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STUDIES ON THE INCIDENCE AND EVALUATION OF BACTERIA INPROCESSED AND UNPROCESSED MILK

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ABSTRACT

Introduction: Milk contain important nutrients such as minerals, vitamins, proteins and lipids and are consumed by all age group of human around the world. Since the presence of nutrients makes it hard to prevent milk from becoming contaminated with microorganisms. The microbial content of milk can be used to judge its quality. **Aim:** The current study is to identify the incidence and to evaluate the bacteria in processed and unprocessed milk. **Methods and Materials:** Nine distinct types of milk samples were collected and diluted with selenite F broth, normal saline, and macconkey broth. The samples were grow on blood agar, macconkey agar, nutrient agar, saboraud dextrose agar, thiosulfate citrate bile salt agar and deoxycholate citrate agar and observed for the growth in the form of turbidity following a 24-hour incubation period. Next, the developed organism is identified by biochemical analyses and the gram stain. By measuring the dye reduction time and analysing the milk grade in the samples, the MBRT test is used to determine the quality of the milk. **Results:** The isolated organisms were *E.coli, Kelbsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter, S. aureus, Micrococcus, Bacillus, Spingomonas paucimobils.* When compared to processed milk, the unprocessed milk had a higher bacterial burden, indicating the need for appropriate sterilisation before consumption. **Conclusion:** The results of this study will be useful in evaluating the microbiological safety of both pasteurised and raw milk as well as informing public health risk assessments. Therefore, it is imperative that the control methods be followed in order to increase the microbiological quality of milk.

KEYWORDS: *E.coli, Kelbsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter*, reductase test, pasteurized milk.

INTRODUCTION

Milk is the lacteal secretion of the mammals. It is the first natural food of all young mammals immediately after birth.[1] Milk is commonly considered a complete food as it contains all six essential food stuffs. Milk protein is excellent quality and promotes growth and maintenance of the body tissue.[2] Therefore milk is an ideal food for infants and children.[3] Milk is virtually a sterile fluid when secreted into alveoli of udder however, beyond this stage of production, microbial contamination might generally occur within the udder, exterior to the udder and from the surface of milk handling and storage equipment but the surrounding air, feed, soil, faeces and grass are also possible source of contamination.[4] The number and types of microorganism in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health. It is hypothesized that difference in feeding and housing strategies of course may influence the microbial quality of milk.[5] Rinsing water for milking machine and milking equipment washing also involve some of the reasons for the presence of higher number of microorganism including pathogens in raw milk.[6] All types of milk contain the sugar lactose, and when we consume milk, the lactase enzymes (β – galactosidase) hydrolyzes it into glucose and galactose.

Raw or processed milk is a well known good growth medium that supports the growth of several microorganisms because of its high water content, nearly neutral pH and variety of available essential nutrients that renders it as one of the good media for microbial growth and multiplication.[7] Microorganism may contaminate in milk at various stages of procurement, processing and distribution. Presence of saprophytic bacteria in raw milk might change the milk composition and influence the quality of the product.[8]

The natural inhibitory systems in milk may prevent a significant increase in microbial loads within the first 3-4 hours after harvesting milk at ambient temperature.[9] Sometimes, however, the iron-chelating property of lactoferrin results in the death of some bacteria but encourages the growth other bacteria with low iron requirement for growth.[10] Another mechanism for the antimicrobial property of lactoferrin is the direct interaction of intact or partially hydrolysed lactoferrin with lipopolysaccharide of the microbial cell, which may disrupt the cell wall integrity through dispersion of lipopolysaccharides, resulting in cell lysis.[11] Lactoferrin was found to inhibit the growth of *E.coli* and *P.aeruginosa* at concentrations of 0.67mg/mL, 1.67mg/mL and 2.67mg/mL but not *S.aureus, K. pneumoniae* and coagulase-negative *Staphylococci* isolated from a mastitis bovine udder.[12] Heavy bacterial load and coliforms present in the milk indicate the hygienic level and poor quality of milk. So, there is an urgent need to follow the control measure to improve microbial quality of milk. Hence, the attempt was made to analyse the processed and unprocessed milk for bacterial contamination using various milk samples.

METHODOLOGY

MATERIALS AND METHODS: The prospective study was conducted with 9 different milk samples namely raw cow milk, Aavin orange, Aavin blue, Aavin green, Heritage tonedmilk, Hatsun, Heritage full cream milk, Thirumala milk and Arokya milk.

MEDIA USED

Nutrient agar, Macconkey agar, Saboraud dextrose agar, Blood agar, Normal saline, Macconkey broth and selenite F broth were used for the isolation of colonies.

DILUTION OF TEST SAMPLES

Selenite F broth, normal saline and 2 different types of macconkey broth are used for the isolation of colony. Methylene blue reductase test was performed to determine the quality of processed and unprocessed milk pathogens, which is based on the dye reductase time.

ASSESSMENT OF ISOLATED ORGANISMS

The pathogenic organisms were isolated and identified using a variety of biochemical assays, including the Indole, Methyl Red, Voges-Proskauer, Urease, Catalase, Citrate Utilisation, Oxidase, and Hanging Drop Motility Testing tests.

GRAMS STAINING: Using the gram staining method, the distinct pathogenic bacterial colonies were distinguished microscopically as gram positive and gram negative.

RESULT

Isolated Microorganisms from different varieties of Milk Samples

Among the 9 different varieties of milk samples collected**,** 30 isolated organisms have been identified. From these, 9 isolates were *E.coli*, 9 isolates were *Pseudomonas aeruginosa*, 6 isolates were *Klebsiella pneumoniae*, 2 isolates were *Acinetobacter baumanii*, 1 isolate belonged to different bacterial spp., namely *S.aureus*, *Micrococcus*, *Bacillus* and *Spingomonas paucinobilis*. Different dilution of milk samples is shown here in the Fig 1. Staining image of isolated pathogens was depicted in Fig 2 with cultural identification is given in Fig 3. The graph represents the number of organisms isolated in the study and its total percentage (Fig 4).

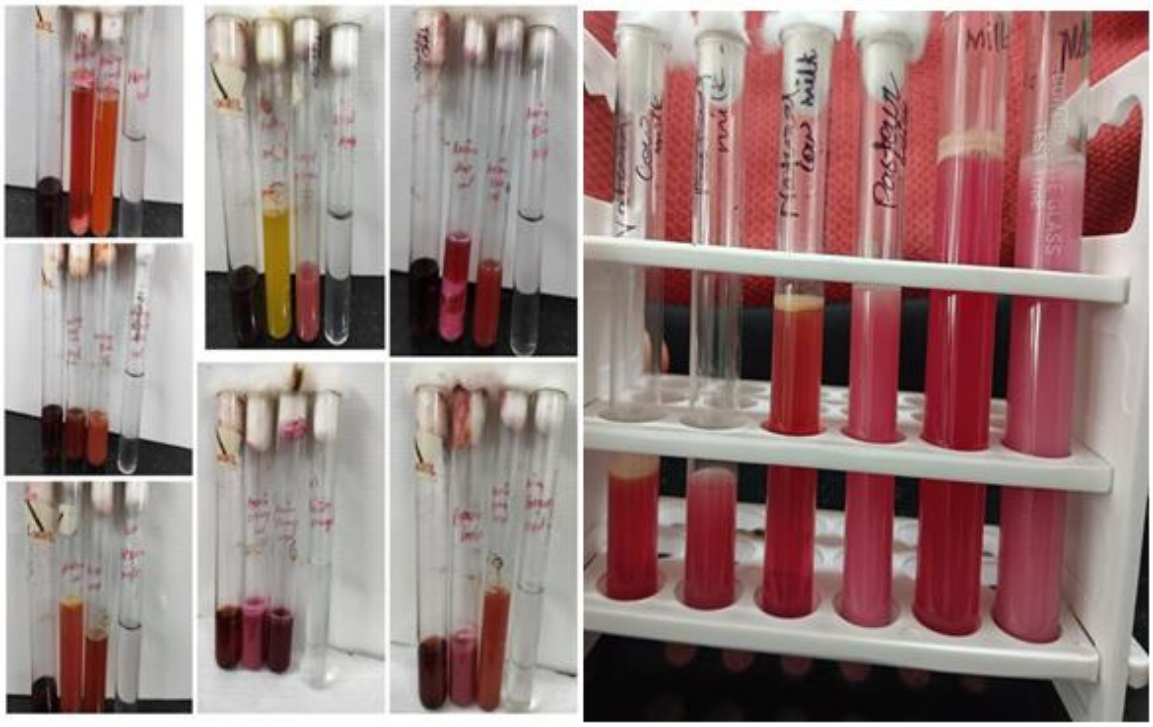
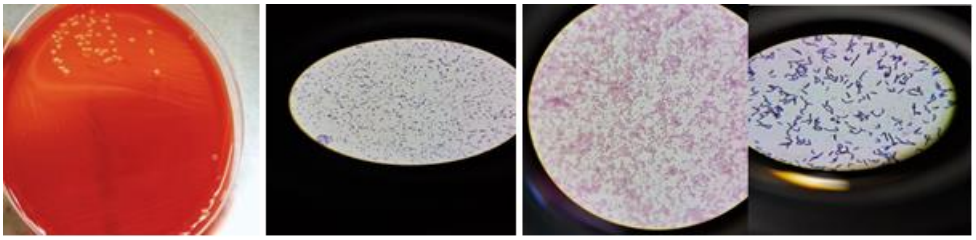
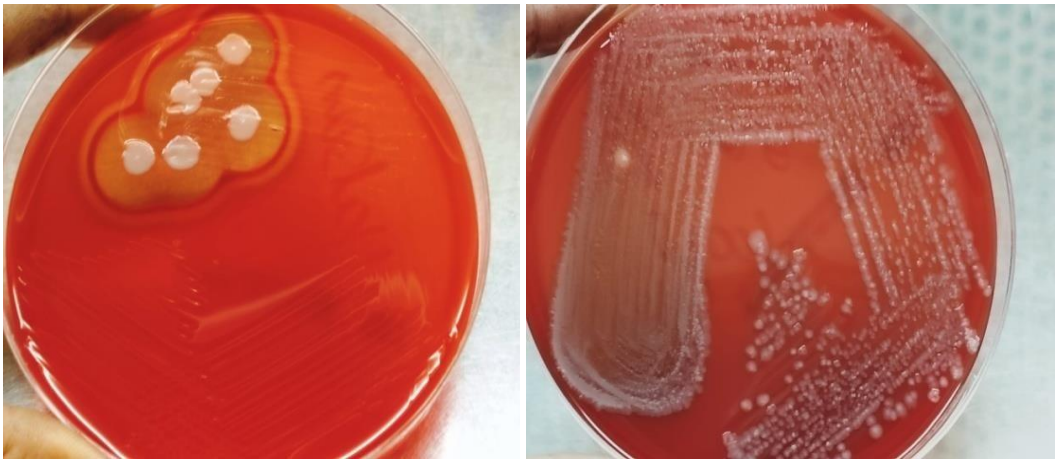


Fig. 1: Different Dilution of Milk Samples.

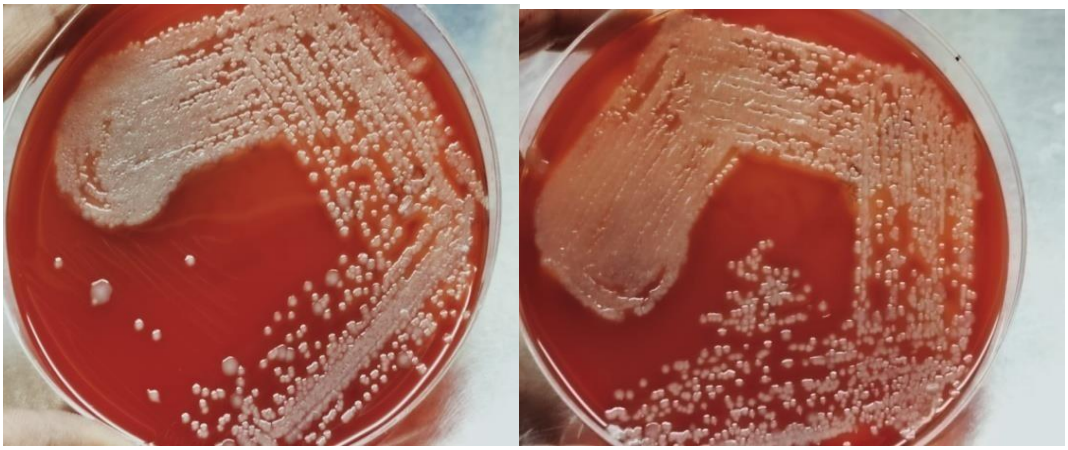


Sphinongomonas Paucimobilis Gram Positive Cocci Gram Negative Bacilli Gram Positive Bacilli

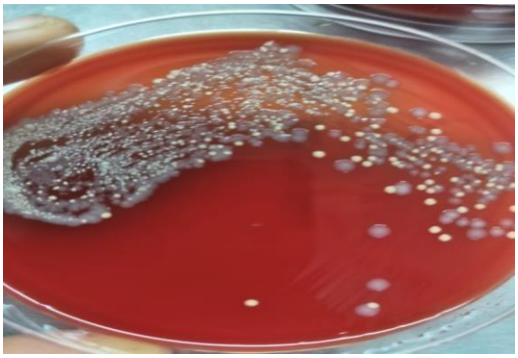
Fig. 2: Staining Images Of Isolated Bacteria.



E.COLI **ACINETOBACTER BAUMANII**



PSEUDOMONAS AERUGINOSA **KLEBSIELLA PNEUMONIAE**



STAPHYLOCOCCUS AUREUS

Fig 3: ***Isolated pathogens from different Milk sources.***

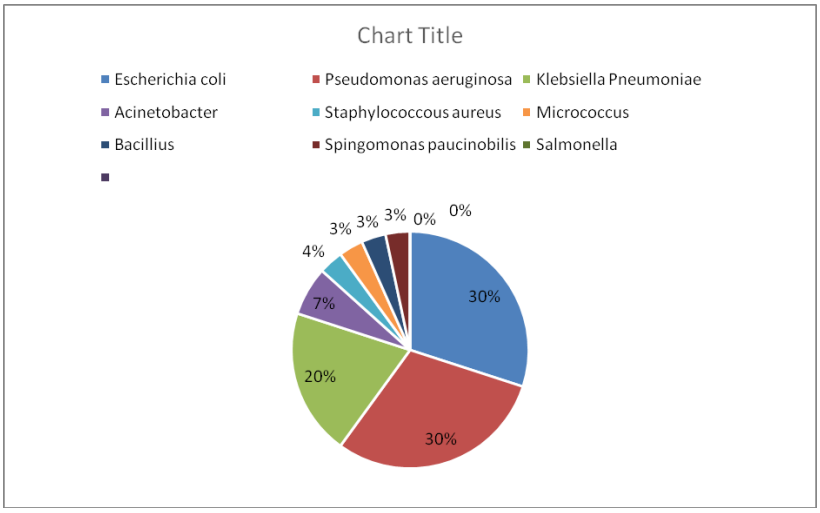


Fig. 4: Microorganisms isolated from 9 different varieties of Milk samples.

Biochemical Identification

From the 30 isolates, 5 organisms were identified using a gram strain and biochemical reaction *Staphylococcus aureus* is a gram positive coccus arranged in clusters, it has shown negative for indole and oxidase test and positive for methyl red, critrate, urease, catalase and coagulase, it produce acid slant and acid butt in TSI.

Escherichia coli is a gram negative rod, showed positive result for methyl red, indole and negative result for coagulase, catalase, urease, citrate and oxidase and produced acid slant and acid butt in TSI.

Pseudomonas aeruginosa is a gram negative bacillus showed negative result for indole, methyl red and coagulase and Positive result for citrate, oxidase and catalase, produced alkaline butt, alkaline slant in TSI.

Klebsiella pneumoniae is a gram negative baccilus, showed negative result for indole, oxidase and methyl red, positive result for urease, citrate, and catalase and produced acid butt and acid slant in TSI.

Acinetobacter baumanni is a gram negative cocco bacillus, it showed negative result for oxidase, indole, methyl red and urease positive result for catalase, citrate and produced alkaline butt and alkaline slant in TSI. (Table 1).

Table 1: Biochemical characterization of the isolated colony.

Properties	*Staphylococcu saureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Acinetobacter baumanii*
Indole	_	+	_	_	_
Methyl red	+	+	_	_	_
Citrate utilization test	+	_	+	+	+
Urease test	+	_	_	+	_

Table 2: Determination the quality of milk by MBRT method.

Triple sugariron	Acid /Acid	Acid/Acid	Alkaline /Alkaline	Acid /Acid	Alkaline/Alkaline
Catalase	+	+	+	+	+
Coagulase	+	_	_	_	_
Oxidase test	_	_	+	_	-
Gram stain	+	_	_	_	_

The methylene blue reductase test (MBRT) is performed to check the quality of milk through the disappearance of the color of methylene blue. The colour change from blue to white due to reduction reaction occurs very quickly in one hour itself for Raw milk whereas the it was about 5hrs and 45 mins for Thirumala milk and Arokya milk. All the other varieties of milk shown moderate timings for the color change, based on the time taken for color change quality of each milk sample determined was poor to excellent. (Table3)

4.0 DISCUSSION

The aim of this study is to assess the microbial quality of raw milk and pasteurized and to detect the incidence of different strains of bacteria. At the time of milking, milk has a low bacterial count but after milking the bacterial load increase due to various external and internal contamination. Milk is a highly nutritious food, ideally suited for growth of pathogenic and spoilage milking organism when suitable condition exists. Microbial load of milk is a major factor in determining its quality,

microbial analysis of processed and unprocessed milks, which are sold in milk dairies and rural venders.[13] Microbiological analyses of milk in the study detected the presence of pathogenic organisms like *E.coli*, *Pseudomonos aeruginosa*, *Klebsiella pneumoniae*, *Micrococcus, Lactobacillius*, *Spingomonas paucinobilis* and *Acinetobacter baumanii*. Based on the above results, various spp of bacteria were isolated from the 9 different varieties of milk samples. The samples are inoculated in two dilution of macconkey broth and selenite f broth. Single dilution of normal saline is used. Maintaining good quality of milk is a main challenge in dairy sectors worldwide, where production of milk and its products take place in unhygienic condition.[14] In the present study 1 cow milk and 8 pasteurized samples were collected from the rural vendors and milk dairy to checked milk quality by MBRT TEST and culturing milk samples into the plates and detect different types of bacteria were isolated. Out of one cow milk and eight Pasteur milk sample in selenite F broth shows no growth and other media shows growth. There are 30 bacterial colonies where selected from in 9 samples including 1 gram positive and 7 gram negative bacteria. However, in this study of bacteriological quality of raw milk and pasteurized milk is determined by MBRT method. The test is performed to check the bacteria contamination in milk it will visually indicate the presence of bacteria in a 9 milk samples, and it indicates the level milk quality. The bacterial count was analyzed by colony forming unit (cfu/ml) and results shown highly significant.

5.0 CONCLUSION

This study would be helpful to convey public about the health risk causing pathogens and to evaluate the safety of pasteurized and raw milk. So, there is an alarming need to followthe control measures to improve quality of milk by eliminating the pathogenic microbes.

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