International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

To Check the Quality of Raw Milk & Presence of Pathogenic and Non - Pathogenic Bacteria in Milk Sample

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Abstract: ***Objective: To evaluate the hygienic quality by determining the presence of predominant pathogenic microbial contaminants (contagious or environment) contained in unpasteurized milk samples collected from mammalians (cow, buffalo, goat). Methods: Raw milk samples were collected in June, 2023 from different area of District Bareilly, Town Nawabganj and cultured on the specific media plates according to the manufacture instructions to observe pathogenic microbial flora and confirm it with ravelment biochemical tests to specify bacterial specie. Results: Milk samples analyzed on Mac - Conkey, MSA, Nutrient media were found contaminated mostly with coliform, Staphylococcus aureus, Proteus vulgaris, Streptococcus and Enterobacter aerogenes. Conclusion: Microbial contamination of milk is mainly accredited to the scrupulous unhygienic measures during processing of milk exhibiting a wide array of hazardous impacts on human health.***

Keyword: Unpasteurized Milk, Chemical analysis and Quality control, Microbial - flora.

1. Introduction

Milk is a complete food that includes all of the necessary nutrients. For nutritional reasons, people typically want milk and its products with great biological potential, enhanced nutritional qualities, and no health risks or dangers [1]. Raw milk varies in composition depending on the species, but it contains considerable amounts of water, carbohydrates, minerals, enzymes, calcium, protein, and saturated fats in addition to vitamin C [2, 3]. The pH of cow's milk ranges from 6.4 to 6.8, making it rather acidic [4].

However, the main factors that contribute to milk's susceptibility to spoilage are the presence of microorganisms and toxins, improper handling and storage conditions, the amount of light and oxygen the milk is exposed to, the cleanliness of the equipment, seasonal variations, the state of the soil, and the health of the animals [5]. In its unprocessed or raw form, milk can be found in nature. It also acts as a medium for the growth of numerous pathogenic microbes, including *Streptococcus*, *Enterobacter*, *Staphylococcus*, and *Proteus* species [6 - 8].

When eaten, a number of zoonotic illnesses, including undulant fever, dysentery, gastroenteritis, food poisoning, and intoxication, can be brought on by the presence of bacterial species in raw milk samples through the formation of enterotoxins [9]. Physical hazards, microbiological hazards, chemical contamination, and veterinary medications are the categories of risks associated with raw milk [10].

Good, safe meals with a lengthy shelf life are in high demand. However, because milk and its derivatives are biochemically unstable, they spoil quickly. The food business uses many approaches for quality and safety control [11]. These systems are highly efficient despite being costly and sophisticated. These systems include Total Quality Management (TQM), Hazard Analysis and Critical Control Point (HACCP), ISO 9000, and others [12]. If milk is handled carelessly or manufactured in an unhygienic manner, it can easily become contaminated and spoil quickly [13]. Milk stems for the proliferation of microorganisms. Globally, food - borne illnesses rank among the top causes of concern for public health.

Each and every day, millions of people drink raw milk. Numerous people suffer various food - borne illnesses as a result of consuming contaminated milk, either from diseased cows or during the milk manufacturing process [14]. These illnesses include Q - fever, typhoid fever, nausea, and dehydration [15].

Numerous scientific studies were conducted to identify pathogenic germs in milk, beneficial microorganisms present in milk, and milk quality control. The microbiological quality of milk was influenced by the raw milk's original flora. Milk is typically required for nutritional reasons because it has a strong biological potential, increased nutritional qualities, and no health concerns or dangers [16].

Milk is an essential component of a healthy diet for growing children, expectant moms, and nursing mothers. mostly bacteria, such as Lactobacillus, Proteus, Bacillus species, Staphylococcus, and Streptococcus [17 - 19]. Aside from being a vital and nourishing nourishment for people. The various causes of microbial contamination of raw milk include the air, soil, feed, grass, faeces, and milking equipment.

2. Methodology

2.1. Sample Collection

Milk samples were taken straight from the several dairy farms in the vicinity (Bareilly, Nawabganj) and placed into sterile containers. The dates of the isolation collection were June 2023–August 2023. Up until the study or subsequent subcultures, all cultures were routinely subcultured on Nutrient agar slant and kept at 4°C in the refrigerator at Mahatma Jyotiba Phuley Rohailkhand University Bareilly's Department of Microbiology. After being appropriately labelled, the samples were delivered to the lab for examination. Further, the molecular biology studies were carried out at Somics Lifesciences Pvt. Ltd. Laboratory, Bareilly.

2.2. Quality analysis of collected milk samples

COB (clot on boiling) test [20], Fehling's test [21], MRBT test [22], and alcohol test [23] were carried out to determine the stability of milk for heat processing and to detect abnormal milk such as colostrum's or mastitis milk.

2.3. Isolation of bacteria from samples

Initially, the samples were diluted in normal saline and then spread over sterilized nutrient agar plates and then incubated at 37℃ for 48 hours. The isolated colonies were shortlisted on the basis of their morphological parameters. The selected isolates were then streaked on sterilized selective media incubated at 37℃ for 48 - 72 hours to obtain proper bacterial growth [24].

2.4. Biochemical identification of bacterial isolates

Biochemical tests were performed for the identification of the bacterial culture depending on Bergey manual, including staining and microscopy techniques like gramme and endospore staining, as well as biochemical tests like catalase, oxidase, MR - VP, Indole, and urease tests [25].

2.5. Molecular characterization for the identification of culture

27F and 1492R primers were used to amplify a fragment of the 16S rDNA gene. On an Agarose gel, a single discrete PCR amplicon band of 241 bp was seen. To get rid of impurities, the PCR amplicon was cleaned. Using the BDT v3.1 Cycle sequencing kit on an ABI 3730xl Genetic Analyzer, the forward and reverse DNA sequencing reaction of the PCR amplicon was performed using forward and reverse primers. Using aligner software, a consensus sequence for the 16S rDNA gene was produced from forward and reverse sequence data. BLAST was performed using the NCBI genbank database and the 16S rDNA gene sequence. The first ten sequences were chosen based on the maximum identity score, and Clustal W, a multiple alignment software programme, was used to align them. The phylogenetic tree was constructed using MEGA 7 [26].

2.6. Antibiotic sensitivity test

The antibiotic susceptibility testing of bacterial isolates were carried out through agar disc diffusion method. Were the isolates were spread over sterilized media and then the antibiotic discs were placed over it. The plates were incubated at 37℃ for 48 - 72 hours. Clear zone indicates the inhibition of bacterial growth [27].

3. Results

3.1. Sample collection and quality analysis

A total 25 raw milk sample such as cow milk (8), buffalo milk (10), goat milk (7) each of 100 ml were collected from the dairy farms, and packaged milk (6) were collected from milk points in autoclaved bottles. In packaged milk, 2 samples of amul brand, 2 prayag brand and 2 of ananda brand were collected. The sample was processed on the same day of collection or was stored at 4°C in case delay.

Figure 1: Collected milk sample

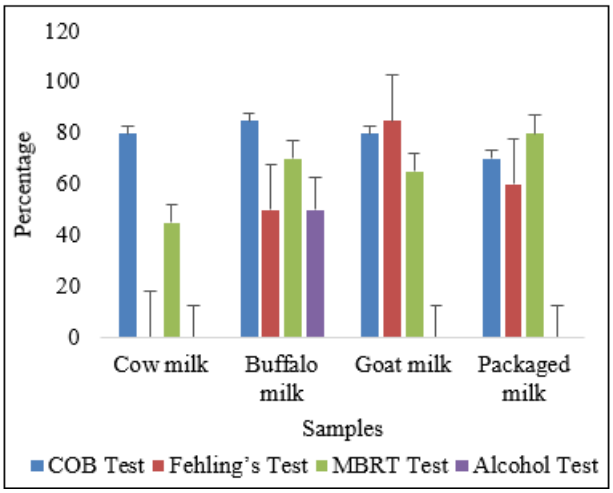

Figure 2: Graphical illustration of collected loose and packed milk samples quality.

3.2 Isolation and identification of bacterial cultures:

Total 70 bacterial isolates were obtained form 25 raw milk sample and 6 packed milk sample as mentioned in figure 4. The isolates were successfully obtained after serial dilution, spreading and colony morphological studies. The identification of the isolates was carried out through various biochemical characterization as given in table 1. Where maximum staphylococcus species and streptococcus species were isolated from the collected samples. The isolate Staphylococcus species was confirmed by phylogenetic analysis after sequencing during molecular characterization and found that the isolate was S. aureus as shown in figure 5.

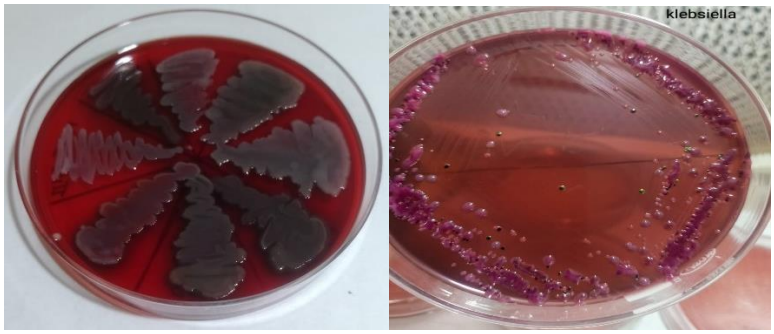

Figure 3: Isolated cultures from milk sample

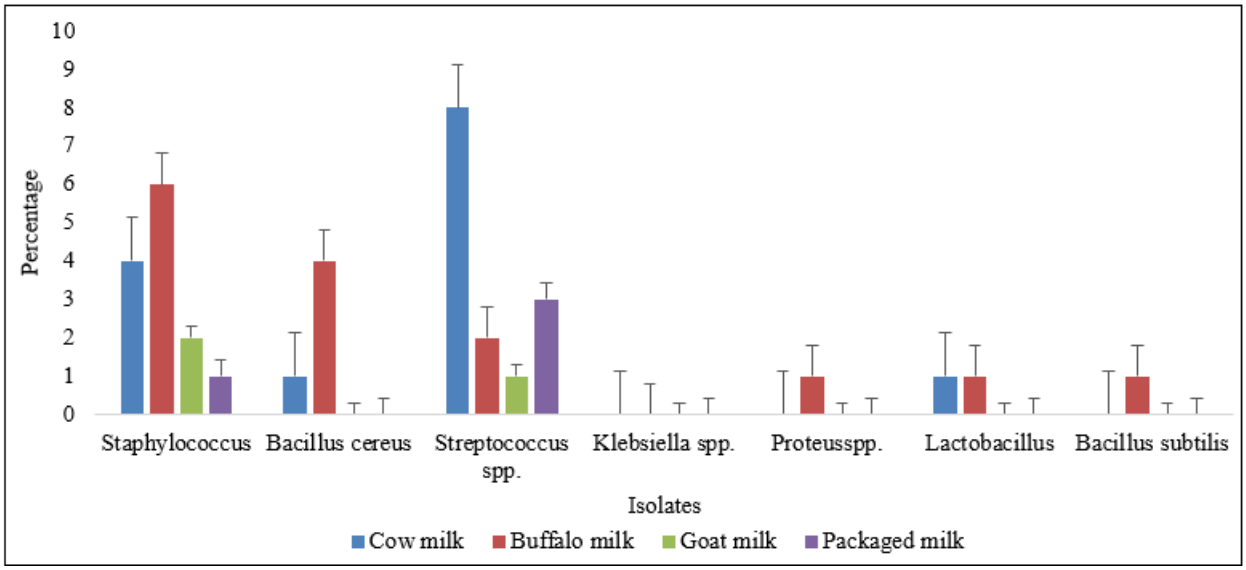

Figure 4: Percentage of bacterial culture isolated from collected loose and packed milk samples.

Table 1: Biochemical identification of bacterial isolates

Bacteria	Catalase Test	Oxidase Test	Indole Test	MRTest	VPTest	Citrate Test	Urease Test
Staphylococcus	Positive	Negative	Positive	Positive	Positive	Positive	Positive
Bacillus cereus	Positive	Positive	Negative	Positive	Positive	Positive	Positive
Streptococcus spp	Positive	Negative	Negative	Positive	Negative	Positive	Negative
Klebsiella spp	Positive	Negative	Positive	Negative	Positive	Positive	Positive
Proteus spp.	Negative	Positive	Positive	Positive	Negative	Positive	Positive
Lactobacillus	Negative	Positive	Negative	Negative	Negative	positive	Negative
Bacillus subtillis	Positive	Positive	Negative	Negative	Positive	positive	Positive

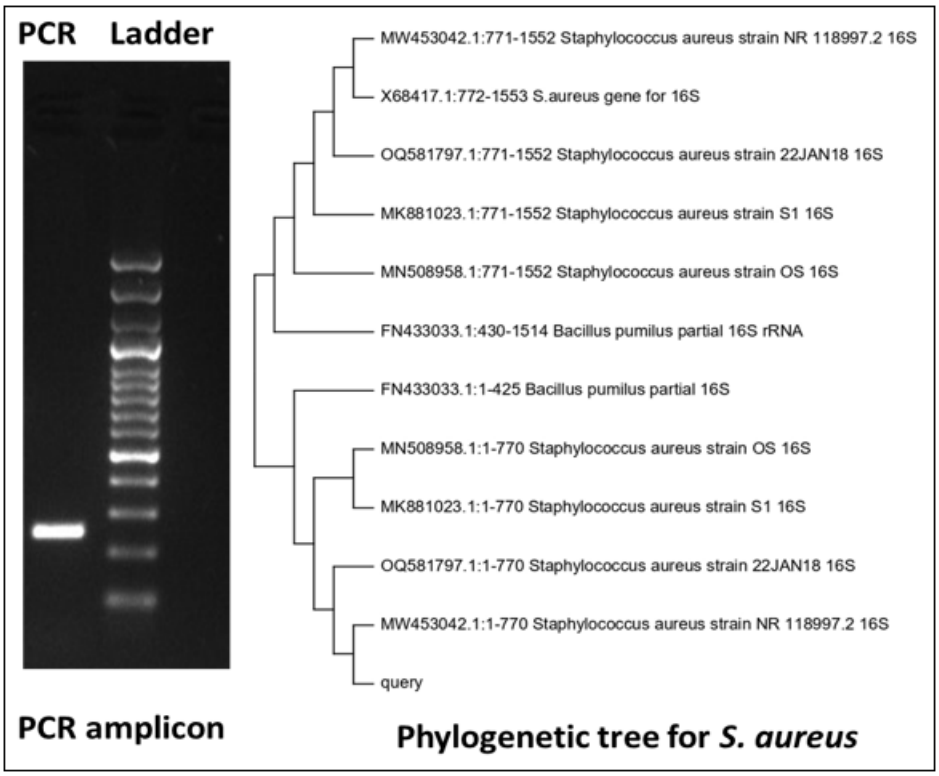

Figure 5: Molecular characterization of Staphylococcus strain

3.3. Antibacterial analysis of antibiotics against *S. aureus*:

The agar disc diffusion test was used to studied the antibacterial properties of the antibiotics against *Staphylococcus aureus*, the clear zone indicates the positive result while showing the efficacy to inhibiting the bacterial growth as shown in figure 6.

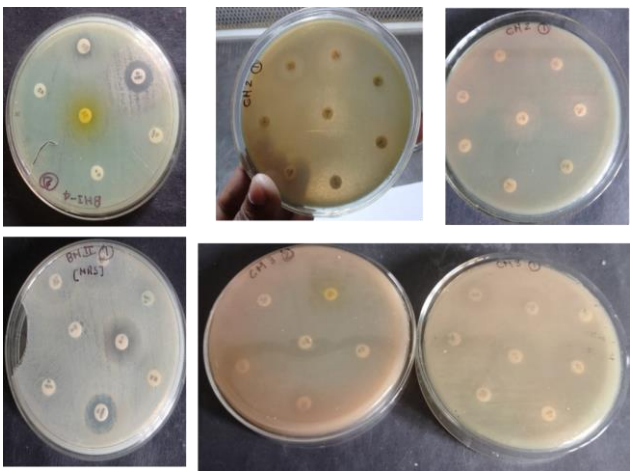

Figure 6: Antibacterial studies of the antibiotics against isolated *S. aureus*

4. Conclusion

Upon analysing milk samples using Mac - Conkey, MSA, and Nutrient medium, it was discovered that the majority of the contaminants were Enterobacter aerogenes, Proteus vulgaris, Staphylococcus aureus, and coliform. The primary cause of milk microbial contamination is the meticulous lack of hygiene during milk processing, which can have a variety of harmful effects on human health. Numerous scientific investigations were carried out to determine the presence of beneficial bacteria in milk, harmful germs in milk, and milk quality management. The initial flora of raw milk has an impact on its microbiological quality. The research conducted revealed the presence of S. aureus in both packaged and loose milk, demonstrating the detrimental impact on human health. Because of its high nutritional value, powerful biological potential, and lack of risks or health concerns, milk is usually necessary for nutritional reasons.

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