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Microbial Load, Microflora and Quality of Pasteurised Milk

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Abstract: - For obtaining clean milk effective sanitary measures are taken starting from the initial withdrawn of the milk from the cow up to it reaches the consumers. The shelf life of milk may it be raw or pasteurized is only increased if the total microbial load in the milk is low at the start of critical points. In the paper, preliminary screening was carried out to discover the physicochemical characteristics and nutritional quality of milk samples. The results that are obtained from the tested milk samples are rich in nutrition and unto the recommend nutritional level. These may be helpful to the organized sector to check the progress of the milk products in the market and also necessary measures should be taken to increase the quality of milk in the market.

Keywords:- *Microbial Load In The Milk, Nutritional Quality Of Milk, Quality Of The Milk Products, Pasteurized Milk.*

I. INTRODUCTION

Milk is a nutrient-rich white liquid food produced by the mammary glands of mammals. Milk consist of milk fat, proteins, vitamins, pigment, **phospholipids, minerals, Lactose (Milk Sugar)** and the milk that are derived after complete milking of healthy milk animal, is full of nutrients if it is obtained before or after 10 days of calving after that it gets contaminated.

❖ *The Present Study Was Carried Out, With The Following Objectives:*

- To determine the physiochemical parameters in raw and pasteurized milk
- To estimate the microbial load in both types of milk.
- To isolate and identify bacterial pathogens, in the milk sample.

➢ *Milk Processing:*

The milk that is drawn from the cow is unprocessed milk and it contains small dirt particles invisible to the naked eye. For removing these particles the milk has to be processed. In industries, various processes are undertaken to process unprocessed milk

➢ *Raw milk reception:*

The raw milk is received from the dairy federation tankers is high fat processing is chilled and stored in the Raw Milk Silos. When a Silo is full, then a sample is taken in the processing lab and is tested for its constituents.

➢ *Bacteriological Quality Tests:*

Sanitary methods of handling milk must be strictly adhered to rigidly in order to provide safe milk for human consumption. Furthermore, since milk is a good growth medium, even a small number of non-pathogens can multiply considerably if the milk is not kept refrigerated. Because the consumer has no way of knowing whether or not the milk delivered to the home or purchased in the store is contaminated, a number of standard tests are carried out periodically on milk in that area. From the results of these tests, milk is classified into grades designated as A, B, and C (Volk and Wheeler, 1980). This Tests commonly used to know the quality of milk.

➢ *Dye-Reduction Tests:*

These tests are the less precise criterion for classifying raw milk according to its bacteriological quality. This calls for the need to periodically verify the quality of milk with more precise microbiological tests such as standard plate count.

➢ *Methylene Blue Reduction Test:*

Methylene Blue Dye Reduction Test is used to check the microbiological quality of raw and pasteurized milk. This test is based on the e blue color of the dye solution added to the milk get decolorized. When the oxygen present in the milk get exhausted due to microbial activity. The sooner the decolorization leads to the presence of more inferior bacteria of milk and more inferior is the bacteriological quality of milk assumed to be.

- *Standard Plate Count (SPC):*

The Standard Plate Counts test is to identify both total aerobic bacterial and total mold present in the milk at the time of pick up. Milk samples are plated on standard plate count agar media and then incubated for 48hrs for bacterial growth. When Single bacteria or clusters growth of bacteria become visible in colonies then they are counted. All plate counts are expressed as the number of colonies forming units (CFU) per milliliter. Plate counts can be performed by membrane

filtration, pour plates, or spread plate methods. In this project spread, the plate method is used and the test is appropriate for all nonsterile products and provides a standardized means of determining the microbial content of the milk sample.

- *Coliform Bacteria in Raw Milk:*

Coliforms are rod-shaped Gram-negative bacteria commonly found in the environment, including soil, surface water, vegetation and the intestinal tracts of warm-blooded animals and human. They can ferment lactose with the production of acid and gas when incubated at 35-37°C. The coliform bacteria are a collection of strains in the Enterobacteriaceae family. E. coli, Enterobacter spp., Klebsiella spp., and Citrobacter spp. are the most common coliforms.

- *Somatic Cell Counts (SCC):*

The Somatic Cell Count is the main indicator of milk quality. If the somatic cell count increased in the milk that milk is not sold for human consumption. The USA the limit is 750.000 cells/ml. The majority of somatic cells in milk are leukocytes. Other factors can also influence somatic cell count in milk by altered milk composition after calving when cell concentration increases.

➢ *Titrable Acidity Test:*

In order to determine the sourness of milk, we use titration using sodium hydroxide (NaOH) and the degree of sourness is given by Soxhlet-Henkel Degree (SH0). Generally, the sourness of normal milk is 6 to 7 SH0. If the milk sourness is 4 to 5 SH0, it indicates that either the milk is adulterated or there is mastitis.

➢ *Phosphatase Test:*

The phosphatase test is the most important public health measure for controlling the efficiency of pasteurization, hence the safety of milk. Phosphatase is an enzyme, which is normally present in raw milk. When milk is pasteurized by any of the recognized processes, the enzyme is completely inactivated. Therefore, a positive phosphatase test will indicate that the milk is not properly pasteurized. It may mean any one of the following:

- The pasteurization temperature-time combination was not strictly observed or
- The pasteurization equipment was not functioning properly or
- Pasteurized milk has been contaminated by raw milk.

This is important because improperly pasteurized milk still could transmit tuberculosis, brucellosis, and Q fever.

➢ *Organoleptic Tests:*

Microorganisms cause various undesirable and detectable organoleptic and physical changes in raw milk. Generally, when actively growing types of an organism capable of causing changes in flavor and physical appearance reach population levels of 5-20 million per ml organoleptic and physical changes are evident or imminent.

➢ *Clot on the Boiling Test:*

It is a qualitative method for determining the acidity in milk ad used to determine whether milk is suitable for processing, as it indicates whether the milk is likely to coagulate during processing the milk will be clotted if acidity is 0.25% and more

➢ *Catalase Test:*

This measures the activity of the enzyme catalase. The catalase content of milk primarily depends upon the number of cells in milk. Hence the increased activity of this enzyme indicates mastitis.

- *Test:*

The normal freezing reading of milk is between -0.50 and -0.61°C. The soluble constituents, lactose, and ash determine the freezing reading of milk and are responsible for its being lower than that of water. This fact makes it possible to determine whether or not milk has been watered. It had been shown that with the addition of 1% of water to milk, the freezing point is raised approximately by 0.0055˚C.

- *Mycobacterium bovis:*

Mycobacterium bovis causes tuberculosis in both human and domestic animals. Mycobacterium tuberculosis group bacteria are 1.0 - 4.0 µm long by 0.2 - 0.3 µm wide in tissues

M. bovis is *a* potentially serious health hazard infection through milk. The situation in developing countries may be much more demanding because of the higher incidence of *M. bovis* in dairy animals resulting in dissemination of the organism through raw milk. Raw milk consumption is thus the most important channel for the transfer of tubercle bacilli.

- *Salmonella species:*

Salmonella species are intracellular pathogens causes' illness and increasing have been implicated to food containing eggs or poultry products. Natural infections of the udder may occur very rarely and therefore don't play any role in human infections. There are a lot of case reports about epidemics by consumption of raw milk, however, mostly from the seventies and eighties. Contamination of raw milk mostly is due to infected persons and to the environment.

- *Brucella species:*

From the viewpoint of human health, the disease is important because the organism can cause undulant fever or Malta fever or Mediterranean fever in man. The possibility of infection occurring by drinking of infected milk necessitates the pasteurization of milk. Officially approved the method of commercial pasteurization renders naturally Brucella contaminated raw milk safe for consumption.

- *Escherichia coli:*

It is a bacteria which lives in Human intestine. It is also found in the gut of some animals. Most E. coli are harmless and even help keep the digestive tract healthy. *Escherichia coli* occurred commonly in organisms in milk whenever the methods of production, transportation, handling and sale of milk are unhygienic. That causes a great hazard to public health.

- *Listeria monocytogenes:*

It is an anaerobic bacteria where dairy products have been cited frequently as causes of food poisoning and the industry is now required to go to the extensive expense to ensure the dairy products are free from *Listeria monocytogenes. Listeria monocytogenes* is widespread in nature and frequently gains access to the farm milk supply. This can either be from infected animals or more frequently from the environment in which the milk is produced. It causes a serious public health risk, particularly where the dairy industry usage depends upon raw milk. The risk is due to infection of milk contamination. Consequently in order to prevent contamination early detection of cows with *Listeria mastitis* is required. Good hygienic practice during milking and proper use of normal cleaning routines are adequate to limit the number of *Listeria monocytogenes* in milk as it has no special resistance to the routinely used cleaning material. The organism can grow in milk at refrigeration temperature but it has been now demonstrated that all *Listeria species* are destroyed by pasteurization. Problems with *Listeria species* in milk, therefore, are limited to post-pasteurization contamination.

- *Staphylococcus aureus:*

It is a Gram-positive bacteria. Milk does not get contaminated unless good hygiene practice like control occurs on farms, the milk that is appropriately pasteurized should be taken to prevent contamination and subsequent growth of Staphylococci during the manufacturing process and the finished product. It causes mastitis or skin disease in milk-producing animals or lead to foodborne intoxication in milk

II. REVIEW OF LITERATURE

Milk is the dietary food component of many people adequate intake of calcium from dairy food product reduces the risk of osteoporosis and also increasing bone acquisition during growth (Miller GD). Milk that consists of protein has specific peptides associated with casein and that proteins can outstandingly lower blood pressure in the body. In-home mostly milk is consumed by socio-economic strata where higher income groups consume more pasteurized milk than raw milk which is revealed under a survey of National Health and Nutrition Examination Survey 2006. Dairy products will decrease the risk for diabetes in vulnerable populations (Pippus., 1988) and also high dairy intake in pregnancy will increase on bone health of mothers and her offspring. Milk and milk products play an important role in human nutrition and the products that are formed are high hygienic quality. Milk is also a suitable substrate for microbial growth and development. The Physico-chemical properties of equine milk which is potential in human nutrition and leads to distribution of the nitrogenous components in the human body by Daniel M. By verifying the possibility of the presence and percentage of maize in the animal diet on the basis of multi-element (C, N, H, O) isotope ratio investigations of milk give knowledge about the presence of maize in animal diet could be very useful for protecting the consumer who is willing to give additional value to milk using traditional pasture practices (Gianni Colombari., 2008). The compound like Protein, fat, carbohydrate, Ca, P and Fe were present in milk were estimated in samples of the homogenized diets for women (F. C. Aitken). The microbial growth rate depends on the temperature used for pasteurized milk thereafter (Kurwijilla *et al*., 1992). Microbial proteinases helps in storage of raw milk at 4 °C by YvesLe Roux in 1995. Milk contain both pathogenic and nonpathogenic organisms. Pathogenic organisms, which may come directly from the cow's udder, are species of *Staphylococcus*, *Streptococcus*, *Mycobacterium*, *Brucella*, *Escherichia*, *Corynebacterium*, etc...The isolation of lactobacillus was classified as high level resistant to streptomycin.

III. MATERIALS AND METHODS

❖ *Collection of Samples*

A total of 10 samples of milk were collected from for analyzing physiochemical and microbiological criterion of milk. 5 samples of raw milk were collected from a dairy farm and similarly, 5 pasteurized milk samples were collected from different milk vendor store in Hyderabad. These milk samples were analyzed for physical parameters like pH, titratable acidity and specific gravity. Chemical quality of milk was tested by determining the contents of total solids, fat, solids not fat, lactose, protein, and casein in raw as well as in pasteurized milk samples. The physic-chemical properties of raw and pasteurized milk samples were analyzed by the methods mentioned in the previous chapter.

❖ *Testing of Milk:*

A. Chemical Testing

B. Microbiological Analysis.

A. Chemical Testing

Chemical testing includes platform tests and test for adulteration. The microbiological analysis includes conventional methods and rapid methods. Total plate count and Coliform plate count were done using the method and rapid method using petrifilms. Isolation and identification of bacterial agents were done using various media and biochemical tests were performed.

➢ *COB Test:*
COB test for screening of increased acidity.

- *Principle:*

The heating of milk which contains acidity of more than 0.20% will result in clotting.

- *Procedure:*

Taking of about 2 ml milk in a test tube and boiling on the flame of a spirit lamp up to 4 minutes leads to the formation of clots in the test tube indicates COB positive milk and also the presence of 0.20% of acidity is unacceptable.

➢ *Acidity Test:*
Acidity Test is for the screening of milk sample.

- *Principle:*

Titrating of milk sample by using the base like a dye, which changes color at a specific pH, is added to the milk by a small amount until the color changes.

- *Procedure:*

Taking 10ml milk in the 10ml conical flask. Titrate against N/10 NaOH using phenolphthalein indicator to check acidity. Complete the titration within 20 seconds. Acidity above 153% is not acceptable.

➢ *Rosalic Acid Test:*
Rosalic acid test is used for the identification of the presence of neutralizers in milk.

➢ *Reagent:*

- Rosalic Acid Solution 0.05%
- 60% Ethyl Alcohol Solution 100 ml
- Distilled Water 40 ml
- Rosalic Acid Powder 50 mg

- *Procedure:*

To 2ml rosalic acid solution in a test tube containing 2ml of milk. The milk that contains alkali then develops the color change to rose-red color. The color change indicates the presence of in milk and the formation of flakes indicates disturbed salt balance and pure milk shows only a brownish color.

➢ *Alcohol Test:*
Alcohol test is done to know the quality of raw milk.

- *Principle:*

Milk that consists of high-level calcium and magnesium compounds will coagulate by adding alcohol.

- *Procedure:*

Take 2 ml milk in a test tube and then adds 2ml 68% alcohol to the test tube. By adding alcohol leads to the formation of flakes that indicates alcohol-positive milk and such milk is rejected.

➢ *Phosphatase Test:*
Phosphatase test is used for pasteurized milk to check whether the milk is pasteurized are not.

- *Principle:*

Milk contains Alkaline Phosphatase enzyme which is inactivated by heating at the temperature used for the pasteurization of milk

- *Procedure:*

Take two sterilized MBR test tube. In the sample, the bottle draws milk sample from the silo. Take 1 ml of milk in each of the two sterilized MBR test tube. One Tube is acted as a control sample and temperature is maintained by heating. To that tubes add 5 ml of Phosphatase dye and mix well. Incubate at 37°C in a water bath, observe the color change for every 10 minutes and finally after two hours.

➢ *Phosphatase Dye:*
Dissolve 0.15gm of p-nitrophenyl disodium orthophosphate salt in 100ml of buffer solution.

➢ *Buffer Solution:*
Dissolve 3.5gm of sodium carbonate and 1.5gm of sodium bicarbonate to make 1liter of a solution in distilled water. Keep the buffer solution in a cool place at 4°C or less.

➢ *Methylene Blue Reduction Test (MBRT):*
MBRT test is used to test the microbiological quality of raw and pasteurized milk.

- *Principle:*

The microbiological quality of raw and pasteurized milk is tested by using blue color dye solution is added to the milk and milk get decolorized when the oxygen present in the milk get exhausted due to microbial activity. Decolorizing of milk is observed by microbial activity.

- *Procedure:*

Labeled the test tube according to the requirement. Take 10 ml of milk in a sterilized MBR tube. Add 1 ml of MBR dye. Tighten the test tube mouth with stoppers. Invert the tube to mix the contents and Keep the tubes in the water bath at 37 °C. De-coloration of milk take place. Check the tube for the first 10 minutes and subsequently every hour. For pasteurized milk time should be 5hrs or at least 4 hrs. Whereas for raw milk time is 30 min.

➢ *Fat & SNF:*
Solids Not Fat (SNF) does not contain milk fat and water. Solids content contains fat protein, lactose, and minerals.

- *Procedure:*

Take milk sample of 11ml in the test tube and labeled then accordingly. Add 10ml sulphuric acid in butyrometer and closed with a rubber cork and mix the sample thoroughly and incubate it I water bath at 65 °C. The sample was centrifuged in Gerber centrifuge machine for 3 to 5min at 1000 rpm. The fat percentage was noted on the butyrometer scale.

➢ *Lactose:*

Lactose is the naturally occurring sugar supplement found in milk products that can be difficult for some people to digest.

- *Procedure:*

Take 100 ml volumetric flask to that add 20ml of milk sample ad mix well to that sample add 12ml 10% sodium tungstate and also add 15 ml 2/3 N H2SO4 and make the sample to 100 ml with use of distilled water. Then the sample is get filtered by using the titration method and titration is done with using 25 ml benedict's solution. After titration, the sample changes to white color

➢ *Protein & Casein Estimate:*

Milk consists of Casein. That present in form of calcium salt and calcium caseinate.

- *Procedure:*

Take 10ml of milk and pipette out into a flask. Label the flask according to that add 4-5 drops of Phenolphthalein indicator used as an indicator for this method. To that sample add 0.4ml of saturated potassium oxalate solution and keep it aside for 4-5 min without disturbing. Milk sample is titrated by using 0.1 N NaOH and for neutralizing add 2ml of neutral formalin and mix well.

B. Microbiological Analysis

In milk microbiological analysis takes place by following method.

➢ Standard Plate Count (SPC)
➢ Coliform Count
➢ Isolation and identification of Bacterial agents

➢ *Standard Plate Count:*

Standard plate count is for estimation of the presence of aerobic bacteria in the raw milk sample. In this method the samples are plated on agar media for 48hrs at 32 °C for bacterial growth and growth of bacteria is in the form of single or clusters colonies ad it is measured in colonies forming units (CFU) units.

- *Procedure:*

Serial dilution process takes place for Standard plate count. Take 1 ml of well-mixed milk sample to 9 ml diluents with a phosphate buffer solution. Mix the sample properly. From that sample take 1 ml to add to the second tube which consists of 9ml of phosphate buffer solution, it is the second dilution. This process is continued for five times that is five dilutions takes place**.** Then take 5 Petri plates for each dilution and label them accordingly. Take 1ml of a diluted sample in each plate respective and the diluted sample is spread over a plate for testing and it cooled in about 45°C and pour 10 ml of this medium into the Petri dishes. Mix the agar by rotating the plate. Allow the agar to set at 37°C for 48 hours. At the end of 48 hours remove the plate from the incubator and count the colonies.

- *Media Used For Isolation Bacteria*

o *Mac Conkey - 500g*

MacConkey agar is a selective media used for the isolation of gram-negative Enterobacteriaceae.

o *Composition (g/l):*

✓ Peptone from casein 17.0
✓ Peptone From Meat 3.0
✓ Sodium Chloride 5.0
✓ Lactose Monohydrate 10.0
✓ Bile Salt Mixture 1.5
✓ Neutral Red 0.031
✓ Crystal Violet 0.001
✓ Agar 13.5.
✓ Distilled Water Add To Make 1 Liter

o *Preparation:*

Take 50g of the dehydrated medium in 1 liter of distilled water and heating it in boiling water bath will dissolve the medium completely and autoclave 15 minutes at 121˚C for Sterilization and the cool at 45-50°C.

- *Nutrient Agar - 500g*

Nutrient agar media for bacterial growth

o *Composition (g/l):*

Agar 1.5%
✓ NaCl 0.5%
✓ yeast extract 2.0
✓ Peptone 5.0
✓ Distilled water

o *Preparation:*

Suspend 28 g of nutrient agar powder in 1 liter of distilled water. Heat the mixture for the complete dissolving of the mixture. Autoclave the dissolved mixture for sterilization at 121˚C for 15 minutes. The cool the mixture.

- *Tests for Enzymes:*

✓ *Catalase Test:*

The enzyme catalase which breakdown the hydrogen peroxide into oxygen and water. Bubbles occur when a breakdown of hydrogen peroxide. Formation of bubbles leads to the presence of catalase enzyme. The culture should not be

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more than 24 hours old. The production of gas bubbles indicates a positive reaction. It occurs almost immediately.

✓ *Oxidase Test:*

This test depends on the presence of oxidases in the bacteria that will catalyze the transport of electrons between electrons donors in the bacteria and a redox dye tetramethyl-p-phenylenediamine. The dye is reduced to a deep purple color. The dye is used for screening species of *Neisseria, Moraxella, Campylobacter, and Pasteurella (oxidase positive).*

➢ *Coliform Count Plate (CC).*

The CC plate counts for the identification and growth of the members of the family Enterobacteriaceae which ferment lactose to produce gas. E. coli Count plates contain violet red bile nutrients and a cold-water-soluble gelling agent. Organisms which ferment lactose to produce gas will have gas bubbles trapped in the gel next to the colonies. The bile salts in the medium select for the family Enterobacteriaceae and the TTC indicator assists in visualizing the colony. Incubation is at 37degrees for 24 hours. Count all colonies which appear red and which are associated with gas bubbles as confirmed coliforms.

IV. RESULTS AND DISCUSSION

The result of physic-chemical tests conducted in raw milk and pasteurized milk sample is given in tables

A. Physical Quality Of Milk:

Mean pH of raw milk obtained from the dairy farm was 6.65 and pasteurized milk obtained from various was 7.18 and both were within the normal range. The titratable acidity of raw milk (0.14) was greater than pasteurized milk (0.12%). The results of titrable acidity and pH values of milk in the present study are not correlating to each other. The reason could be attributed to the addition of water, ice or chemical preservative in pure raw milk to extend its shelf life. Raw milk showed the highest specific gravity (1.31), while the specific gravity of milk obtained from other agencies was lower (1.28). These results suggest the water adulteration in milk from these agencies.

Source of milk	pH	Acidity	Specific gravity
Raw milk	6.65	0.14	1.031
Pasteurized milk	7.18	0.12	1.28

Table 1:- Mean Values of Various Physical Parameters in Raw and Pasteurized Milk

B. Chemical Quality Of Milk:

Total solids of raw milk in the present study averaged 16.3% when as total solids content of milk collected from different agencies averaged from 13.45% which is lower than that of raw milk solids not fat (SNF) content of raw milk obtained from dairy farm averaged 9.79% whereas pasteurized milk had an average of 8.25% SNF. The fat content of raw milk had a mean value of 6.57% whereas that of pasteurized milk had a lesser mean value of 5.20 %. The average protein content of raw milk obtained from the dairy farm was 4.35% which was greater than the protein content of pasteurized milk. Similarly casein content and lactose content of raw milk had a mean value of 3.56% and 4.53% respectively. However in comparison, the casein and lactose content in pasteurized milk had lesser values of 2.70% & 3.65% respectively

Source of milk	Total Solids %	S N F %	Fat Content %	Protein Content %	Casein Content %	Lactose Content %
Raw milk	16.30	9.79	6.51	4.35	3.56	4.53
Pasteurized milk	13.45	8.25	5.20	3.85	2.70	3.65

Table 2:- Mean Values of Various Chemical Parameters in Raw and Pasteurized Milk

C. Microbiological Analysis

Source of milk	**phosphatase**	MBRT	TPC (CFU/ml)	Coliform count (CFU/ml)
Raw milk	+ve	$\leq$ 2 hrs.	$1.5x10^8$ (8.17)	$2.8x10^4$ (4.44)
Pasteurized milk	-ve	$\geq$6hrs	$1.25x10^7$ (7.09)	190 (2.27)

Table 3:- Result of Quality Testing of Milk

Log $_{10}$ values of the total plate and coliform count Based on the results of MBRT and phosphatase tests given in the table, raw milk was found to be of poor quality. Whereas pasteurized milk was of good quality.

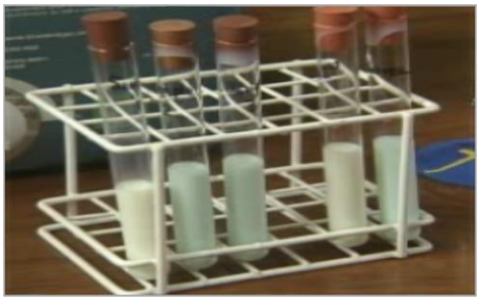

Fig 1:- MBRT Test Results of Raw and Pasteurized Milk

Raw milk showed color change within 2 hours whereas pasteurized milk was stable without any color change for greater than two hours indicating that the huge presence of micro-organisms in raw milk.

❖ *Phosphatase Test Results of Raw and Pasteurized Milk Samples*

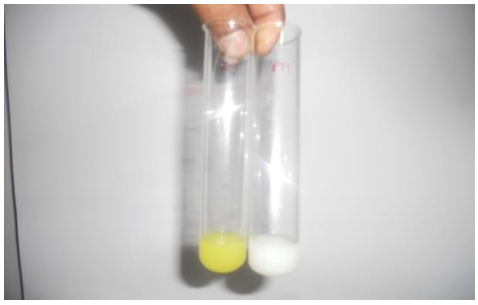

Fig 2

➢ *Result:*

The response of raw milk was positive in raw milk indicating the presence of micro-organisms which degraded the dye within half-an-hour and pasteurized milk sample showed negative results by remaining stable for greater than two hours having no color change.

- *Total Plate Count*

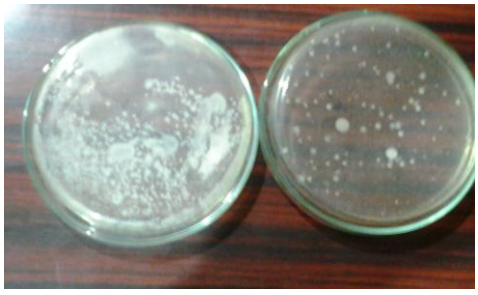

Fig 3

Depicts the mean values for total aerobic plate count of raw and pasteurized milk. The mean values for total aerobic and coliform counts were considerably increased in raw milk when compared to pasteurized milk

- *Coliform Count by VRB (Violet Red Bile Agar) Media:*

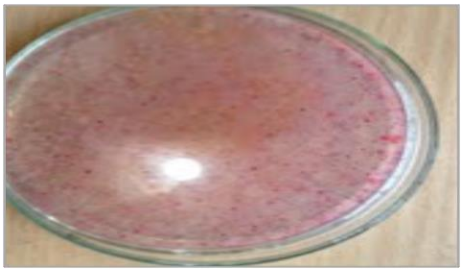

Fig 4:- Pasteurized Milk Sample

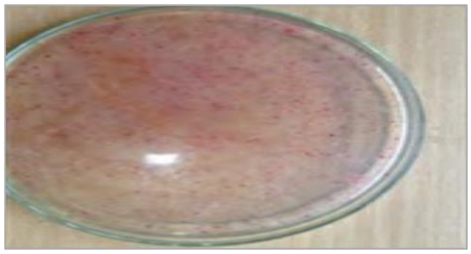

Fig 5:- Raw milk sample:

➢ *Result:*

In the case of the coliform count, raw milk samples had counts > $2.8x10^4$(4.44)CFU/ml which is higher than the given international standard set for the acceptable coliform count. Whereas, pasteurized milk samples had counts of 190 (2.27)CFU/ml.

- *Coliform Activity by BGBB Broth:*

Fig 6:- Brilliant Green Bile Broth

➢ *Result:*

Raw milk sample showed the presence of microbes by gas production, as a result, the Durham tube moved upwards whereas pasteurized milk showed nill gas production, as a result, Durham tube remained in the bottom of the tube indicating the absence of micro-organisms.

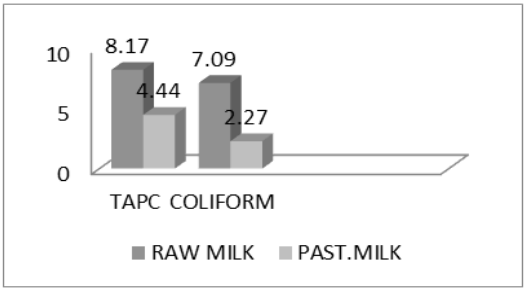

Fig 7:- Total Plate and Coliform Count in Raw And Pasteurized Milk

D. Isolation And Identification Of Bacterial Species

Gram staining	Morphology	Catalase	Coagulase	Sugar fermentation		
				Mannitol	Maltose	Trehalose
Gram +ve	Cocci	-	-	-	+	-

Table 4:- Differential tests used for ***Staphylococcus sp***.

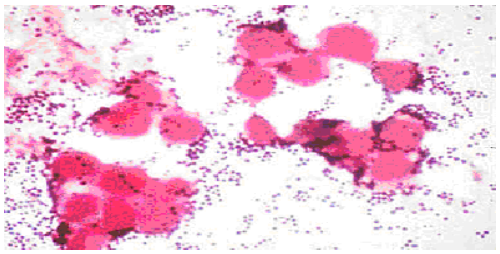

Fig 8:- Differential tests used for lactic acid bacteria

Gram Staining	Morphology	Catalase	Sugar fermentation						
			Sucrose	**Maltose**	**Lactose**	**Mannitol**	**Glucose**	**Galactose**	**Fructose**
+ve	Spherical short chains	-	+	+	+	-	+	+	+
+ve	rods	-	+	+	+	-	+	+	+

Table 5:- Differential Tests Used For ***Bacillus Sp***

Fig 9

➢ *Result:*

Gram staining	Morphology	Citrate	Voges-Proskauer	Arabinose	Mannitol
+	rods	-	-	-	-

Table 6:- *Bacillus species* showed negative results for the Voges-Proskauer test

Gram staining	Morphology	Catalase	Sugar fermentation			
			Glucose	Lactose	Maltose	Trehalose
+ve	rod	+	-	-	-	-

Table 7:- Differential tests for ***corynebacterium sp***

Fig 10

➢ *Observation:*

After 48 hours of incubation when four drops of hydrogen peroxide were added to the slants slow appearance of gas bubbles was observed. This is an indication of a positive test. Hence *corynebacterium sp*ares positive for catalase.

Fig 11:- Differential tests used for *Actinomycetes Sp*

➢ *Result:*

After 24 hours of incubation it was observed that there is a change in the medium color to blue. From the above observation, it is said PNP degrading organism to this positive for citrate utilization test.

Bacteria isolated	Number of isolates %	
	Raw milk	**Pasteurized milk**
Streptococcus lactis	**40**	**19**
Lactobacillus acidophilus	***36***	***12***
Bacillus stearothermicus	**5**	**5**
Cornyn bacterium freundi	**6**	**-**
Actinomycetes pyrogera	**9**	**-**
Staphylococcus epider minus	**40**	**20**

Table 8:- Bacterial isolates from raw & pasteurized milk samples

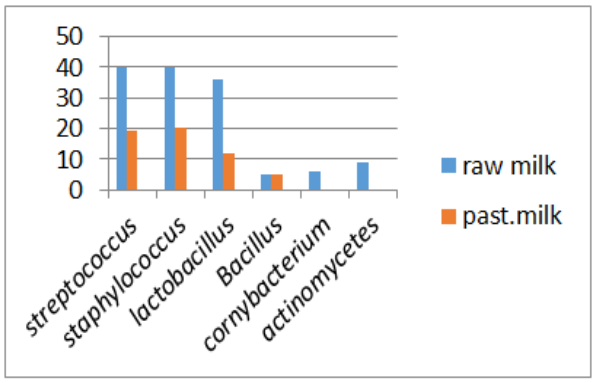

Fig 12:- Bacterial isolates from raw & Pasteurized Milk Samples

Source of milk	Rosalic acid	Hydrogen peroxide	Formamide	Cane sugar	Starch	Glucose	Urea	Added water
Raw milk	-	-	-	-	-	-	-	-
Pasteurized milk	-	-	-	+	-	-	-	-

Table 9:- Result of Adulteration tests

Fig 13

➢ *Result:*

The results of tests for milk adulteration in raw and pasteurized milk are given in the table. The results indicate that no adulterants were added to pasteurized milk. However, cane sugar was found in pasteurized milk samples.

E. Coliform Count:

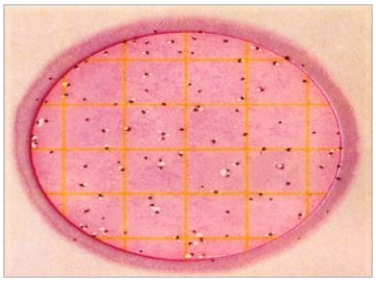

Fig 14

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➢ *Result:*

Total coliform count in raw milk was found to be 2.4 *10ˆ4/mg/ml indicating the presence of coliforms.

- *Coliform Count In Pasteurised Milk Sample*

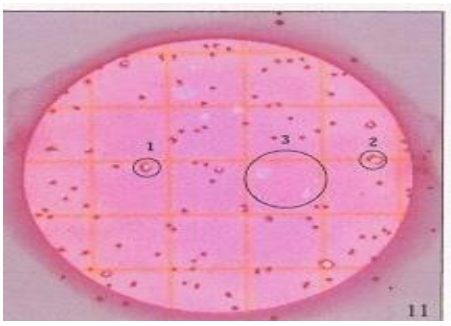

Fig 15

➢ *Result:*

Coliform count in pasteurized milk sample was very less in the range of 150 /mg/ml proving its quality.

- *coli count in raw milk:*

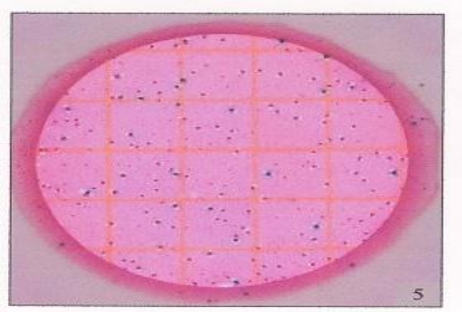

Fig 16

➢ *Result:*

E.coli count in raw milk was found to be 3*10ˆ4 mg/ml, this result inferred that raw milk is highly contaminated with microbes especially coliforms and e.coli.

- *E.coli count in pasteurized milk sample:*

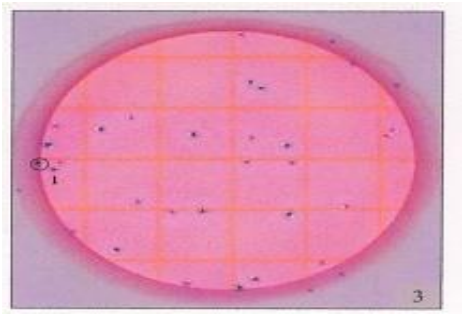

Fig 17

➢ *Result:*

Pasteurised milk sample showed the presence of very few e.coli in the range of 32 /mg/ml

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