International Journal of Advances in Engineering and Management (IJAEM)
Volume 2, Issue 1, pp: 610-617 www.ijaem.net ISSN: 2395-5252

Study of Microbiological Quality of Raw Milk from Local Vendors at Dehradun.

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Date of Submission: 19-06-2020 Date of Acceptance: 05-07-2020

ABSTRACT
The study was conducted to evaluate bacteriological quality of raw milk at Dehradun. Milk samples were collected from local vendors of premnagar(Dehradun).
Raw milk can carry harmful bacteria & other germs that can make you very sick. It is to get food borne disease from many different foods; raw milk is one of the riskiest of all. The contamination level of sample was estimated by MBRT test & standard plate count. Isolated colonies of bacteria were identified by observing culture characters, microscopy &IMViC test. In the present study the outcomes of thirteen samples revealed that the out of thirteen samples many were found to be contaminated with *staphylococcus, streptococcus lactis, Bacillus cereus, Micrococcus, Alcaligenes*and one were found to be contaminated with *Escherichia coli*. It indicates a need for more strict hygienic practices. To ensure that only good quality milk is sold, for this there should be a proper control system. This will improve the quality of milk and protect the health of consumers.
KEYWORDS: Raw milk, Dehradun, Microbiological quality, *Escherichia coli, Staphylococcus, Streptococcus lactis*.

I. INTRODUCTION

Milk is a nutrient rich, white liquid food produced by the mammary glands of mammals. Early lactation of milk contains colostrum's which carries the mother's antibodies to its young and can reduce the risk of many disease. It contains many other nutrients including protein and lactose (Perry et al., 2003).

India is the world's largest producer of milk (DairyCow - Datum World Cow Numbers, 2014) and is the leading exporter of skimmed milk powder (Kumar 2013). In India Dairy farms produced about 730 million tons of milk in 2011(global market analysis, 2012.) from 260 million dairies cattle

Milk is a complete food:

Milk from the cow is processed; it is not an engineered food. The fat portion of the milk contains fat soluble vitamins. Other than fat, milk also contains proteins, carbohydrates, water soluble vitamins, and minerals. These nutrients are present in the milk and make it nature's nearly perfect food. The products of milk contain high quality proteins. Casein is a protein found only in the milk and it contain all of the essential amino acid. (Dr AnjuSood et al;2018).

Composition of Milk:

1. Water-87-88%
2. Carbohydrate-5%
3. Lactose-4.8%
4. Fat-3-4% in wholemilk
5. Proteins-3-4%
6. Minerals-0.8%

Microbial Activity of Milk:

Since milk a rich food which contains protein, fat, sugar, vitamins and minerals hence susceptible for microbial contamination. Contamination of raw milk may occur during handling, transportation, storing, processing and other activities.

As we know that milk is a nutritious food for humans instead of it provides a favorable environment for the growth of microorganism. various types of bacteria and yeast can grow in milk at a temperature 16C. Isolate from human sources such as *salmonella species, shigella species, and streptococcus species* can also be presented in milk. Microorganism are the most important group of microbes present in dairy milk. Major sources of contamination of raw milk are water, air, dust, equipment's, insects, and rodents. contamination of raw materials can also occur from soil, external surface and internal organs of

animals. Microbial growth of organism can be controlled by cooling the milk. Most of the microorganism in colder environment can reproduce slowly. Bacteria can multiply very rapidly in warm milk and milk gets rapidly sour.

II. MATERIALS & METHODS

All the chemicals, reagents and culture media used in present study were HIMEDIA, Mumbai, India. The common equipment's used were Autoclave, BOD incubator, Colony counter, Laminar air flow, Microscope etc. All the glassware's were used of Borosilicate quality.

A. Place ofStudy:

The study was carried out in the Department of Microbiology, TaqGene Training & Research Institute (TGTRI), Dehradun, Uttarakhand.

B. SampleCollection:

A total of 13 Samples (S-1, S-2, S-3, S-4, S-5, S-6, S-7, S-8, S-9, S-10, S-11, S-12 & S-13) were collected randomly from Dairy shops at Nanda- ki-chowki, Prem Nagar &Sudhowala in Dehradun city, India. The samples were carried in sterilized bags so that the action of microorganisms cease to reduce the chances of contamination & prepared immediately for the MBRT Test.

C. MBRTTest:

In the methylene blue reduction test, 10 ml of the raw milk sample was added to each test tube. One ml of methylene blue thiocyanate was added to each tube, close the tube tightly &shake well. Keep the tube at room temperature & note the time taken by blue color to change into white.

Classification: The suggested classification is given below
Class 1: Excellent, not decolorized in 8Hr.
Class 2: Good, decolorized within 6Hr.
Class 3: Fair, decolorized in less than 2Hr.
Class 4: Poor, decolorized within ½Hr.

D. Standard plate count:

Standard plate count of samples of raw milk were tested by spread plate method. Each sample was diluted by serial dilution method. One ml of sample was added in subsequent test tube containing 9 ml of distilled water. From each dilution of tube 0.1 ml of inoculums was transferred on surface of nutrient agar medium in petri plates. All plates were incubated at 37±1°c for 18 hours in an inverted position in BOD incubator. The bacterial growth was observed in each petri plate & isolated colonies were counted as colony forming unit (CFU).

Preparation of pure culture of bacterial isolate:

Theisolatedcoloniesshowedsimilarculturec haractersinthepetriplatewereobserved&then purifyonfreshlyprepared nutrientagar medium by streak plate method. Plates were incubated at 37° for 18 hours in inverted position. The isolated colonies were transferred from streak plate to agar slants with the help of inoculating wire loop. The agar slants were incubated at 37±1°c for 18 hours & well grown cultures was observed in slants & slants were preserved at 4°c in a refrigerator for furtheruse.

1. CultureTest:

Pure culture character of each bacterial isolate was observed carefully on nutrient agar medium.

2. Microscopy:

Morphological characteristics of isolated colonies of bacteria were observed by standard method of gram staining. Motility of pure culture of bacterial isolates was observed on cavity slides by standard procedure of hanging drop method.

3. BiochemicalTest:

The IMViC test is a group of individual tests used in microbiology lab testing for identification of specific bacteria. The IMViC series include four tests:

A. INDOLETEST:

In a distilled water tryptone was added to make a broth. Isolated colony was inoculated in a tryptone broth & incubated for 24-48 hours. After incubation period, kovac'sindole reagent was added & after that result is read. The positive test results is indicated by the crimson red layer at the top of tube. A negative result is indicated by the lack of color change at top of test tube.

B. MRTEST:

The isolated colonies were inoculated in methyl red broth test tube with the help of inoculating wire loop & incubated for 24-48 hours at 37°c. After incubation period methyl red reagent was added & after that result is read**.** The positive test is indicated by the formation of red color in the test tube. While the negative test is indicated by no color change.

C. VPTEST:

For VP test, empty test tube was taken & one ml of VP media was pipetted out into test tube. In one ml of VP, 0.6 ml of alpha - naphthol was added & the 0.2 ml of KOH solution was added into test tube. The positive test results is indicated by red brown

International Journal of Advances in Engineering and Management (IJAEM)
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color. The negative test is indicated by nocolor.

D. CITRATE UTILIZATIONTEST:

The test is performed on simmon citrate agar. Bacterial colony was suspended in the medium by streaking. It was incubated for 24- 48 hours. The positive test is indicated by growth & a blue color change. The negative test is indicated by lack of growth & color change.

E. TRIPLE SUGAR IRON (TSI)TEST:

TSI was inoculated by first stabbing through the center of the medium to the bottom of the tube & streaking on the surface of the agar slant. The tube was incubated at 37°c for 18 hours. Phenol red indicator turns into yellow both in butt & slant. It is possible only when lactose is fermented. The positive test is indicated when lactose, sucrose, glucose is not fermented both in butt & slants & turns red. If H2s is produced the black color at the bottom is seen.

F. CATALASETEST:

A clean slide was taken & suspended a drop of VP culture media on it. A drop of H2O2 was added to the slide & Observe the bubble formation. Positive test shows immediate bubble formation. Negative test shows no bubble formation.

III. RESULT & DISCUSSION

In the present study, 13 samples of raw milk were collected from different areas of Dehradun, India for the analysis of microbiological quality. These samples were tested for MBRT Test & Standard plate count. Color disappearance time for samples of raw milk in ethylene blue reduction test were varies between 1/2-hour, 2-hour, 6 hour & more than 8 hours. Milk samples of S-3, S-11 were decolorized within 2 hours & for most of the samples of raw milk the disappearance time was above 8 hours & some were disappeared within 6hours.

Table 1: MBRT test of raw milk Sample

Sample no	Sample -1	Sample -2	Sample -3	Sample -4	Sample -5	Sample -6	Sample -7	Sample -8	Sample -9	Sample -10	Sample -11	Sample -12	Sample -13
Decolorization Time	Above 8 Hrs.	Above 8 Hrs.	Within 2 Hrs.	Above 8 Hrs.	Above 8 Hrs.	Above 8 Hrs.	Above 8 Hrs.	Within 6 Hrs.	Above 8 Hrs.	Above 8 Hrs.	Within 2 Hrs.	Within 6 Hrs.	Within 1 Hrs.
Grade	Excellent	Excellent	Fair	Excellent	Excellent	Excellent	Excellent	Good	Excellent	Excellent	Fair	Good	Poor

All the samples were found contaminated by a variety of bacteria The highest bacterial load was 12.2 × 10^5cfu/g & lowest was 0.6×10^2cfu/g respectively. The increase value of standard plate count may be due to change in climatic conditions, contamination on dairies, storage & during the transportation of milk. From the 13 sample of raw milk a total of 21 isolates were observed & identified by pure culture, microscope & biochemical test.

Table-2: Standard Plate Count of Raw Milk Sample:

S. No	1	2	3	4	5	6	7	8	9	10	11	12	13
Sample	S -1	S -2	S -3	S -4	S -5	S -6	S -7	S -8	S -9	S -10	S -11	S -12	S -13
Bacterialcount (cfu/ml ofmilk)	7.1×10²	6.9×10²	1.1×10⁴	0.6×10²	10.5×10²	3×10³	7.8×10²	14.2×10³	2.2×10²	5.9×10²	6.1×10⁴	3.52×10³	12.2×10⁵

Culture character of isolates of bacterial colony:

Samples were shown with a no of varieties in the culture characters. Bacterial colony color varies from white, pale yellow, off white etc. Colonies of sample I-2, I-8, I-9, I-14, I-15, I-16, I-17, I-18, I-20 & I-21 were white. While some were pale yellow (I-1, I-5, I-11 & I-12) and some were yellow (I-3, I-4, I-10 & I-19) and some were off white (I-13). There was slightly difference in the size of the colony size varies from 0.1 to 0.8 & no pigmentation was seen. Elevations of the most of the colonies were convex. Some colonies with rough margin as well as smooth margin. Optical density of some colonies was opaque whereas others were transparent and translucent.

Microscopy:

Among all the 13 samples of raw milk, bacterial colonies were categorized as motile & non-motile. Except I-3, I-4, I-9, I-10, I-13, I-15, I-17 & I-19

International Journal of Advances in Engineering and Management (IJAEM)
Volume 2, Issue 1, pp: 610-617 www.ijaem.net ISSN: 2395-5252

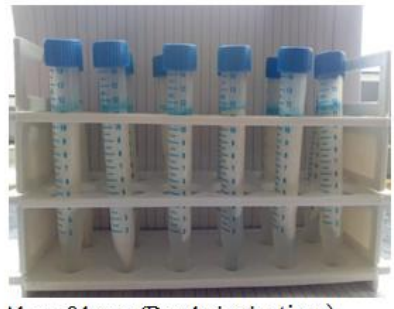
others were non- motile.
Isolate I-1, I-2, I-5, I-6, I-7, I-8, I-11, I-12, I-14, I-16, I-18, I-20 & I-21 were found cocci shaped & gram positive. Bacterial isolate I-3, I-4, I-9, I-10, I-13, I-15, I-17 & I-19 were found rod shaped & gram negative. There were slightly variationsin their cell arrangement that is some were arranging in bunches & some were inchain.

Biochemical Test:
Pure culture of isolated colonies of bacteria showed variations in their IMVIC & other biochemical test. Isolates I-1, I-2, I-3, I-4, I-5, I-6, I-8, I-9, I-10, I-11, I-12, I-13, I-14, I-15, I-16, I-17, I-18, I-19, I-20 & I-21 were MR positive while others were
negative. Colonies of all isolate I-1, I-2, I-5, I-6, I-15 & I-17 were VP positive & others were negative. The variation was seen in citrate utilization test & TSI test of bacterial isolates. Colonies of isolate I-3, I-5, I-12, I-14 & I-17 were citrate positive & others were negative. In TSI test isolate I-1, I-2, I-3, I-4, I-5, I-6, I-8, I-11, I-13, I-15, I-16, I-17, I-18, I-20 & I-21 were utilized all three sugars whereas others were utilized in the form of partially or utilization ofsugar.
On the basis of culture test, microscopy & biochemical test bacterial isolate were identified as *Streptococcus spp., Staphylococcus aureus, Bacillus cereus, staphylococcus spp., Alcaligenes spp., Micrococcus spp., Enterobacteraerogenes, Escherichia coli.*

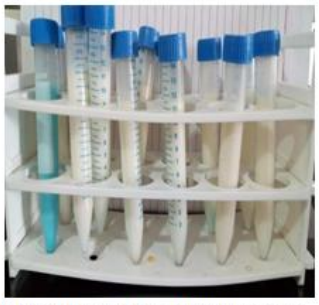
Detection of *Escherichia coli* in sample is of great health concern as it indicates contamination with the faucal materials. In some samples *Lactococcuslactis*were also found which is crucial important in manufacturing dairy product.

Table-3: Identification of bacterial isolates

S .no	Isolates	Bacteria	Positive samples
1	I -1, I-3, I- 15	*Streptococcus* spp.	S-1, S-2, S-8
2	I-2, I-6	*Staphylococcus aureus*	S-1,S-3
3	I-4, I-17	*Bacillus Cereus*	S-2, S-10
4	I-5	*Staphylococcus* spp.	S-3
5	I-7	*Alcaligenes*spp.	S-4
6	I-8, I-11, I- 16, I-18,	*Streptococcus lactis*	S-4, S-6, S-9, S-11, S-12, S-13
7	I-14, I-19	*Micrococcus* spp.	S-8, S-11
8	I-13	*Enterobacteraerogenes*	S-7
9	I -9	*Escherichia coli*	S-5


Above 8 hours (Decolorizationtime)


Within ½ hours(DecolorizationTime)


Within 6 hours(Decolorizationtime)

Figures:1 MBRT Test for Raw Milk

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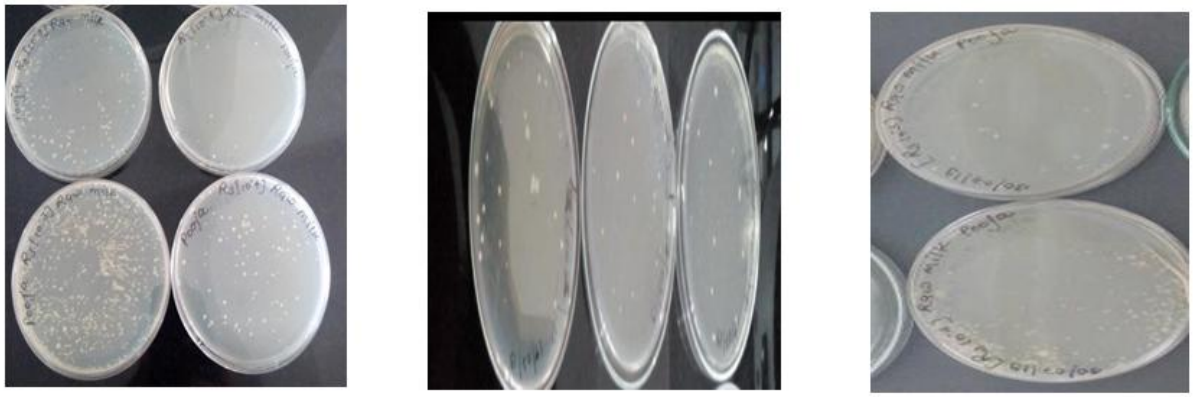
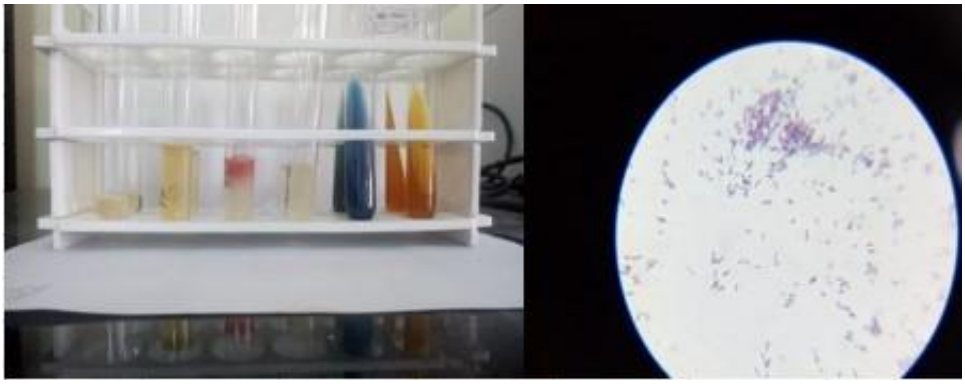


Figure 2: Standard plate count of sample

a) Biochemical (IMVIC) test (left) and grams staining(right) of streptococcusspp.

b) **Biochemical (IMVIC) test (left) and grams staining(right) of *Staphylococcusaureus***



c) **Biochemical (IMVIC) test(left)and grams staining(right) of *Bacillus cereus***

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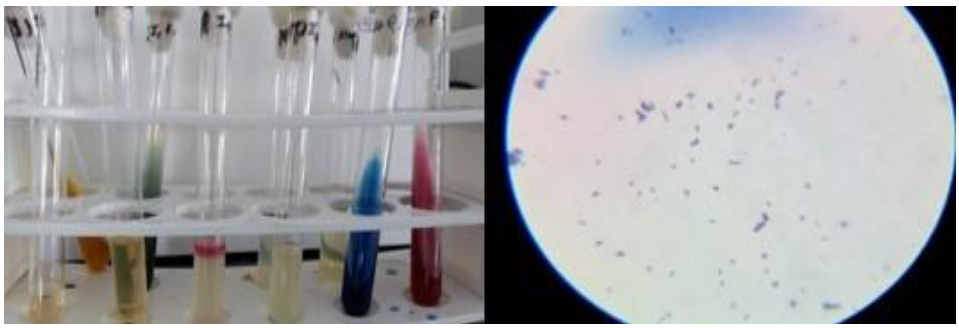
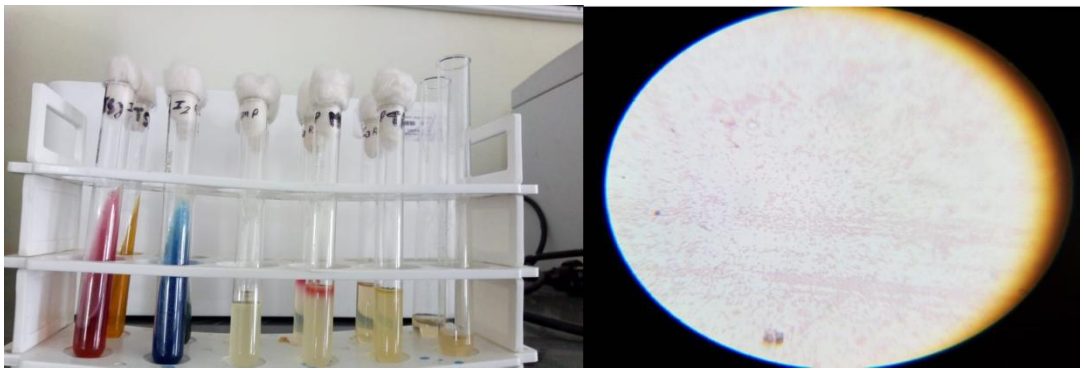
d) **Biochemical (IMVIC) test(left)and grams staining(right) of *Alcaligenes spp.***

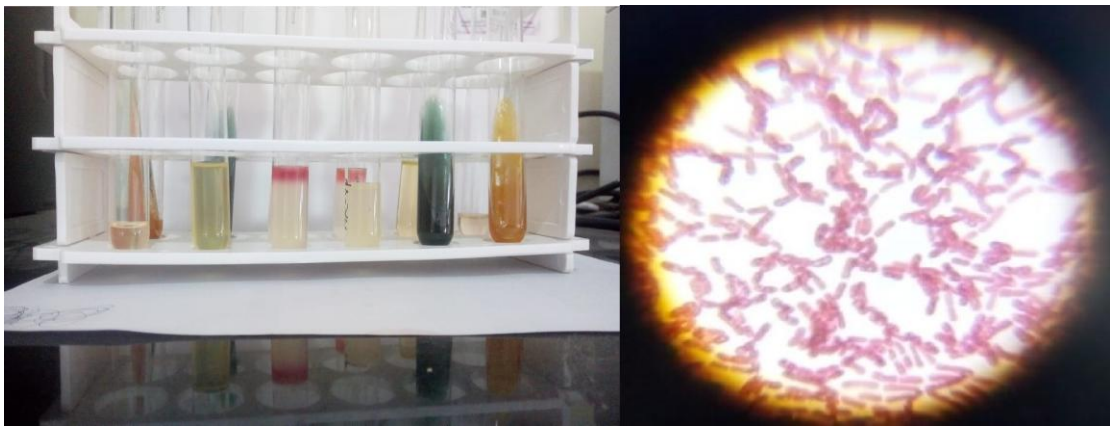
e) **Biochemical (IMVIC) test(left)and grams staining(right) of *Streptococcus lactis***



f) **Biochemical (IMVIC) test(left)and grams staining(right) of *Micrococcus spp.***



g) **Biochemical (IMVIC) test(left)and grams staining(right) of *AlcaligenesFaecalis***



h) **Biochemical (IMVIC) test(left)and grams staining(right) of *Escherichiacoli***

i) **Biochemical (IMVIC) test(left)and grams staining(right) of *Enterobacteraerogenes***

Figure-3: Biochemical Identification & Microscopy of Bacterial Isolates

IV. CONCLUSION

The present study of raw milk provides an important information regarding the presence of pathogenic microorganism in the vendor milk. It is serious public health concern for the infants, children, adults & elder people.

The current study clearly requires the attention regarding the hygiene condition at milk processing dairies. Efforts should be made to maintain the hygienic condition from suppliers to consumers. Milk vendors should be educated to implement the hygienic condition during milking processing.

It is recommended that simple households' steps like good hygiene, use of clean utensils, boiling the milk before consumption & refrigerator for the storage of milk should be undertaken to improve the microbiological quality of raw milk.

So from this study it was concluded that the samples of raw milk from the surrounding areas of Dehradun were of good quality & safe for the consumption.

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International Journal of Advances in Engineering and Management
ISSN: 2395-5252

IJAEM

Volume: 02 Issue: 01 DOI: 10.35629/5252

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