RESEARCH ARTICLE

Isolation and identification of microorganisms from cow milk

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ABSTRACT

Milk being an important source of nutrition, its quality hygiene is the prime factor from communal view point. The present study was undertaken to estimate the incidence of opportunistic pathogens in cow milk from various dairies in Meerut city. 10 milk samples were collected from different dairies & were inoculated on the relevant bacteriological media. The quality of milk was assessed by viable bacterial count by Standard Plate Count (SPC) method, Mastitis test, and Methylene Blue Dye Reduction (MBRT) test. The SPC shows that large no. of microorganisms was present in the milk samples. The MBRT test performed for cow milk samples shows that out of ten samples, 3 samples were of poor quality & 7 were of fair quality. Mastitis test was performed which showed that 3 samples were infected. The bacterial identification was carried on differential media which shows the presence of different bacterial strains including *E. coli, E. aerogenes, S. aureus, M. luteus P. aeruginosa*. Further these were confirmed by various biochemical tests. The presence of these pathogens shows that the hygiene practices are poor & hence adequate sanitary measures should be taken at stage of from production to consumption.

Key Words: *Mehtylene Blue Dye Reduction (MBRT) test; Standard Plate Count (SPC); Milk quality; Bacteria isolation and identification*

INTRODUCTION

Milk is an important food of diet of vast population on earth, due to its high nutritional value for human beings. Milk is an excellent growth medium of microorganism when suitable temperature exists. If it is produced un hygienically and handled carelessly, it gets contaminated very easily leading to its early spoilage. Many milk-borne epidemics of human diseases have been spread by contamination of milk by spoiled hands of dairy workers, unsanitary utensils, flies and polluted water supplies. The same thing can be said for improper handling of foods in the home, restaurants, hospitals and other institutions.

The quality of milk is determined by aspects of composition and hygiene. Due to its complex biochemical composition and high-water activity milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms.

The contamination of milk and milk products is largely due to human factor and unhygienic conditions. Usually, milk gets contaminated with different kinds of microorganisms at milk collecting places. Approximately 50% of the milk produced is consumed as fresh or boiled, one sixth as yoghurt or curd and remaining is utilized for manufacturing of indigenous varieties of milk products such as Ice cream, Butter, Khoa, Paneer, etc. The manufacture of these products is based on traditional method without any regard to the quality of raw material used and the hygienic quality of the products. Under such conditions many microorganisms can find access to the milk products.

Microbiological examination of milk is essential to find the degree of contamination and enumeration of indicator organisms. The coliform bacteria are able to grow well in a variety of substrates and to utilize a number of carbohydrates and some other organic compounds as food for energy and a number of fairly simple nitrogenous compounds as a source of nitrogen. Coliforms are considered as normal flora of intestinal tract of human and animals [1]. They have been used as indicator organisms for bacteriological quality of milk and its products. The present study has been designed to assess the milk quality of different milk animals with special reference to coliforms.

Coliform count is always being taken as a definite index of fecal contamination of milk and its products, that besides the possible presence of enteric pathogens which may constitute health hazards to the consumers. The most important index of microbiological quality is total bacterial count, coliforms, yeast and molds count and detection of specific pathogens and their toxins.

Among all micro-organisms *Escherichia coli* is frequently contaminating organism in food and is reliable indicator of fecal contamination and generally present due to insanitary conditions of water, food, milk and other dairy products. Recovery of E. coli from food is an indicative of possible presence of enter pathogenic or toxigenic micro-organism which could constitute a public health hazard. Coliforms particularly Escherichia coli are frequently used in the microbiological analysis of food as an indicator of poor hygienic condition.

DIFFERENT BACTERIAL STRAINS FOUND AS CONTAMINANTS IN MILK

Bacillus cereus produce a toxin that can cause diarrhea and vomiting. The spores of *B. cereus* are heat-resistant and may survive pasteurization. There have even been very rare cases linked to dried milk and dried infant formula. *Brucella* is bacterial microbe that is found in unpasteurized dairy products. Brucella infection, or Brucellosis, has also been called "undulant fever" because of the regular recurrence of fever associated with the disease. *Campylobacter jejuni* is the most common bacteria to cause diarrheal disease in the U.S. and is found in raw milk and poultry. It has an increased chance of causing disease when consumed in milk, because the basic pH of milk neutralizes the acidity of the stomach.

S. aureus can cause disease Skin infections, food poisoning, and achieves this by generating toxins in the human body. Its incubation period lasts one to six hours with the illness itself lasting from 30 minutes to 3 days. Preventive measures one can take to help prevent the spread of the disease include washing hands thoroughly with soap and water before preparing food. Stay away from any food if ill, and wear gloves if any open wounds occur on hands or wrists while preparing food. If storing food for longer than 2 hours, keep the food below 40°F or above 140°F (4.4 or 60°C).

Enterobacter aerogenes can cause gastrointestinal infections, Urinary Tract Infections (UTIs), skin and soft tissue infections, respiratory infections, and adult meningitis.

E. coli 0157:H7 particular strain of *E. coli* has been associated with a number of food-borne outbreaks and is the cause of bloody diarrhea, frequently associated with dairy cattle, microbial contamination of raw milk and soft cheeses can result in disease.

Listeria monocytogenes is a common bacterial pathogen that is found in soft cheeses and unpasteurized milk. It can even survive below freezing temperatures and can therefore withstand refrigeration. It is particularly dangerous to individuals who have weakened immune systems, including

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pregnant women, AIDS patients, and the very young and very old.

Mycobacterium tuberculosis cause of "consumption," a horrific wasting disease that first affects the lungs, *Mycobacterium bovis* is associated with consumption of raw milk and was one of the most common contaminants prior to the practice of pasteurization. *M. bovis* causes tuberculosis in cows and can be passed to humans via unpasteurized cow's milk, causing a disease that very similar to *M. tuberculosis*.

Salmonella contamination of raw milk and milk products has been the source of several outbreaks in recent years. Symptoms include diarrhea and high fever.

The hands of a person milking cows can become contaminated with mastitis-causing pathogens, either from handling dirty equipment or from contact with contaminated milk from infected cows. Some microorganisms prefer living and growing on skin, whether it is the cow's teat skin of the milker's hands. Today, most milking operations will have the milkers wear disposable latex gloves. These are replaced periodically through the milking process [2].

Literature review

Cow's milk is considered one of the most perishable agricultural products because it is so easily contaminated with bacteria. Its nutrient composition makes it an ideal medium for bacterial growth, and therefore it can be highly susceptible to spoilage in the home and commercial production process.

Fahey et al. told that the correct pasteurization reduces the prevalence of disease generally associated with raw milk, especially raw milk produced and handled under unhygienic condition [3]. The most important control measures to ensure milk safety are proper pasteurization and avoiding post-pasteurization contamination.

Milk is heated to a temperature sufficient to kill all pathogenic bacteria and most spoilage organism's contamination has taken place. Holsinger et al. said that pasteurization is the most common process used to destroy bacteria in milk [4].

Raw milk may contain microorganisms pathogenic to man which originate either from within or outside the udder. Fahey et al. observed the most of the outbreaks of disease after consuming pasteurizations or post pasteurization contamination in a study of cows and their milk [3].

Adesiyun et al. showed that in the Survey of raw milk samples in Trinidad, they were of a poor bacteriological quality [1]. Between 20% and 75% of milk samples tested positive for *E. coli* and between 94% and 100% contains *S. aureus.*

Raw milk supports the growth of several pathogenic microorganisms that can lead to spoilage and infections in consumers [5]. Raw milk is colonized by a variety of zoonotic foodborne pathogens such as *Campylobacter jejuni, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus and Yersinia enterocolitica*.

MATERIALS AND METHODS

Collection of samples

All samples were collected under aseptic conditions in sterile containers and transported into ice boxes and processed immediately after delivery to the laboratory.

Determination of pH

The pH of all the samples was checked by measuring through digital pH meter.

Methylene Blue Reduction Test (MBRT)

Methylene blue test is a rapid and an inexpensive way of indicating quality of milk that had been unrefrigerated. In this test, methylene blue, which is colour sensitive to oxygen concentration, is added to the milk. This indicator is blue in the oxidized state and leuco or white in the reduced condition. Grading of milk samples on the basis of MBRT in different samples of Cow milk was done.

Conformational test for mastitis

Chloride test: Take 1 ml of milk in test tube and add 5 ml silver nitrate solution and add two drops of potassium chromate solution Observe for the change of color. If yellow color will appear it indicates positive cases of mastitis and if brownish red color will appear indicates no mastitis.

Standard Plate Count (SPC)

It is a routine procedure used to determine the number of microorganisms in milk. It is an agar plate method for estimating population of bacteria. Serial dilutions were prepared of milk samples and 1ml of each dilution was transferred to petri plate. Melted cooled agar medium was added to the plate and rotated gently. All the plates were incubated at 38°C for 48 hours.

Isolation of microorganisms

Milk samples were collected from different dairies for isolation of microorganisms. The samples were stored under refrigerated condition.

PROCEDURE

Milk samples were aseptically opened. During the mean time the Nutrient Agar Media (NAM) was prepared followed by autoclaving and poured into the petri plates which are then allowed to solidify. The streaking of each sample was done on the solidified media. The plates were incubated at 37°C for to 48 hours. After incubation, the plates were observed with distinctive colonies. The slants were made of each sample and incubated at 37°C for 18 to 24 hours. The slants of each sample were preserved in freezer under -20°C for further examination. The morphology of different colonies was noted.

Identification of microorganisms

Microscopic examination

To identify the culture, first of all the cultures were examined microscopically. It was done by gram staining. First of all, the air-dried smear of bacteria on slide was stained with crystal violet for 30 seconds followed by washing with distilled water (gentle stream) to remove excess stain. Smear was then stained with iodine (mordant) for 60 seconds follow by addition of 95% ethyl alcohol drop by drop for 10 seconds -20 seconds. Washing with distilled water was followed by counterstaining with safranin for 30 seconds. The gram-positive bacteria stain violet whereas the gram-negative bacteria stain pink.

Identification of the selected culture

At very first instant, grow the bacteria on the Nutrient agar media. After that streaking on different selective media of various above-mentioned samples. After culturing put the samples in incubator at 37°C. After that growth appeared on petri plate colonies were identified with the following methods.

Growth of samples on Eosin Methylene Blue Agar (EMB)

Now these samples were streaked on other media name Eosin-methylene blue agar and then the petri plates were kept for incubation at 37°C for 24-48 hours and growth of the colonies were observed.

Growth of samples on Mannitol Salt Agar (MSA)

These samples were streaked on Mannitol salt agar and then the petri plates were kept for incubation at 37°C for 24 hours -48 hours and growth of the colonies were observed.

Biochemical tests

IMViC test

This biochemical test consists of a combination of tests that is used for the identification of bacteria in coliform group. The tests include: indole test, methyl red test, Voges Proskauer test and citrate utilization test.

Indole test

The test organism is inoculated into (1%) tryptone broth, a rich source of the amino acid tryptophan. Indole positive bacteria produce tryptophanase, an enzyme that cleaves tryptophan, producing indole and other products. When Kovac's reagent (p-dimethyl amino benzaldehyde) is added to a broth with indole in it, a dark pink color develops.

The indole test must be read by 48 hours of incubation because the indole can be further degraded if prolonged incubation occurs. The Kovac's reagent is added after 48 hours of incubation.

Methyl red and Voges-Proskauer tests

Preparation of MRVP broth tubes: Inoculate the tubes with the selected bacterium and keep one tube as uninoculated comparative control. Incubate all the tubes for 48 hours. Then add five drops of methyl red indicator to the tubes of each Set. Observe the change in colour of methyl red for MR test. Add 12 drops of V-P reagent and 2 drops-3 drops of V-P reagent 2 to

the other set of tubes as well as to control tube. Shake the tubes gently for 30 seconds with the caps off to expose the media to oxygen. Allow the reaction to complete for 15 minutes -30 minutes. Observe the tubes for change in colour for the VP test.

Fermentation of different carbohydrates

Fermentative degradation of various carbohydrates such as Glucose, Sucrose, Lactose, and Dextrose by microbes under aerobic conditions is carried out in fermentation tube. For the preparation of media, add 20g of sugar in 800ml of fermentation media. Phenol red was used as an indicator. Broth taken into fermentation tubes is autoclaved at 121°C and at 15 psi pressure for 15 minutes. Inoculate sugars fermentation broth with each bacterium and keep one inoculated tube of each fermentation broth as a comparative control. Incubate the culture at 37°C and observed the change in color and gas production.

Catalase test

The catalase test was performed as per slide method. Using an inoculating needle, culture from a well isolated colony was placed onto a clean glass slide. A drop of 3% hydrogen peroxide solution was added to this culture and closely observed for the evolution of bubbles.

RESULTS

Table 1 gives the complete information about the pH, reduction time and the quality of the milk samples collected from different regions of the city, Meerut.

The pH has been determined using digital pH meter. The reduction test is based on the oxidation-reduction (O/R) activities of the bacteria present in the milk. The more bacteria present, the faster the reduction. This is determined by the MBRT test (Figure 1).

Conformational test (chloride test) had been performed to check for mastitis. This test was conducted in all the milk samples by adding 5 ml silver nitrate solution and adds two drops of potassium chromate solution of the milk sample.

C-A, C-E & C-H Milk samples showed positive for the mastitis test. While the other samples were negative for the mastitis test. Then the plates were examined for viable bacterial colonies & the number of colonies on each plate was counted by using Quebec Colony counter (Figures 2 and 3 Table 2). The SPC of milk gives the indication of total no of aerobic bacteria present in milk, at the time of pickup.

The different types of colonies from the SPC plates were picked and transferred on the slants for further identification. Different types of colonies were observed on SPC plates from each sample (Table 3). Different types of isolates were isolated on nutrient agar medium and maximum no of isolates were seen.

All the 41 isolates were studied for their gram reaction properties and cell morphology using gram staining techniques. When the slides were observed under compound microscope with immersion oil then the following results were observed, shown in table 3.

TABLE 1
Data of the collected samples: different r egions o f t he city (Meerut, Uttar Pradesh, India), pH, reduction time and quality of milk.

Sample no.	Collection area	pH	Reduction time	Quality of milk
C-A	Ganga Nagar	7.17	2.5 hours	Fair
C-B	Meerut Cantt	6.86	5.0 hours	Fair
C-C	Lohiya Nagar	6.92	5.0 hours	Fair
C-D	Garh Road	6.72	5.5 hours	Good
C-E	Shastri Nagar	7.32	4.5 hours	Fair
C-F	Rishi Nagar	6.82	4.0 hours	Fair
C-G	Mangal Pandey Nagar	6.71	1.5 hours	Poor
C-H	Partapur	6.8	4.0 hours	Fair
C-I	Kanker Khera	7.01	5.5 hours	Good
C-J	Jagriti Vihar	6.68	3.0 hours	Poor

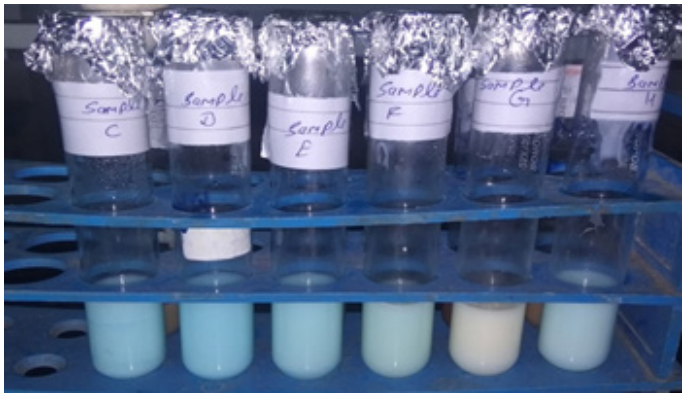

Figure 1) *Methylene blue reduction test*

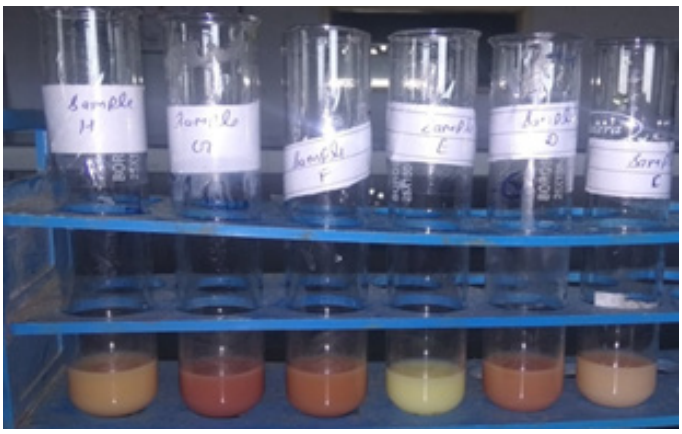

Figure 2) *Results of mastitis test*

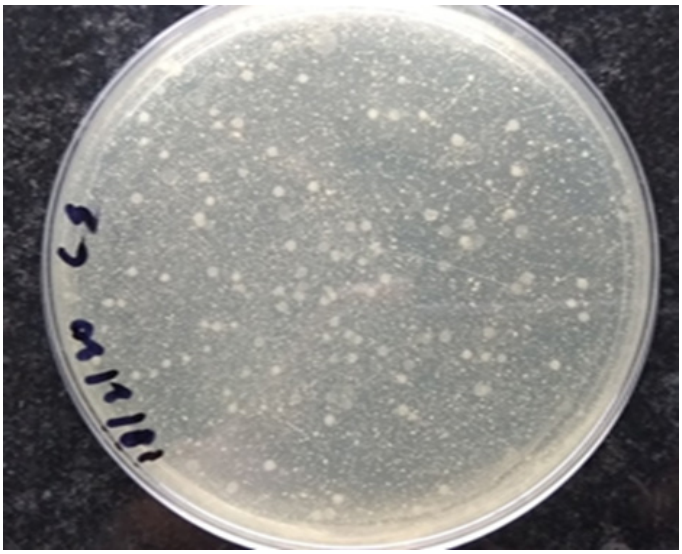

Figure 3) *Standard plate count: plate showing bacterial colonies present in milk sample (C-8) of 10-4 dilution*

TABLE 2
Results of mastitis test and serial dilutions

Sample no.	Mastitis	Number of colonies (cfu/ml)		
		10-2	10-4	10-6
C-A	+	438	256	107
C-B	-	310	184	85
C-C	-	336	171	82
C-D	-	354	156	77
C-E	+	652	434	309
C-F	-	454	208	109
C-G	-	465	234	146
C-H	+	667	484	313
C-I	-	250	146	76
C-J	-	354	157	94

TABLE 3
Different samples (C-A to C-J) containing different type of colonies (sub-samples)

Sample no	Sub sample no.	Gram Staining	Cell Morphology
C-A	A1	-	Rod
	A2	-	Rod
	A3	+	Rod
	A4	-	Cocci
	A5	+	Coccus
	A6	+	Rod
C-B	B1	+	Rod
	B2	_	Coccus
	B3	_	Rod
	B4	+	Rod
	B5	_	Cocci
	B6	_	Rod
C-C	C1	_	Cocci
	C2	+	Coccus
	C3	_	Rod
	C4	+	Coccus
	C5	+	Rod
	C6	+	Coccus
C-D	D1	+	Coccus
	D2	+	Rod
	D3	+	Coccus
	D4	+	Coccus
	D5	+	Rod
	D6	+	Rod
C-E	E1	_	Rod
	E2	_	Rod
	E3	_	Rod
	E4	+	Coccus
C-F	F1	+	Rod
	F2	+	Coccus
C-G	G1	+	Coccus
	G2	_	Rod
C-H	H1	+	Rod
	H2	_	Rod
	H3	_	Rod
	H4	+	Cocci
C-I	I1	+	Rod
	I2	+	Coccus
C-J	J1	_	Rod
	J2	+	Coccus
	J3	+	Cocci

On the basis of gram staining, microorganisms were classified as gram positive and gram negative and accordingly microorganisms were also classified as cocci and rods (Figure 4).

It was found that out of 41 isolates, 26 isolates were gram positive and 15 isolates were gram negative, out of which 10 isolates were gram positive rods and 13 isolates were gram positive cocci and the remaining isolates were negative rod.

Growth on EMB

EMB is selective-differential plating medium for the detection and isolation of gram- negative bacteria. It is especially used in isolation of coliforms. Growth of gram-positive bacteria is inhibited by the eosin and methylene blue dyes in the media. Metallic sheen was observed in sample no. C-A1, C-B3, C-B5, C-D4, which confirms the presence of *E. coli* in these samples. In sample no C-A3, C-C5, pink colonies with blue center dot were seen which shows the presence of *Enterobacter aerogenes* in these samples. Metallic sheen was observed in sample no. C-H2 and C-J1 which confirms the presence of *E. coli* in these samples. In sample no. C-G2 and C-H3 Pink colonies with blue center dot were seen which shows the presence of Enterobacter aerogenes in these samples (Figure 5 and Table 4).

Growth on mannitol salt agar

MSA contains a high concentration (˜7.5%-10%) of salt (NaCl), making it selective for gram positive bacterium, since this level of NaCl is inhibitory to most of other bacteria. It is also a differential medium for mannitol fermenters, containing mannitol and the indicator phenol red. In sample no C-A1, C-A2, C-A5, C-B1, C-B5, C-C1, C-C5, C-D2, C-D5, Yellow colour

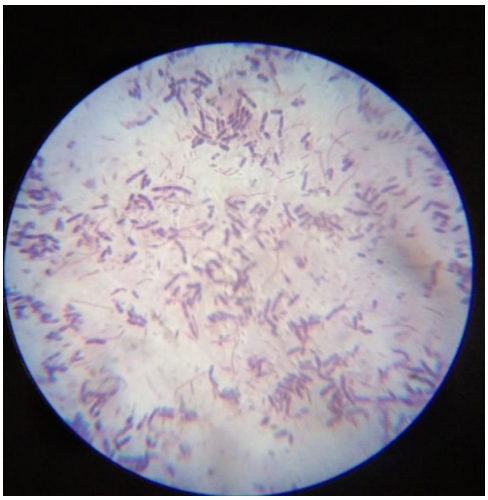

Figure 4) *Gram staining of microorganisms*

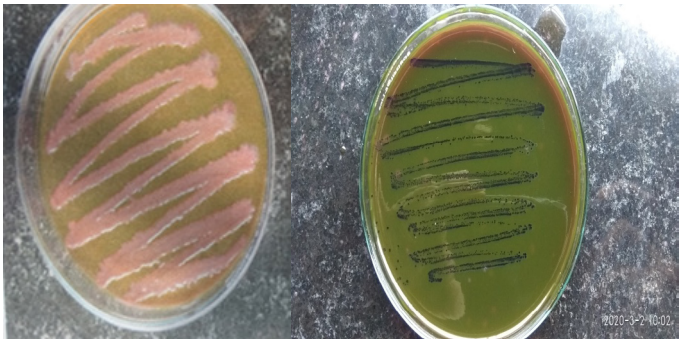

Figure 5) *Green metallic Sheen on EMB of E. coli and Pinkish Mucoid Colonies on EMB of Enterobacter aerogene*

TABLE 4
Results representing bacteria growth on different media (EMB& MSA) and IMViC test to identify coliform group

Sample nO	Sub sample	Growth on EMB	Growth on MSA	Indole	MR	VP	Citrate
C-A	A1	Green metallic sheen	Yellow medium, yellow colony	+	-	+	-
	A2	No colour change poor growth	Yellow medium, yellow colony	-	+	-	-
	A3	Large mucoid pink colonies with blue center	No growth	-	+	-	-
	A4	No colour change	No growth	+	+	-	+
	A5	No growth	Yellow medium, yellow colony	+	-	+	+
	A6	Colour less colony	No growth	-	+	-	-
C-B	B1	No colour change poor growth	Yellow colony	+	+	-	-
	B2	Growth not viable, no change in colour	No growth	+	+	-	-
	B3	Green metallic sheen	No growth	-	+	-	-
	B4	Growth not available, no colour change	No growth	+	+	-	-
	B5	Green metallic sheen	Yellow medium, yellow colony	-	+	-	-
	B6	Colour less colony	pink medium	+	+	-	-

C-C	C1	No growth, no colour change	Yellow medium, Yellow colony	-	+	-	-
	C2	Growth not viable, no change in colour	No growth	+	+	-	-
	C3	None, poor	No growth	-	+	-	-
	C4	Colour less colony	No growth	-	+	-	-
	C5	Large mucoid pink colonies with blue center	Yellow medium, yellow colony	+	+	-	-
	C6	Colour less colony	No growth	-	+	-	-
C-D	D1	Colour less colony	No growth	-	+	-	-
	D2	No growth, no colour change	Yellow medium, yellow colony	-	+	-	-
	D3	None, poor	No growth	-	+	-	-
	D4	Green metallic sheen	No growth	-	+	-	-
	D5	Colour less	Yellow medium, yellow colony	-	+	-	-
	D6	Colour less	No growth	-	+	-	-
C-E	E1	Green metallic sheen	No growth	+	+	-	-
	E2	Large mucoid pink colonies with blue center	Yellow medium, yellow colony	-	-	+	+
	E3	Pinkish mucoid	No colour change	-	-	-	+
	E4	Growth not viable, no change in colour	Yellow medium, yellow colony	-	+	-	-
C-F	F1	None, poor	No colour change	-	-	+	-
	F2	Growth not viable, no change in colour	Yellow medium, yellow colony	-	+	-	-
C-G	G1	Growth not viable, no change in colour	Yellow medium, yellow colony	-	+	-	-
	G2	Large mucoid pink colonies with blue center	Yellow medium, yellow colony	-	-	+	+
C-H	H1	None, poor	No colour change	-	-	+	-
	H2	Green metallic sheen	No growth	+	+	-	-
	H3	Large mucoid pink colonies with blue center	Yellow medium	-	-	+	+
	H4	No growth, no change in colour	Pink medium, colony yellow	-	-	-	+
C-I	I1	None, poor	No colour change	-	-	+	-
	I2	Growth not viable, no change in colour	Yellow medium, yellow colony	-	+	-	-
C-J	J1	Green metallic sheen	No growth	+	+	-	-
	J2	Large mucoid pink colonies with blue center	Pink medium	-	-	+	-
	J3	Growth not viable, no change in colour	Pink medium, colony yellow	-	-	-	+

growth was observed & the medium also turns to yellow which confirms the presence of *Staphylococcus aureus* in these samples. In sample no.C-F2, C-G1 and C-I2 Yellow colour was observed & the medium also turns to yellow which confirms the presence of staphylococcus aureus in these samples (Figure 6).

Biochemical tests

The IMViC tests consist of four different tests: Indole production, Methyl-red, Voges-Prokauer, citrate utilization. Different results are observed in different types of colonies (Figure 7).

Fermentation of carbohydrates

It involves fermented degradation of various carbohydrates such as lactose,

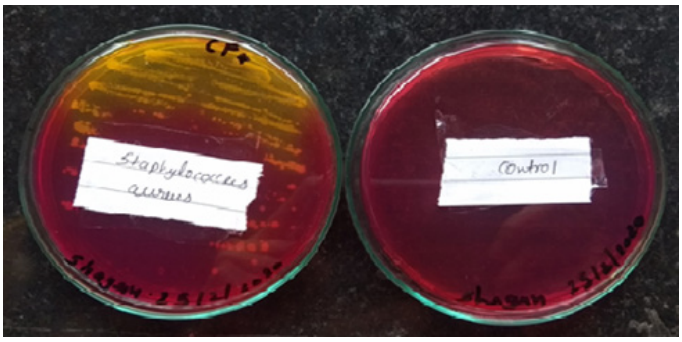

Figure 6) *Growth on MSA: yellow medium with yellow colony indicates S aureus, Medium with no colour change indicate no growth*

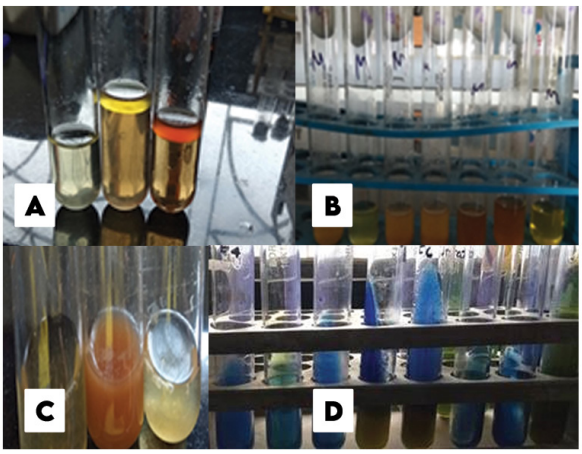

Figure 7) *(A) Tubes with deep red top layer indicate positive indole test (B) Tubes turning red throughout: positive MR test tubs turning yellow throughout, negative MR test* (C) *Tubes showing red colour indicate positive VP test, tube with no colour change indicate negative VP test* (*D) Tubes with blue colour indicate positive citrate test, tubes with green colour indicate negative citrate test*

dextrose and sucrose by microbes, under anaerobic condition (Table 5). In some samples only acid is produced, which is confirmed by the colour change in test tube. Some of them showed the gas production also. A is Acid only i.e., broth has turned yellow. AG is acid and gas i.e., change in colour and appearance of bubbles (due to production of acid and gas (Figure 8).

Catalase test

The enzyme catalase catalysis the breakdown of H2O2 solution. A catalase positive culture will produce bubbles of oxygen within one minute after addition of H_2O_2 absence of any bubbles is indicated as negative for catalase enzyme (Figure 9 and Table 5).

8 Samples i.e., C-A1, C-A2, C-A5, C-B2, C-B3, C-C1, C-C2, C-D2, C-D3, C-D4, C-D5, showed positive catalase test, while the remaining samples were negative. 8 Samples i.e., C-F2, C-G1, C-G2, C-H1, C-H3, C-I2, C-J1, C-J2, showed positive. Catalase test, while the remaining samples were negative.

Total number of bacterial species identified

The graph is plotted against the bacterial species and the total no. of species isolated. Figure 10 shows that which isolates in more number.

DISCUSSION

Pathogenic bacteria in milk have been a major factor for public health concern since the early days. Therefore, the microflora of raw cow milk was isolated and further identified 10 milk samples were collected from various dairies in Meerut. Out of 10 milk samples, C-5, C-6, C-7, C-8 and C-10), while the colony content was low in sample no. C-9. The methylene blue test performed for raw milk samples revealed that out of 10 milk samples, 3 samples were of poor quality (C-1, C-5, and C-8) and the rest samples were of fair quality. The raw milk contained higher number of microflorae probably due to contamination from the animal, especially the exterior of the udder and the adjacent areas [6-8]. Bacteria found in manure, soil and water may enter milk due to dairy utensils and milk contact surfaces. If the milk contact surfaces are inadequately cleaned, bacteria may develop in large numbers.

Figure 8) *Change in colour and appearance of bubbles*

TABLE 5 Fermentation results

Sample No		Carbohydrates			Catalase Test
		Lactose	Dextrose	Sucrose	
C-A	A1	AG	-	A	+
	A2	A	-	A	+
	A3	AG	AG	A	-
	A4	-	-	-	-
	A5	-	A	A	+
	A6	-	-	A	-
C-B	B1	-	-	-	-
	B2	A	A	A	+
	B3	AG	AG	A	+
	B4	-	-	-	-
	B5	AG	AG	A	-
	B6	A	A	AG	-
C-C	C1	-	-	-	+
	C2	A	A	A	+
	C3	-	-	-	-
	C4	AG	AG	A	-
	C5	A	A	A	-
	C6	A	AG	A	-
C-D	D1	A	A	A	+
	D2	-	-	-	+
	D3	AG	AG	AG	+
	D4	AG	AG	A	+
	D5	A	AG	AG	+
	D6	-	-	-	+
C-E	E1	AG	AG	A	+
	E2	AG	AG	AG	+
	E3	-	-	-	+
	E4	A	A	A	+
C-F	F1	-	A	A	_
	F2	A	A	A	+
C-G	G1	A	A	A	+
	G2	AG	AG	AG	+
C-H	H1	-	A	A	-
	H2	AG	AG	A	+
	H3	AG	AG	AG	+
	H4	-	-	-	-
C-I	I1	-	A	A	-
	I2	A	A	A	+
C-J	J1	AG	AG	A	+
	J2	A	A	A	+
	J3	-	-	-	-

Figure 9) *Showing the catalase test observed for the evolution of bubbles*

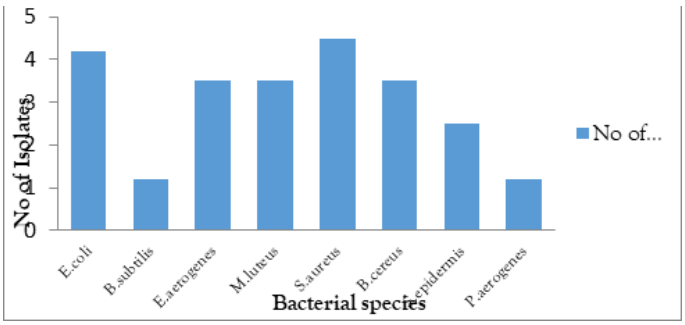

Figure 10) *A Graphical representation of the total number of bacterial species*

Bacterial colony was found to be opaque and metallic sheen in colour in 5 cow milk samples which confirmed it being E coli. The bacteria were found to be gram negative in nature and were small straight roads. EMB agar was used as media because it contains methylene blue which inhibits gram positive bacteria. Gram negative lactose fermenters (coliforms) that grow on this medium will produce "nucleated colonies (dark center) Colonies of E. coli and Enterobacter aerogenes can be differential on the basis of size and presence of greenish metallic sheen [9,10].

Biochemical tests showed that the bacteria were indole positive, methyl-red positive, Voges-Proskauer test negative, citrate utilization test negative. Lactose fermentation positive, dextrose fermentation positive & sucrose fermentation positive. Enterobacter aerogenes was observed in 4 samples, which was identified on the EMB plates as "pink colonies with blue dots". Yellow media with yellow colonies, observed in 6 milk samples, confirmed the presence of S aureus. These organisms may have entered the milk from hands' skin and clothing of handlers during milking processes. Milk is an excellent growth medium of microorganism when suitable temperature exists. If it is produced unhygienically and handled carelessly, it gets contaminated very easily leading to its early Spoilage [11-15]. The introduction of the few pathogens into the milk becomes a much more serious problem because of the ability of these substances to support tremendous increases in bacterial number [16,17]. Microbiological examination of milk is essential to find the degree of contamination with the diction and enumeration of organisms.

CONCLUSION

The microbiological quality was only marginally acceptable with respect to the total SPC. The presence of pathogenic and indicator bacteria such as *E. coli*, Coliforms, *S. aureus* indicate that the growth of these organisms may lead to hazard against public health. Therefore, practice and regulation, such as on-site pasteurization following established standard, should be introduced to facilitate the production of cow milking operation, however, milk may be exposed to contamination from the animal, especially the exterior of the udder and adjacent areas. Such contamination can be reduced by clipping the cow and washing the udder with water or a germicidal solution before milking. Contamination of cow with manure, soil and water may also be reduced by paving and dining barnyards, keeping cows from stagnant pools and cleaning manure from the barns of milking parlors. Pasteurization kills pathogens that may enter the milk and improve the keeping quality of milk. Information given by the obtained results allows concluding that strict hygienic measures should be applied during production, processing and distribution of cow milk and its products to avoid contamination. Periodical inspection must be done by specialists on the dairy farms to minimize milk contamination with different type of microorganism. Efficient cleaning and sanitization of farm dairy utensils must be done to improve the quality of cow

milk and consequently the related dairy products. The cow milk and milk products should be kept under refrigeration at all times and the practice of display at room temperature should be discouraged.

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