicity is low so the Contamination level can be quite justifiable. Simialar study were done by Torkar and Teger 2008 [13].

So far as the phosphatase test is concerned this enzymes are naturally present in milk Adequate pasteurization of milk can be determined by measuring the phosphatase enzymes present in milk. If the pasteurization process may not be done properly 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. During pasteurization pathogenic bacteria and the enzyme is generally that gives negative phosphatase test. Similar study were reported by Parekh and R Subhash, 2008. So as shown in the table 3 Milk from various region have different enzyme activity. It is quite less for Amul and other branded milk but for Mahua bagh region it have high value signifies a good number of microbes in the sample.

Table 1: Enumeration of microorganisms in different milk samples by standard plate count method

S.N	Sample	1/1000 Cfu/ml	1/10,0000 cfu/ml
1	Amul milk	133	40
2	Sudha milk	210	44
3	Mother Dairy Milk	187	42
4	Raj Dairy Milk	230	70
5	Milk (Jagedeopath-1	300	120
6	Milk(Jagdeopath-2)	335	132
7	Mahua Bagh-3	451	166
8	Mahua Bagh-4	450	154
9	Mahua bagh-5	552	168

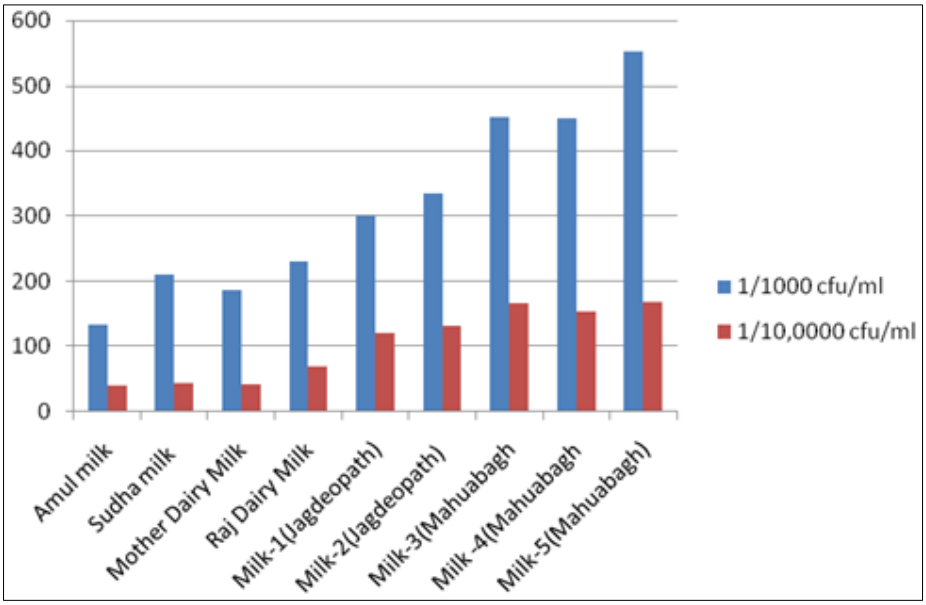

Fig 1: Microbial load of different samples obtained from different area of Patna

Table 2: Microbial quality according to MBR Test

S.N	Sample	MBR (Reduction Time hr)
1	Amul milk	14 h
2	Sudha milk	13 h
3	Mother Dairy Milk	14 h
4	Raj Dairy Milk	12 h
5	Milk (Jagedeopath-1	10 h

6	Milk(Jagdeopath-2)	11 h
7	Mahua Bagh-3	2 h
8	Mahua Bagh-4	3 h
9	Mahua bagh-5	3h

Table 3: Phosphatase test

Samples	Disc Reading after 2h incubation at 37ºC		Remarks
1	Amul milk	7	Properly pasteurized
2	Sudha milk	9	Properly pasteurized
3	Mother Dairy Milk	7	Properly pasteurized
4	Raj Dairy Milk	10	Properly pasteurized
5	Milk (Jagedeopath-1	10	Properly pasteurized
6	Milk(Jagdeopath-2)	10	Not pasteurized
7	Mahua Bagh-3	47	Not pasteurized
8	Mahua Bagh-4	48	Not pasteurized
9	Mahua bagh-5	49	Not pasteurized

Conclusion

Obtaining hygienic milk is a combination of package and practices that implies proper pasteurization and cleaning at each and every point from production to consumption of milk. Milk born disease may have significant role on our health system therefore management of farm to consumers is necessity of the population. Various test are available from which we can grade the milk or select the milk suitable for consumption. In addition awareness to the population about the hygienic and clean milk production is must to obtain satisfactory result at national level.

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