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Evaluation of Milk Borne Pathogens and Their Antibacterial Sensitivity

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ABSTRACT

In the present study 200 different samples *viz.,* raw milk,, boiled milk, pasteurized milk and skimmed milk were collected from local market of Durg, Rajnandgaon and Khiragad areas of Chattishgarh. All these milk samples were analysed for microbial count and antibiotic sensitivity test. Four types of bacteria were identified in 200 milk samples of three cities. The bacteria identified were *Streptococcus, Staphylococcus, Bacillus and E. Coli*. Highest mean count was detected in raw milk followed by pasteurized milk. In antibiotic sensitivity test, inhibition zone of Chloramphenicol and Ofloxacin was maximum against *Streptococcus* (26mm) and *Staphylococcus sp*.(30mm). The results indicated that analysed milk could contribute a potential risk for public health in the cases that if it was consumed or used in the production of dairy products without being pasteurized or being subjected to a sufficient heat process.

Key Words: Antibiotic sensitivity test, Methylene blue reductase, Milk borne pathogens.

INTRODUCTION

Despite the nutritional and health benefits, outbreaks of human infection associated with the consumption of fresh raw milk or minimally pasteurized milk or its products have increased in recent years. Milk freshly drawn from a disease free udder contains small numbers of bacteria (500-100 bacteria/ml) which derive from organisms colonizing the teat canal. Milk quality starts to deteriorate immediately after milking due to bacteria entering the milk from a wide variety of sources. Bacteria may originate from soil, water and faeces that present on the skin of the cow and unavoidable end up in the milk. Once micro-organism gets in to the milk they multiply readily. The speed at which milk quality deteriorates 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 treatment at a factory or sale to the consumer. There have been numerous outbreaks of milk borne diseases in humans with pathogens such as *S.aureus, E.coli, Campylobacter, Salmonella, Listeria and Yesinia sp.* Being indiscriminate during the past century, especially sick mass production came in to effect most of these outbreaks have occurred in raw milk (Bauer and Hermann, 1996), but there have also been outbreaks of disease after consuming pasteurized milk due to failure in the pasteurization system or post-pasteurization contamination. Hence, there is the need for instituting effective control measures to protect public health which includes mandatory milk pasteurization by traders and improved hygienic handling of the commodity during milking.

MATERIALS AND METHODS

Fifty milk samples for each of category *viz.,* raw milk, boiled milk, pasteurized milk and skimmed milk were collected from different places of local market of Durg, Rajnandgaon, Khiragad areas of Chattishgarh. All these two hundred milk samples were analysed for microbial count and antibiotic

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sensitivity test was also performed in the laboratory of ABIS dairy, Rajnandgaon. Quality of milk was assessed by Methylene Blue Reduction Test.

Methylene Blue Reduction Test

Quality of milk was determined by Methylene blue reductase test (Atherton, 1997). Each sample of raw, boiled, pasteurized and skimmed milk was thoroughly mixed separately and 10 ml of each milk sample was transferred to sterilized labelled test tube using sterilized pipet. One ml of Methylene blue was added to each test tube, mixed, closed and incubated in a water bath at 37±2°c for 6 hr.

Enumeration of bacterial population

Nine ml of distilled water was taken in the test tubes and sterilized at 121 °c for 15 minutes. After cooling 1 ml 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 in to plates. Media was poured and allowed to solidify. The plates were incubated at so as to prepare 37±2°c for 24-48 hr. 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 (1ml)

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 were done to differentiate among groups of coliform bacteria.

Antibiotic sensitivity Test

Susceptibility of identified culture were tested by using various antibiotics disc simultaneously at a given time to see the resistance of the micro-organisms isolated thereby indicating their clinical significance.

RESULTS AND DISCUSSION

All the four categories of milk samples were found to have bacterial load. The bacteria identified were *Streptococcus, Staphylococcus sp, Bacillus sp and E. Coli*. Highest mean count was detected in raw milk followed by pasteurized milk. Boiled milk and skimmed milk were reported to have less mean count which has been demonstrate in the sample testing. Bacterial population isolated from milk samples were identified on the basis of biochemical tests.

Milk quality

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

Table 1. Observation table of MBRT

Time interval (hr)

Sample	1 hr	3 hr	4 hr	6 hr
Raw milk	-	-	++	+++
Boiled milk	-	-	-	-
Pasteurized milk	-	-	+	+++
Skimmed milk	-	-	-	-

(-) means blue color dye was not reduced (good quality);
(+) means blue color of dye was reduced within 4 hours (poor quality milk);
(++) and (+++) means blue color of dye was reduced within 6 hours (very poor quality milk).

Table 2. Quality of milk samples by comparing it with standard table of MBRT.

Sample	Methylene blue reaction time	Classification of milk samples
Raw milk	Within 4 hr	Poor quality of milk
Boiled milk	More than 5 hr	Good quality of milk
Pasteurized milk	Within 4 hr	Poor quality of milk
Skimmed milk	More than 5 hr	Good quality of milk

Table 3. Number of bacterial colonies per dilution of milk.

Dilution	Raw milk	Boiled milk	Pasteurized milk	Skimmed milk
10^{-1}	210±0.12	110±0.8	192±1.3	110±0.8
10^{-2}	200±0.6	72±1.5	179±0.1	96±0.8
10^{-3}	119±0.1	50±2.2	141±0.3	81±0.9

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 pathogen. This test provides the knowledge about minimum inhibitory concentration (MIC) to antibiotics. The MIC is the lowest concentration of antibiotics which inhibits the growth of given strain of microbes under controlled condition. 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 shown in Table 4 .

Table 4. Antibacterial activity of antibiotics against bacteria.

	Zone of inhibition in (mm)			
Antibiotics	A	B	C	D
Chloramphenicol	22	19	25	17
Ofloxacin	22	30	21	22
Ciprofloxacin	20	27	25	22
Control	-	-	-	-

A-Bacillus sp; B-Staphylococcus sp; C Streptococcus sp; D-E Coli, (-) no growth

Raw milk is considered as highly nutritious food and serves as an ideal medium for bacterial growth. Several factors contributes to milk contamination such as poor hygienic milking conditions, contaminated equipments, milking utensils and milk handler's poor personal hygiene. (Nan Li *et al,* 2018). High incidence of bacterial infection in market milk may be due to that bulk farm milk is transported directly to the dairy plant for processing while market milk is usually collected from small farm of farmers therefore it will be liable to cross contamination by different ways as mixed fresh clean milk with unclean milk by hands of workers, container of transportation or contaminated water used for cleaning utensils could be source of contamination (Murphy and Boor, 2000).

In 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 analysed. 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* (2010) also reported considerable higher level of total bacterial count in raw milk having 5.96 log cfu/ml. The presence of pathogenic bacteria, *Staphylococcus aureus, Streptococcus and colliform* including *E coli* which may be considered an indicator micro-organism of faecal contamination (Thomson *et al,* 1971) and can produce many systemic infections after consumption makes it unfit for drinking.

The antibiotic sensitivity of the organisms isolated was checked by Kirby-Bauer method based on the standard reference produced 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* (25 mm) and *Staphylococcus sp* (30mm).

CONCLUSION

From the study it was found that microbial loads of raw milk and pasteurized were not satisfactory. Presences of *Bacillus, Staphylococcus,*

Streptococcus and E. coli were also of importance from the angle of public health, therefore measures should be taken to maintain the minimum legal standards and farmers should be trends in aspects of clean milk production, so that milk reaches to the consumers.

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