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Quantitative and qualitative bacterial examination of milk samples

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Abstract
Milk has an outstanding nutritional quality but it is also an excellent medium for bacterial growth and an important source of bacterial infection when or to the hot pasteurization Raw milk collection and its transportation to the processing centers present several technical, economical, and organizational problems in most developing countries. Many bacteria could get easy access to milk and milk products such as *E.coli*, and coliform and they are often your used as indicator organisms to confirm the bacterial contamination of milk. It may cause due to their contact with microorganisms during their exposure to environment. Milk provide suitable environment for the survival and growth of many types of microbes especially bacteria. Several bacteria have been isolated and identified based on morphology of colony, staining procedure and also by shape and arrangement of bacteria. The observed bacteria includes E.*coli*, Lactococcus, and *Salmonella*. Methylene Blue Dye Reductive Test was done for testing the quality of milk which may be useful for the milk processor to take a decision on further processing of milk.

Keywords: Raw milk, pasteurized milk microbiological quality assessment, coliforms

Introduction

Milk and milk products are excellent high-quality foods providing both nutritional and culinary values. However, milk is extremely susceptible to spoilage by microorganisms and the microbiologist plays a major role in the dairy industry in quality control of milk. Cow's milk consists of a variety of nutrients such as fats, proteins, minerals, vitamins, carbohydrates and water and thus it serves as an excellent medium for bacterial growth.

The appropriate conditions milk can act as a carrier of disease-causing microorganisms Mycobacterium bovis, *Brucella* species, *Streptococci* and *Coxiella burnetti* from infected cattle. Agents from human sources such as Salmonella species, Shigella species, Corynebacterium diphtheria and Streptococcus species can also be presented in milk. According to Gunasekerall, psychrotrophic microorganisms are the most important group of microbes present in milk and dairy products. The microbe Pseudomonas spp. is considered as the most important psychrotroph contributing to milk spoilage through the production of lipolytic and proteolytic enzymes.

Raw milk comes from cows, goats inform which is free from microorganisms, but the treatment processes milk and makes it harmful or unhealthy reference contaminated with various sources such as closed milk systems, improper bulk tanks, unising storage equipment, unclean processing areas and other factors Mungai EA *et al*. Type of bacterial species can contaminate milk in different ways, including contaminated organisms, gram-positive, gram-negative etc., in which raw milk is treated at high temperature to reduce or eliminate the bacteria from milk and make the milk suffer for consumers desmosomes. Pasteurized milk is considered to be safe for a person because the heat treatment process kill the bacteria and other organisms such as salmonella, *Campylobacter*, *E.coli*, etc. Pasteurized milk does not nutritive value of milk. Basically, the pasteurization process, milk and quick cooling lead to certain bacteria's elimination. Best result pasteurized milk heated at the high temperature of 145 degrees Fahrenheit for 30 minutes. That is also called batch pasteurization.

Contamination occur from various factors such as diseased vichar suffered from mastitis (an animal disease). From improper cleaning of milky equipment and milk tanks in which milk is collected, stored and transported Deshmukh AM *et al*.

Most common cause of raw milk contamination is soil teats, udders, and trails of animals. Milk is an enrichment medium for the development of microbes.During the transportation of milk at normal temperature, the microbes multiply and decline the quality of milk. Study was conducted to detect and analyse the pathogenic microbes among different collected samples of raw and pasteurized milk.

Temperature is an important factor for the prevalence and proliferation of microorganisms in milk. When milk is subjected to temperature abuse, microorganisms can multiply at higher level and may produce toxins. For it is interesting to evaluate the bacteria logical quality of milk based on storage temperature at different collection points.

Materials and methods

Three type of unused and fresh milk samples combining of Aavin, Arokya, and Cow milk were collected from Krishnapuram, Tirunelveli.

Fig 1

Preparation of agar medium

A gelatinous substance obtained from certain seaweeds and used in biological culture media and as a thickener in food. Nutrient agar and EMB agar have choosen for bacterial culture.

Nutrient Agar Medium Preparation: Take 2.8 grams of Nutrient agar and dissolve it in 100 ml of distilled water and sterilize it in autoclave at 15lbs pressure (121°C temperature) for 15 minutes.

Isolation of bacteria

Serial Dilution is done at different concentrations it is a stepwise dilution of a substance in a solution. The dilution factor at each step is constant resulting in a geometric progression of the concentration in a logarithmic fashion.

Culture medium

The sterilization of the laminar airflow chamber must be done using UV light prior 30 starts of work. The Petri plates, prepared agar media, prepared milk samples, and other required materials were sterilized in an autoclave and they were taken to the laminar airflow chamber. Ethanol is used for further maintenance of sterilization condition. Allow the agar media to get cooled but molten for pour plating A 100µL of selected dilutions of each milk suspension was poured over labeled nutrient agar (NAM) plates from each selected dilution (Pour plate method or streak plate method).

Pour plate

It refers to a plate prepared by mixing the inoculum with cooled out still molten medium before pouring the latter into the Petri dish. It is the method of choice for counting the number of colony-forming bacteria present in a liquid specimen.

Streak plate

It is a rapid qualitative isolation method. The techniques commonly used for isolation of discrete colonies initially require that the number of organisms in the inoculums be reduced. It is essentially a dilution technique that involves spreading a loopful of culture over the surface of an agar plate.

Incubation

The inoculated petri plates were incubated at 37°C for 24 hours to allow bacterial growth. Consequently, bacterial colonies were subcultured, maintained, and stored on NAM slants at 40°C for further use.

Colony counting

After 24 and 48 hours of incubation, bacterial growth was observed. They were counted and tabulated. Further, the morphological analysis was conducted to determine different bacterial colonies and to observe morphological variation of bacterial growth.

Identification of bacteria

Morphological analysis was observed to determine type of bacteria based on colonies and morphological variation of bacterial growth. Gram staining was conducted to study the cellular morphology of isolated bacteria.

Simple staining

The simple stain can be used as a quick and easy way to determine the cell shape, size, and arrangements of bacteria. It is a very simple staining procedure involving a single solution of stain. Any basic dye such as methylene blue, safranin or crystal violet can be used to color the bacterial cells.

Gram staining

Gram staining is a differential method of staining used to assign bacteria to one of two groups (gram-positive and gram-negative) based on the properties of their cell walls.

Procedure

Place a small drop of bacterial sample on a slide. Heat fix the bacteria to the slide by passing it through the flame of a Bunsen burner three times. Applying too much heat or for too long can melt the bacteria's cell walls, distorting their shape and leading to an inaccurate result. If too little heat is applied, the bacteria will wash off the slide during staining. Use a dropper to apply the Primary stain (crystal violet) to the slide and allow it to sit for 1 minute. Gently rinse the slide with water no longer than 5 seconds to remove excess stain. Use a dropper to apply gram's iodine to the slide to fix the crystal violet to the cell wall. Let it sit for 1 minute.

Rinse the slide with alcohol or acetone about 3 seconds, followed immediately with a gentle rinse using water. Apply the secondary stain, safranin, and allow it to sit for 1 minute. Gently rinse with water no longer than 5 seconds. The gram-negative cells should be stained red or pink, while the gram-positive cells will still appear purple or blue. View the slide using the microscope

Methylene blue reductase test (MBRT)

The test is based on the principle that METHYLENE BLUE (an oxidized-reduction test) which is blue in its oxidized state is reduced to a colorless compound (leucoform) as a result of the metabolic activities of bacteria in milk.

When a solution of methylene blue is added.The organism present in milk consumes the dissolved oxygen and lowers the Q-R potential to a level where methylene blue and similar indicators are reduced as decolorised.The time taken for the reduction of the dye (methylene blue reduction time) is influenced by the number and type of bacteria growing in the milk.The greater the number of the organisms presents in the milk and the greater their activity, the more rapidly is the dye reduced. The methylene blue reduction time thus gives an indication of bacterial numbers and activity in milk.

Procedure

Take test tubes and pour 10 ml of milk sample in each test tube. Then the test tubes add drops of Methylene Blue Dye in each test tube. Next, incubate the test tubes at 37 C in a water bath for 15 minutes. Absorb the color of the test tubes concerning times. If the color is changed its means that the milk sample having contamination (Load of Microbes), and the quality of milk is not good, while if there is no color changed in the test tubes, it means the quality of the concerned milk sample is good.

Results

In this study, various bacteria were isolated and identified from 3 different samples such as Arokya, Aavin and Cow milk (Fig: 1) which are collected from Krishnapuram, Tirunelveli.The isolation of bacteria have been done by pour plate, streak plate methods in Nutrient agar using 3 different samples of milk at different concentrations and the Methylene Blue Dye Reductive Test method was also done for assessing the microbial milk quality.

The culture of bacteria in various milk samples was observed after 24 and 48 hours Table 1(Fig: 2) and the total bacterial count is done using a colony counter. The different concentrations showed varied bacterial growth.As numeral bacterial growth were observed in 10-2 concentration which is not feasible for counting.

According to classical bacteriology,most species of bacterial isolate can be differentiated based on simple gram staining technique.Gram positive bacteria were found in Arokya and Aavin and gram negative bacteria have been found in Aavin and Cow milk (Fig: 5). Gram stain reaction is based on the difference in the chemical essence of bacterial cell walls.Gram positive cells have thick peptidoglycan layer whereas in gram negative cells it is much thinner and contains outer lipid layers.In results,Gram positive bacteria appear purple,because iodine and crystal violet precipitate in the thickened cell wall and they are not eluted by the alcohol.But in gram negative bacteria the crystal violet will be eluted from the bacteria and it will appear in pink colour.

Based on the morphology such as shape, size, texture, arrangement and colour of bacterial isolates they have been grouped due to their similarity, the morphology of bacterial colony were observed and noted.In table 3, the shape and arrangement of bacteria were observed and recorded.

The observed morphology of colony for sample Arokya was whitish, flat, smooth, large in size.Sample Aavin was observed as creamy yellow, filamentous, smooth appearance. Sample cowmilk is observed as creamy white, yellowish colour, small in size and irregular colonies.

From all these data (stain type,colony morphology and size and arrangement).the bacteria have been predicted.In Arokya sample,bacteria observed was Lactococcus.The Aavin sample was observed with Salmonella.The bacteria observed in Cow milk sample was E.coli.(Table 2) (Fig: 5)

Methylene Blue Dye Reductive Test was done for testing the quality of milk which may be useful for the milk processor to take a decision on further processing of milk.Large number of bacteria present in milk indicates poor methods of production or handling.From the time the milk leaves the udder,until it is dispensed into containers,everything which comes in contact with potential source of contamination.Bacteria present in the milk utilize the oxygen present in the sample thus lowering oxidation – reduction potential due to the exhaustion of dissolved oxygen.It loses its colour from (Table 3)(Fig:7)we came to know that the sooner the decolorisation,more inferior is the bacteriological quality of milk assumed to be(Fig:8).Based on that the sample Aavin is interpretate as excellent quality. Cowmilk is interpretate as good quality and Arokya is considered as poor quality.

Table 1: Total bacterial count in nu

Type of milk	Concentration	Total no. of colonies (CFUs/ml)	
		24 hours	48 hours
Arokya	10-2	Too numerous	Too numerous
Arokya	10-3	$5.17x10^5$	$6x10^5$
Arokya	10-5	$4x10^7$	$4.98x10^7$
Aavin	10-2	Too numerous	Too numerous
Aavin	10-3	$4.08x10^5$	$5.8x10^5$
Aavin	10-5	$2.32x10^7$	$4.04x10^7$
Cow milk	10-2	Too numerous	Too numerous
Cow milk	10-3	$7x10^5$	$8.09x10^5$
Cow milk	10-5	$5.8x10^7$	$7.82x10^7$

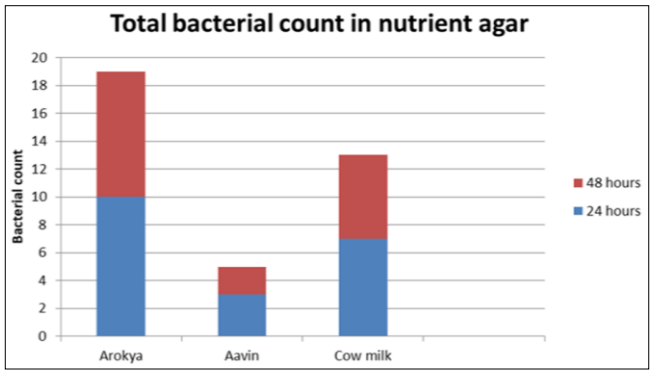

Fig 2

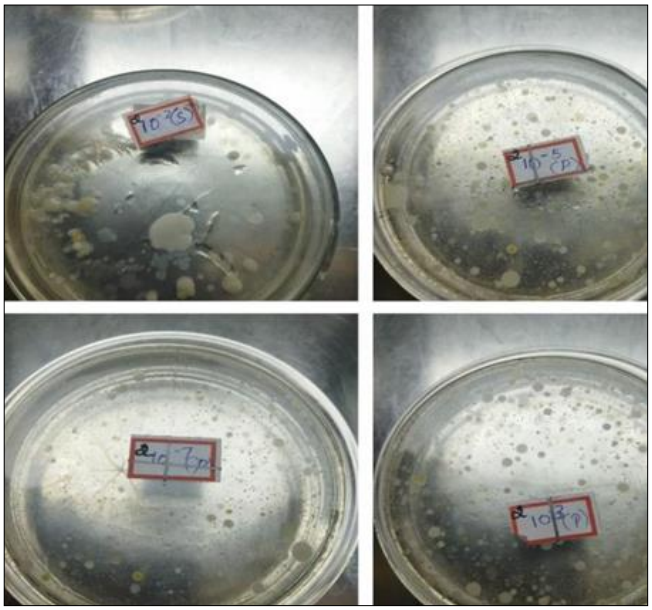

Fig 3: Bacterial growth on nutrient agar in 24 hours

Fig 4: Bacterial growth on nutrient agar in 48 hours

Table 2: Identification of bacteria

Type of milk	Gram type	Shape and arrangement	Bacteria predicted
Arokya	Positive	Spherical or ovoid,non motile	*lactococcus*
Aavin	Positive and negative	Rods and coccoid shape, branched	*Salmonella*
Cowmilk	Negative	Rod shaped, Straight, arranged singly	*E.coli*

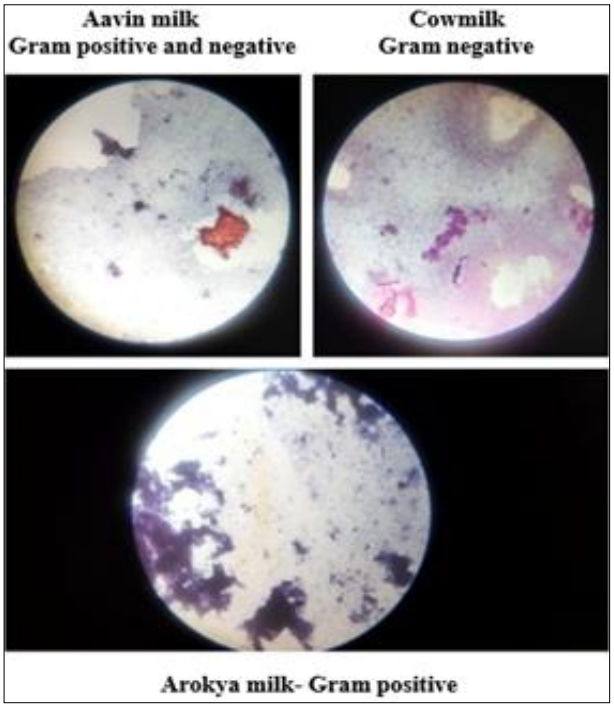

Fig 5: Gram staining in 3 samples

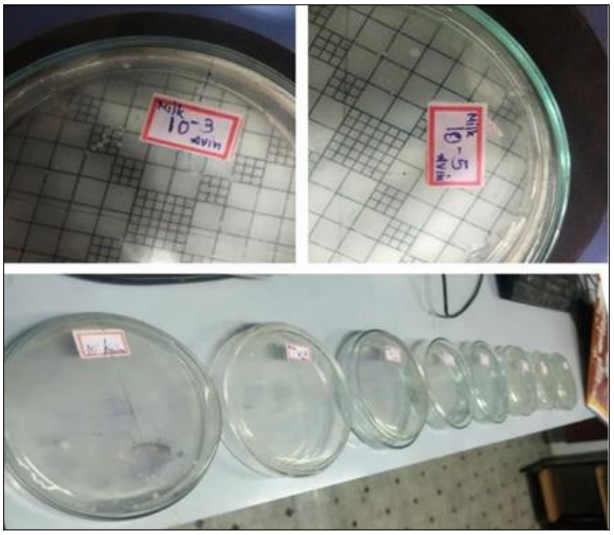

Fig 6: Bacterial colony counting

Table 3: MBRT

Arokya	Changes to white colour after ½ hours
Aavin	Remains blue after 2 hours
Cowmilk	Changes to light blue colour after 1 hour

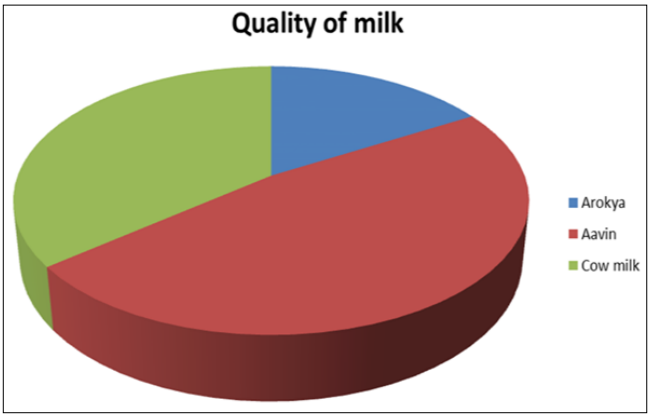

Fig 7

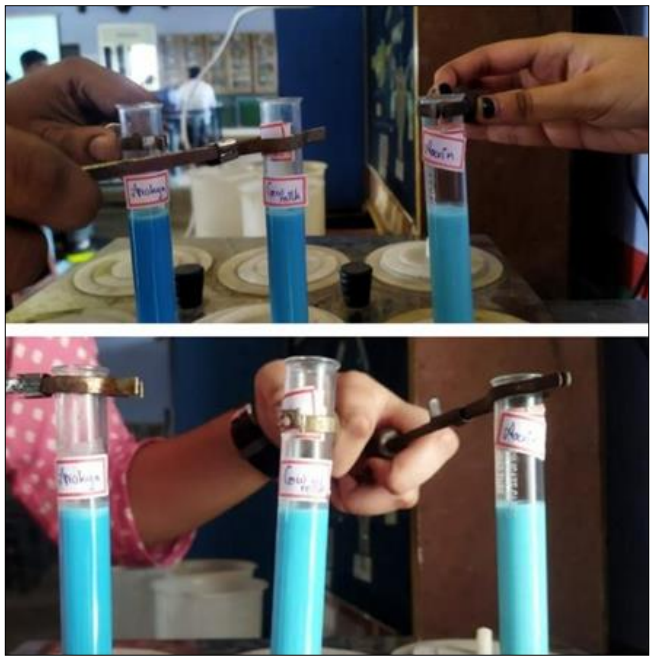

Fig 8: Milk decolorization phase by MBRT

Conclusion

As from the above observations as numeral bacterial growth were observed in 10^{-2} concentration which is not feasible for counting. From all these data (stain type, colony morphology and size and arrangement).the bacteria have been predicted. In Arokya sample, bacteria observed was *Lactococcus*.The Aavin sample was observed with *Salmonella*.The bacteria observed in Cow milk sample was *E.coli*. Methylene Blue Dye Reductive Test was done for testing the quality of milk. Based on that the sample Aavin is interpretate as excellent quality. Cowmilk is interpretate as good quality and Arokya is considered as poor quality.

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