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MICROBIAL EXAMINATION OF MILK SAMPLE FROM NAGPUR REGION WITH REFERENCE TO COLIFORM

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Abstract- The study was undertaken to investigate the microbiological quality of raw milk samples and pasteurized samples were used to study from surrounding area of Nagpur and Wardha district, 10 raw sample and 10 pasteurized samples used. Bacteriological identification reveals a definite dominance of *E. coli and E. aerogene* sp. beside its, the other genera *Salmonella typhi* were isolated on selective agar medium. The microbial colonies were found to be high in six sample and colony content low in rest four sample. In pasteurized sample the coliform colonies were less in number as compared to raw milk sample. Bacteriological colonies were found to be opaque and metallic sheen in coloured prepared. Biochemical test were used for the of identified *Escherichia coli and Enterobacter arogene*.
Key words- Raw milk, Pasteurized milk, Methylene blue reduction test, Standard plate count, Coliform

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Introduction

Milk is an essential part of diet for the expectant mothers as well as growing children. Milk being nutritious food for human being, also serves as a good medium for the growth of many microorganism, especially *E. aerogenes specious* [8], Bacterial contamination of raw milk can originated from different sources such as air, milking equipment, feed, soil, faeces and grass. The number and type of microorganism in milk immediately often after milking are affected by a different factors such as animals and equipment cleanless, season feed, animal health.

It is hypothesized that differences in feeding and housing statergies of cows may influence the microbial quality of milk. Rinsing of milking machine and milking equipment with a unclear water may also be one of the reason for the presence of number of microorganism including pathogen in a raw milk [4]. The presence of these pathogenic bacteria in a milk often emerge as a as a major public health concern, especially for those individuals. Keeping a fresh milk at an a elevated temperature together with unhygienic practices in the milking process may also results in microbiological inferior quality.

Coliform (*Escherichia coli)* are frequently used in the microbiological analysis of milk is essential to find the degree of contamination with diction and enumeration of indicator organism. The colliform bacteria are able to grow well in a variety of substrate and to utilize a number of carbohydrate and some other organic compound as a food for energy and number of fairly simple nitrogenous compound as a source of nitrogen [9].

Milk and dairy product harbour a naturally microbial flora and other microorganism, which vary within the wide range of product available on market. The origin of contamination by pathogenic bacteria, various within the product and a mode of production and processing. Contamination of milk and dairy product by pathogenic microorganisms can be an endogenous origin, following excretion from the udder of an infected an animal, through the direct contact with infected herds or through the environment [10]. Treatment and processing of milk an inhibitor encourage the multiplication of microorganism. The bacteria most frequently involved coliform. Nagpur and wardha region is suitabled in the center of India. Nagpur having a zeromile stones of India. The present study has been designed to asses, milk quality with reference to coliforms.

Materials and Methods

Sample Collection

The 10 raw samples were collected from (Cow and Buffelo), milk vendor and 10 Pasteurized milk sample were collected from (Milk vendor, milk society shops, processing unit etc), in a sterile screw cap bottle. These sample collected from Wardha and Nagpur region. These sample were serially diluted, It spread on Eosine methylene blue media. Bismuth sulfide agar media for isolation of *E.coli, Enterobacter aerogen and S.typhi.*

Isolation of Microorganisms from Milk Sample

The milk sample were mix well before dilution. The samples were serially diluted in 1:1000 by using sterilized phasphate buffer wa-

ter. The diluted sample 0.1 ml spread on the Eosine methylene blue agar, which inhibits gram positive bacteria [1], Gram negative lactose fermentation (coliform) thats grow in this medium will produce "nucleated colonies" (dark center) colonies of *Escherichia coli and Enterobacter aerogene* can be differentiated on the basis of size and the presence of greenish metallic sheen.
After solidification the plate were incubated at 37^{0}C for 24 hour. After incubation the colonies were counted by standard plate count method [5] and the result were recorded.

Methylene Blue Reduction Test
1ml of methylene blue (1:2500) is added to 10 ml of milk. The tube were sealed with rubber stopper and slowly inverted 3 time to mix. Tubes were place in water bath at 35^{0}C and examine at interval upto 6 hour. Thn check the time to change for methylene blue to become colourless is the methylene blue reduction time (MBRT). The shorter the MBRT lower the quality of milk.The grading of milk sample on the basis of methylene blue reduction test in different milk samples are raw milk sample.

Preservation Test of Microorganism
Eosine Methylene Blue agar slant were used for maintaining pure culture of microorganism for subculturing purpose. Agar slant provided more surface area for growth of *E.coli* and easily stored transfer than petriplate. In a slant slope surface was easier for streaking than horizontal surface. preserve microorganism 4^{0}C.

Identification of Microorganisms from Milk Product
Identification was based on growth on Eosine Methylene Blue agar.
Colony morphology, Gram's reaction, Gram staining, Acid fast staining, Negative staining, Endospore staining, Capsule staining, Motility, as well as the biochemical test were used to indetify coliform are:-
Indol production test [2]
Methylene red test [3]
Vogous proskear test [7]
Carbohydrate fermentation test
Lactose fermentation test,
Citrate utilization test,
Urease test,
Hydrogen sulfide test, [6]
Starch hydrolysis test,

Results and Discussion
The microflora of raw cow milk is differences among milk from different region of cow raw milk were studied. The variation of microflora of raw milk and pasteurized milk are observed. The coliform were detected of structural morphology and biochemical test.

Table 1- Grading of milk samples on the basis of methylene blue reduction test (in different milk sample)

Quality of Milk	Decolourization Time
Excellent	More than 8 hour
Good	Between 6 hour and 8 hour
Fair	Between 2 to 6 hour
Poor	Less than 2 hour

Table 2- Enumeration of microorganisms in different milk sample by standard plate count method

Sample number	Colony Forming Unit (CFU)	
	Raw milk (1/10,000)	Pasterurized milk (1/10,000)
N-1	213	26
N-2	156	16
N-3	162	34
N-4	100	17
N-5	180	66
N-6	240	60
N-7	340	44
N-8	200	61
N-9	280	62
N-10	300	74

Table 3- Decolorization time and grading of milk sample collected from different part of Nagpur regions

SAMPLE	Raw milk		Pasteurized milk	
	Decolourization Time	Grade	Decolourization Time	Grade
N-1	1.30 h	Poor	7.00 h	Good
N-2	3.00 h	Poor	7.30 h	Good
N-3	2.50 h	Poor	12.00 h	Excellent
N-4	4.00 h	Good	12.54 h	Excellent
N-5	3.00 h	Good	2.00 h	Poor
N-6	1.00 h	Poor	7.00 h	Good
N-7	2.00 h	Poor	1.00 h	Poor
N-8	1.30 h	Poor	8.00 h	Good
N-9	1.00 h	Poor	2.00 h	Poor
N-10	1.30h	Poor	2.30 h	Poor

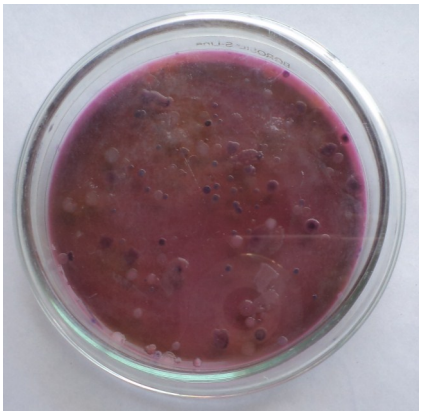

Fig. 1- Microbial colonies on Eosine Methylene Blue Agar plate (Raw milk sample)

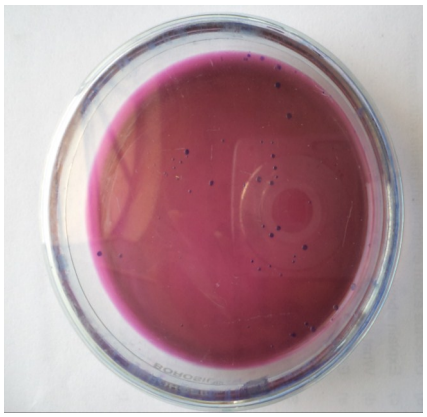

Fig. 2- Microbial colonies Eosine Methylene Blue Agar plate (pasteurized sample)

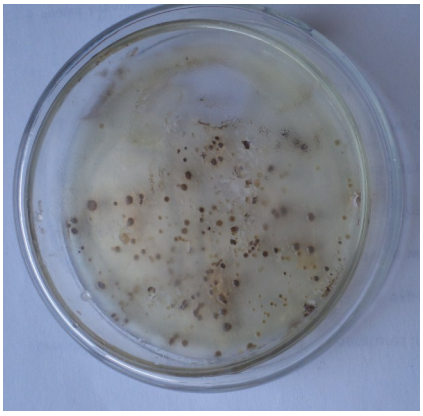

Fig. 3- Microbial colonies on Bismuth Sulphite Agar plate. (Raw milk sample)

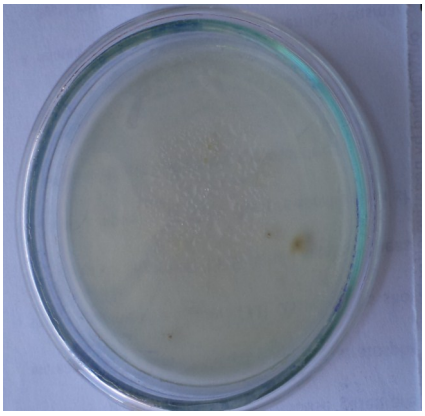

Fig. 4- Microbial colonies on Bismuth Sulphite Agar Plate. (Pasteurized milk sample)

Discussion

Out of 10 Raw milk sample, the microbial colonies were found to be high in six sample. (N-1, N-2, N-6, N-7, N-9, N-10) Table 2. and colonies content were low in four sample (N-3, N-4, N-5 N-8). Table 2. In pasteurizedm milk sample the colonies were low in 5 samples (N-1, N-2, N-3, N-4, N-7). Table 2. The methylene blue reduction test perform for raw milk sample reveal that out of 10 sample. The seven samples showed poor quality and two sample are good quality.

Out of 10 pasteurized samples four sample were poor quality and four sample are good quality and two sample are excellent.

The raw milk contained higher number of microflora a broadly due to contamination from animal Fig. 1. Bacteria are found in manure, soil and water may enter milk due to dairy utensils and milk contact surface. If the milk contact surface inadequetly cleaned, bacteria may develope in large number. Present study showed that 60% of the raw milk sample were of poor category. But in case of Pasteurized milk sample 100% of sample of good quality due to killing of contamination of microorganism by pasteurization. Fig. 2. Bacteria cololnies were found to be opaque and metallic sheen in a colour raw milk samples. The bacteria were found to be Gram negative in nature and were small straight rod. Light microscopic observation reveal the motile nature of these bacteria. Biochemical test showed that the bacteria were Indol positive, Methyl red positive, Voges-Proskaur- *E.coli* negative*, Entertobacter aerogene* positive, Citrate utilization- *E. Coli* negative, *Enterobacter aerogene* positive, Glucose ferment test positive, Lactose fermentation test positive, Starch hydrolysis test positive, Urease test negative.

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