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COMPARATIVE ASSESSMENT OF MILK BORNE PATHOGENS AND THEIR ANTIBACTERIAL SENSITIVITY

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ABSTRACT

Milk is considered as a highly nutritious and valuable human food, consumed by the people in a variety of different products. The present work is an effort to have a survey of number of bacteria present in different milk samples viz., Raw, Boiled, Pasteurized and Skimmed milk collected from local market of Haridwar and its adjoining areas by serial dilution method and the quality of milk assessed by MBRT. The highest mean count was detected in raw milk having 211±0.8 cfu/ml. Among the four types, boiled milk appeared as of best quality. The major microbial contaminants were found to be *Bacillus* sp, *Staphylococcus* sp, *Streptococcus* sp, *E. coli* and *Micrococcus* sp. During the present investigation the antibiotic sensitivity test was also performed against all the five genera isolated. It was found that inhibition zone of Chloramphenicol and Ofloxacin was maximum against *Streptococcus* sp. (24 mm) and *Staphylococcus* sp. (30mm).

INTRODUCTION: Cow's milk has been considered as a highly nutritious and valuable human food, and is consumed by millions daily in a variety of different products. Its nutrient composition makes it an ideal medium for bacterial growth and therefore, it can be considered as one of the most perishable agricultural products because it can be very easily contaminated [1, 2].

Many contaminating organisms not only spoil the product, but reduce its shelf life too. Some bacteria such as lactic acid bacteria, are useful in milk processing, causing milk to sour naturally but others such as *Staphylococcus aureus*, *Brucella* are pathogenic to man and can transmit disease if the milk is consumed untreated [3, 4].

Unlike meat and meat products, milk is less likely to be subjected to any subsequent heating by the consumer before consumption and contaminated milk is therefore, potentially more dangerous [5].

Raw milk of good hygienic quality is necessary to produce milk products of good quality and adequate shelf life to provide a safe, sound and wholesome food for the consumer. Since, milk is a liquid, it is in contact with some type of equipment or surface from the time it is drawn from the cow's udder till it is consumed.

Milk freshly drawn from a disease free udder contains small numbers of bacteria (500-1000 bacteria per ml) which derive from organisms colonizing the teat canal [2]. Milk quality starts to deteriorate immediately after milking due to bacteria entering the milk from a wide variety of sources.

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These bacteria may originate from soil, water and faeces that collect on the skin of the cow and unavoidably end up in the milk.

Once micro-organisms get into the milk they multiply readily. The speed at which milk quality declines depends on the hygiene of the milker, milk equipment and bulk tank, as well as the temperature and the length of time that milk is stored, before sale to the consumer or treatment at a factory [6].

Pathogenic bacteria may also be present in raw milk as a direct consequence of clinical or subclinical mastitis. In 1994, Giesecke *et al.,* [7] reported that subclinical mastitis was prevalent in at least 75.5% of South African dairy herds which were affected at levels ranging from moderate to very serious. Mastitis affects a variety of compositional parameters of milk which in turn may affect the dairy technological usefulness, the nutritional and hygienic characteristics of milk.

Among the organisms commonly producing mastitis, *Streptococcus agalactiae, Staphylococcus aureus* and *Escherichia coli* are pathogenic to man. There have been numerous outbreaks of milk borne disease in humans with pathogens such as *S.aureus, E.coli, Campylobacter spp., Salmonella spp., Listeria spp., and Yersinia spp*. Being indiscriminated during the past century, especially since mass production came into effect most of these outbreaks have occurred in raw milk [1, 8], but there have also been outbreaks of disease after consuming pasteurized milk due to failure in the pasteurization system or post - pasteurization contamination [9, 10].

Raw milk may contain micro-organisms pathogenic to man who originated either from within or outside the udder. Human carriers may also be the source of infection in milk – borne outbreaks, as reported for *Salmonella* infections and for cases of scarlet fever or septic sore throat due to *Streptococcus pyogenes* [1, 2].

Thus, despite their nutritional and health benefits, outbreaks of human infections associated with the consumption of fresh raw milk or minimally pasteurized milk and its products have increased in recent years. Bacteriologically safe milk and its products are essential to maximize the health benefit promised by adequate consumption of these produce.

Hence, the present work was undertaken to determine the microbial load of various milk samples in terms of cfu/ml, MBRT and antibiotic sensitivity of isolated milk borne pathogens.

EXPERIMENTAL:

Sampling: Four different types of milk samples viz. Raw, Boiled, Pasteurized and Skimmed milk were collected from local market of Haridwar and were analyzed directly for the microbial population.

Assessment of milk quality: Quality of milk was determined by Methylene Blue Reductase Test (MBRT). Each sample (Raw, Boiled, Pasteurized and Skimmed) was thoroughly mixed and 10 ml of each type of milk was transferred to sterilized labelled test tubes using sterilized pippetes. 1ml of methylene blue was added to each test tube, mixed, closed and incubated in a water bath at 37±2^{o}C for 6 hours.

Enumeration of bacterial population: Nine ml of distilled water was taken in the test tubes and sterilized at 121^{o}C for 15 minutes. After cooling 1ml of sample was transferred to first tube with 9 ml distilled water which is 10^{-1} dilution and mixed well. Now 1 ml of the sample was serially transferred into another tube containing 9 ml distilled water so as to prepare 10^{-2} and further dilutions. 1 ml from each tube was transferred to sterilized, labelled Petri plates. Media was poured and allowed to solidify. The plates were incubated at 37±2^{o}C for 24-48 hours.

After incubation, the colonies were counted by using colony counter, applying the formula:

Cfu/ml of milk = Number of colonies *dilution factor/weigh of milk (1 ml)

Isolation and identification of bacteria: Colonies isolated were further identified by using biochemical tests including Gram's staining, Catalase, Carbohydrate fermentation etc. Indole test, Methyl red test and Citrate utilization test were done to differentiate among groups of coliform bacteria.

Antibiotic sensitivity: Susceptibility of identified cultures were tested by using Kirby-Bauer method [11] using various antibiotic disc simultaneously at a given time to see the resistance of the microorganisms isolated thereby indicating their clinical significance.

RESULTS:

Milk Quality: In case of raw and pasteurized milk, the blue colour of the dye started reducing after 3 hours while in case of boiled milk and skimmed milk, the blue colour of the dye did not reduced till 6 hours (**Table 1**). By comparing the results with standard table for MBRT (**Table 2**), the milk samples were classified as of good and poor quality (**Table 3**).

Bacterial contamination: All the four samples of milk were found to have bacterial load. Among the four samples of milk, the highest mean count was detected in raw milk (211±0.8) followed by Pasteurized milk (200±1.7). Boiled milk and Skimmed milk were reported to have less mean count which has been demonstrated in **Table 4**.

Bacterial populations isolated from milk samples were identified on the basis of biochemical test (**Table 5**). The five most common bacterial genera encountered were *Bacillus*, *Staphylococcus*, *Streptococcus, Escherichia coli and Micrococcus sp.*

TABLE 1: OBSERVATION TABLE OF MBRT

Samples	Time interval (hrs)					
	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
Raw milk	-	-	-	+	++	+++
Boiled milk	-	-	-	-	-	-
Pasteurized milk	-	-	-	+	++	+++
Skimmed milk	-	-	-	-	-	-

(-) means blue colour of dye was not reduced (Good quality); (+) means blue colour of dye was reduced within 4 hours (Poor quality milk); (++) and (+++) means blue colour of dye was reduced within 5-6 hours (very poor quality milk)

TABLE 2: STANDARD TABLE OF MBRT

Methylene Blue Reduction Time (MBRT)	Classification of milk sample	Approximate no. of bacteria/ml
0 to 30 minutes	Very poor quality	>20,000,000
31 to 120 minutes	Poor quality	>4,000,000
121 to 360 minutes	Fair quality	>5,00,000
361 to 480 minutes	Good quality	<500,000.

TABLE 3: QUALITY OF MILK SAMPLES BY COMPARING IT WITH STANDARD TABLE OF MBRT

Samples	Methylene Blue Reduction Time (MBRT) in hrs.	Classification of Milk samples
Raw milk	Within 4 hrs	Poor Quality Milk
Boiled milk	More than 5 hrs	Good Quality Milk
Pasteurized milk	Within 4 hrs	Poor Quality Milk
Skimmed milk	More than 5 hrs	Good Quality Milk

TABLE 4: ENUMERATION OF BACTERIA PER DILUTION OF MILK

Milk Sample	Dilution	*No. of Colonies ± SEM
Raw Milk	10^{-1}	211±0.8
	10^{-2}	183±0.7
	10^{-3}	117±0.9
Boiled Milk	10^{-1}	112±0.8
	10^{-2}	72±1.7
	10^{-3}	52±1.2
Pasteurized Milk	10^{-1}	200±1.7
	10^{-2}	176±0.9
	10^{-3}	146±0.8
Skimmed Milk	10^{-1}	116±0.8
	10^{-2}	98±0.8
	10^{-3}	83±0.9

*Average of triplicates

TABLE 5: SHOWING BIOCHEMICAL TESTS FOR IDENTIFICATION OF BACTERIA

Biochemical test	Microorganisms				
	Bacillus sp.	***Staphylococcus sp.***	***Streptococcus sp.***	***E.coli***	***Micrococcus sp.***
Gram stain	Rod, +ve	Cocci, +ve	Cocci, +ve	Rod, -ve	Cocci, +ve
Catalase test	+	+	-	+	-
Lactose	-	A	A	AG	-
Dextrose	A	A	A	AG	-
Sucrose	A	A	A	A	-
Indole	-	-	-	+	-
MR	-	+	+	+	-
VP	+	+	-	-	-
Citrate	-	-	-	-	+

A-Acid Production; AG-Acid and Gas Production; + indicates positive; –indicates negative

Antibacterial activity: Antibacterial activity of antibiotic was measured by disc diffusion technique. In clinical laboratory, this technique is used to identify the antibiotic sensitivity of the pathogen. This test provides the knowledge about minimum inhibitory concentration (MIC) of antibiotic. The MIC is the lowest concentration of antibiotic which inhibits the growth of a given strain of microbe under controlled conditions. The size of zone of inhibition is inversely related to the MIC i.e., greater the zone of inhibition lesser the MIC and vice versa. The zone of inhibition of different antibiotics is tabulated in **Table 6**.

TABLE 6: ANTIBACTERIAL ACTIVITY OF ANTIBIOTICS AGAINST BACTERIA

Antibiotics	Zone of Inhibition (mm)				
	A	**B**	**C**	**D**	**E**
Chloramphenicol	22	19	24	17	23
Ofloxacin	29	30	21	22	25
Ciprofloxacin	20	27	24	22	24
Control	-	-	-	-	-

A- *Bacillus* sp.; B- *Staphylococcus* sp.; C- *Streptococcus* sp.; D- *E. coli*; E- *Micrococcus* sp.; (-) no growth

DISCUSSION: Milk is a major component in human diet all over the world, but it also serves as a good medium for growth of many microorganisms, especially pathogenic bacteria. Thus, the quality of milk is considered essential to the health and welfare of a community. Also, all cases of dairy illness continued to be of bacterial origin.

In the current study, different milk samples, were assessed for the presence of various milk borne pathogens. By detecting this bacterial load in the milk samples, it apparently gives an idea about the quality of the sample. Total mean count was found to be very high in all the samples analyzed. Among them, the highest mean count was found as 211±0.8 and 200±1.7 cfu/ml in raw milk and pasteurized milk respectively. Ali *et al*., [12] have also reported considerably higher level of total bacterial count in raw milk having 5.96 log cfu/ml. Also, the presence of pathogenic bacteria, *Staphylococcus aureus, Streptococcus* and coliform including *E.coli* which may be considered an indicator microorganism of feacal contamination [13] can produce many systematic infections after consumption makes it unfit for drinking.

The antibiotic sensitivity of the organisms isolated was checked by Kirby-Bauer method based on the standardized reference procedure for the disc system which were published by WHO and FDA and are updated by the NCCLS. The organisms were differently found to be sensitive to all tested antibiotics namely Chloramphenicol, Ofloxacin and Ciprofloxacin. Among them, inhibition zone of Chloramphenicol and Ofloxacin was maximum against *Streptococcus sp*. (24mm) and *Staphylococcus sp* (30mm).

CONCLUSION: On the basis of the data obtained in the present investigation, conclusion may be drawn that being an important food article like milk is consumed by a number of people in different varieties all over the world. Negligence in this area may result in serious contamination that ultimately represents a low quality product to the consumers. Possible reasons for the contaminants could be due to infected udders of the cows, unhygienic milking procedures or equipment or inferior microbiological quality of water used for cleaning utensils animals as well as the milk storage conditions [14]. So, the proper handling and processing of milk should be recommended before consumption of milk.

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