IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

ISOLATION AND IDENTIFICATION OF BACTERIAL CONTAMINANTS IN RAW BUFFALO AND COW MILK

T. Govardhan* and Dr. Madan MohanGunda

Department of Microbiology, Government Degree and P.G. College(A), Siddipet, Telangana, India.

govardhanthodeti850@gmail.com

ABSTRACT

Milk is recognized as a nutritionally rich food that supports the growth of a wide spectrum of microorganisms because of its high moisture content and abundance of essential nutrients. The present investigation was conducted to isolate and identify bacterial contaminants present in raw buffalo and cow milk collected from local sources. Five milk samples comprising three buffalo milk samples and two cow milk samples were analyzed using standard microbiological techniques. Serial dilution followed by inoculation on Nutrient Agar medium was performed to obtain isolated colonies. Morphological characterization of colonies and Gram staining procedures were employed for bacterial identification. The study revealed the occurrence of both beneficial and pathogenic microorganisms in the analyzed milk samples. The bacterial species identified included *Lactobacillus* spp., *Streptococcus* spp., *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas* spp. Buffalo milk samples demonstrated slightly higher microbial diversity compared to cow milk samples. The isolation of coliform bacteria and *S. aureus* highlights potential public health concerns associated with the consumption of untreated milk. The findings of this study emphasize the necessity of maintaining hygienic milking practices, refrigeration, and pasteurization to ensure microbiological safety and improve the quality of dairy products intended for human consumption.

KEYWORDS

Milk Microbiology, Bacterial contamination, Buffalo milk, Cow milk, Gram staining, Food safety, Dairy hygiene

INTRODUCTION

Milk is one of the most valuable natural foods consumed by humans due to its high nutritional value and balanced composition of proteins, carbohydrates, fats, vitamins, and minerals. It serves as an important dietary component for individuals of all age groups and contributes significantly to human nutrition. However, the same nutritional richness that makes milk beneficial also creates favourable conditions for microbial proliferation. As a result, milk can become contaminated by a variety of microorganisms capable of causing spoilage and foodborne diseases.The importance of microorganisms in milk was first demonstrated by Louis Pasteur in 1864, who established that microbial contamination was responsible for milk spoilage and introduced the process of pasteurization to improve milk safety and shelf life.

Later, Sigurd Orla-Jensen (1919) extensively studied lactic acid bacteria present in milk and classified important genera such as *Lactobacillus* and *Streptococcus*, which are beneficial in dairy fermentation. His research contributed significantly to understanding the microbiology of fermented dairy products.

According to William C. Frazier and Dennis C. Westhoff (1988), microorganisms associated with milk can be categorized into beneficial organisms, spoilage organisms, and pathogenic organisms. Beneficial bacteria aid in fermentation, whereas spoilage organisms reduce the quality and shelf life of milk. Pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* species are capable of causing serious foodborne illnesses in humans.

James M. Jay (2000) reported that milk contamination may occur from multiple external sources including animal skin, milking utensils, contaminated water, handlers, air, and storage containers. He emphasized that poor hygienic practices during milking and improper storage conditions significantly increase microbial load in milk.

Fresh milk obtained from healthy animals is generally sterile inside the udder, but contamination frequently occurs during milking, handling, transportation, processing, and storage. Sources of contamination include the udder surface, milking equipment, water, feed, air, storage vessels, and human handlers. The extent of microbial contamination largely depends on environmental sanitation, animal health, storage temperature, and duration of storage.

Microorganisms associated with milk may be classified as beneficial, spoilage-causing, or pathogenic organisms. Beneficial bacteria such as lactic acid bacteria contribute to the fermentation of dairy products including curd, yogurt, and cheese. In contrast, pathogenic microorganisms such as *Escherichia coli* and *Staphylococcus aureus* can pose serious health hazards to consumers. Some Psychrotrophic bacteria, particularly *Pseudomonas* species, are responsible for the deterioration of milk quality during refrigerated storage through enzymatic spoilage.

In developing countries, especially in rural and semi-urban regions, raw milk is frequently consumed without adequate heat treatment. Under such conditions, the microbiological quality of milk becomes a major public health concern. Therefore, routine microbiological examination of milk is essential to assess its safety and suitability for human consumption.

The present study was undertaken to isolate and identify microorganisms present in buffalo and cow milk samples using conventional microbiological techniques including serial dilution, culture on Nutrient Agar medium, colony morphology analysis, and Gram staining.

METHODOLOGY

Collection of Milk Samples

Fresh raw milk samples were collected aseptically from local dairy sources and household animals using sterile screw-capped containers. A total of five samples were included in the study, consisting of three buffalo milk samples designated as B1, B2, and B3, and two cow milk samples designated as C1 and C2. The collected samples were transported to the microbiology laboratory in an insulated ice container and processed within two hours to minimize changes in microbial population.

Figure 1 : Different milk samples collected from different sources

Serial Dilution Procedure

Serial dilution of each milk sample was carried out under aseptic conditions. One milliliter of milk sample was transferred into 9 ml of sterile distilled water to obtain a 10^{-1} dilution. Further serial dilutions were prepared up to 10^{-5} using sterile pipettes and test tubes.

Inoculation on Culture Media

A loopful of diluted sample was inoculated onto sterile Nutrient Agar plates using spread plate technique. Separate plates were prepared for each dilution and incubated under controlled laboratory conditions. All procedures were performed inside a laminar airflow chamber to prevent external contamination.

Incubation

The inoculated plates were incubated at 37°C for 24–48 hours to facilitate bacterial growth and colony development.

Colony Morphology Observation

Following incubation, visible colonies were examined for their cultural and morphological characteristics including:

Shape, Texture, Size, Colour, Elevation, Margin

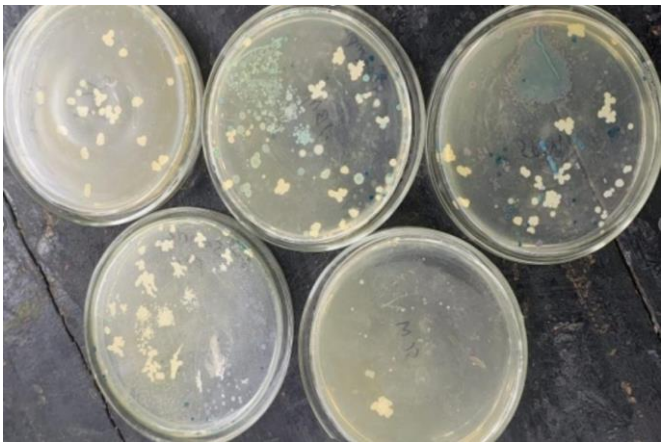

Figure 2: Colony formation on the different milk samples

Gram Staining

Representative bacterial colonies were subjected to Gram staining for microscopic characterization. Smears were prepared on clean glass slides, heat-fixed, and stained sequentially with crystal violet, Gram's iodine, alcohol decolourizer, and safranin. The stained slides were examined under oil immersion microscopy to determine Gram reaction and cellular morphology.

RESULTS

Table 1. Distribution of Bacterial Isolates in Milk Samples

Bacterial species	Buffalo milk(B1-B3)	Cow milk (C1-C2)	Total occurance
Lactobacillus spp.	1	1	2
Streptococcus spp.	2	1	3
Staphylococcus aureus	1	1	2
Escherichia coli	1	1	2
Pseudomonas spp.	1	0	1
Total isolates	6	4	10

Table 2: Percentage distribution of Bacterial Isolates

Bacterial species	Frequency	Percentage(%)
Streptococcus spp.	3	30
Lactobacillus spp.	2	20
Staphylococcusaureus	2	20
Escherichiacoli	2	20
Pseudomonas spp.	1	10
Total	10	100

Table 3 : Microbial diversity between buffalo and cow milk

Milk type	Number of isolates	Percentage(%)
Buffalo milk	6	60
Cow milk	4	40
Total	10	100

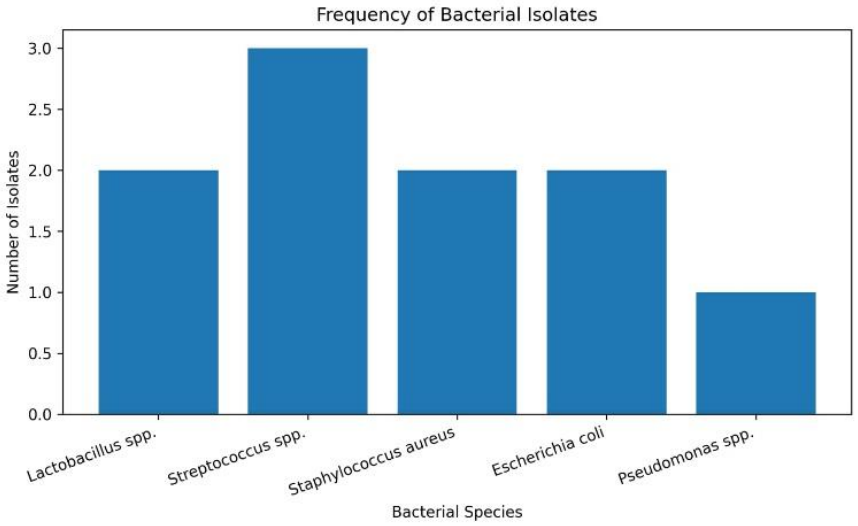

Figure 3: Frequency of Bacterial Isolates

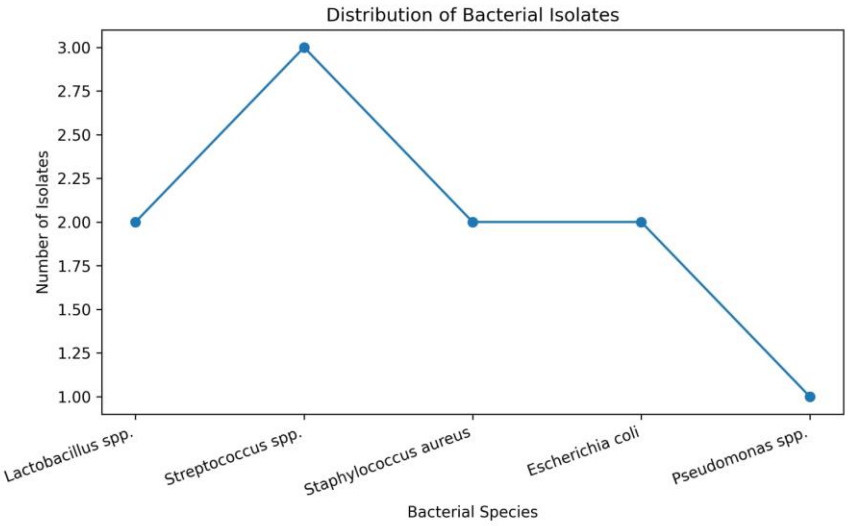

Figure 4 : Distribution of Bacterial Isolates

Microbial growth was observed in all milk samples following incubation. Distinct bacterial colonies with varying morphological characteristics were isolated and identified based on Gram staining and colony morphology.

The bacterial isolates identified in the present investigation included:

Lactobacillus spp.

Streptococcus spp.

Staphylococcus aureus

Escherichia coli

Pseudomonas spp.

Lactobacillus species appeared as Gram-positive rod-shaped bacteria arranged in chains and produced smooth white colonies. These organisms were isolated from buffalo sample B2 and cow sample C1.

Streptococcus species were identified as Gram-positive cocci arranged in chains. They produced small circular colonies and were isolated from samples B1, B3, and C2.

Staphylococcus aureus appeared as Gram-positive cocci arranged in clusters with pale yellow rough colonies. The organism was isolated from buffalo sample B1 and cow sample C1.

Escherichia coli was identified as Gram-negative short rods producing smooth colourless colonies. The presence of this organism was observed in buffalo sample B2 and cow sample C2.

Pseudomonas species were isolated from buffalo sample B3 and appeared as Gram-negative rod-shaped bacteria producing greenish spreading colonies.

Buffalo milk samples showed comparatively higher microbial diversity than cow milk samples.

DISCUSSION

The findings of the present study demonstrate that raw milk obtained from local sources harbours diverse bacterial populations including beneficial and pathogenic microorganisms. Similar observations have been reported in previous microbiological investigations of raw dairy products.

The isolation of lactic acid bacteria such as *Lactobacillus* and *Streptococcus* species is expected because these organisms naturally occur in milk and contribute to fermentation processes. These bacteria are commonly associated with dairy environments and are considered important in the production of fermented milk products.

However, the detection of pathogenic organisms including *Staphylococcus aureus* and *Escherichia coli* indicates inadequate hygienic conditions during milk production and handling. The presence of *E. coli* serves as an indicator of fecal contamination and poor sanitary practices. Similarly, *S. aureus* contamination may originate from infected udders, unclean equipment, or improper handling by workers.

The isolation of *Pseudomonas* species from buffalo milk suggests the possibility of psychrotrophic spoilage during storage. These organisms produce heat-resistant enzymes capable of degrading proteins and lipids, thereby reducing the shelf life and quality of milk.

Buffalo milk samples exhibited a broader range of microbial contaminants than cow milk samples, possibly due to differences in fat content, environmental exposure, and farm management practices. These findings emphasize the importance of proper sanitation, refrigeration, and pasteurization in maintaining milk quality and protecting consumer health.

CONCLUSION

The present investigation successfully isolated and identified several bacterial species from raw buffalo and cow milk samples using conventional microbiological techniques. Both beneficial and pathogenic microorganisms were detected in the analyzed samples. The occurrence of organisms such as *Escherichia coli* and *Staphylococcus aureus* indicates poor hygienic practices during milking and handling procedures.

The study highlights that raw milk can act as a potential vehicle for transmission of foodborne pathogens if proper sanitary measures are not followed. Maintenance of hygienic milking conditions, rapid refrigeration, regular microbiological monitoring, and pasteurization are essential to improve the microbiological quality and safety of milk intended for human consumption.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to the faculty members and laboratory staff of the Department of Microbiology for their valuable support, guidance, and cooperation throughout this work. We are especially thankful to Prof. G. Sunitha, Principal, for providing laboratory facilities, institutional support, and constant encouragement for the successful completion of this study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

REFERENCES

1. Abid, H., Abdul, S., Shahid, M. A., Muhammad, N., & Fahad, G. (2007). Clinical and subclinical Staphylococcus aureus mastitis in dairy buffaloes: Disease characteristics and antibiotic susceptibility profiles of isolates. International Journal of Agricultural Research, 2(9), 804–811.
2. Adesiyun, A. A. (1994). Bacteriological quality and associated public health risk of pre-processed bovine milk in Trinidad. International Journal of Food Microbiology, 21(3), 253–261.
3. Bauer, A. W., Kirby, W. M. M., Sherris, J., &Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45, 225–230.
4. Beizhong, H., Meng, Y., Li, M., Yang, Y., Ren, F., Zeng, Q., &Nout, M. J. R. (2007). A survey on the microbiological and chemical composition of buffalo milk in China. Food Control, 18(6), 742–746.
5. Boycheva, S., Dimitrov, T., Tsankova, M., &Iliev, T. (2002). Investigation on microflora of buffalo milk. Bulgarian Journal of Agricultural Science, 8(2–3), 279–282.
6. Bulla, T. R., Rana, Y. S., Sharma, A., &Beniwal, B. S. (2006). Prevalence of subclinical mastitis in Murrah buffaloes. Haryana Veterinarian, 45, 53–56.
7. Buxton, A., & Fraser, G. (1977). Animal microbiology (Vol. 1, pp. 85–86). Blackwell Scientific Publications.
8. Cheesbrough, M. (1984). Medical laboratory manual for tropical countries (1st ed., Vol. 2, Chapter 35, pp. 40–57). English Language Book Society.
9. Das, P. K., & Joseph, E. (2005). Identification and antibiogram of microbes associated with buffalo mastitis in Jabalpur, Madhya Pradesh, India. Buffalo Bulletin, 24(1), 3–9.
10. Harrigan, W. F., &McCance, M. F. (1976). Laboratory methods in food and dairy microbiology. Academic Press.
11. Kosi, O. H. R., Abdel, E. H., &Saad, A. H. (2000). Fate of enterohaemorrhagic Escherichia coli O157:H7 in buffalo's milk and some of its manufacturing products. Tropenlandwirt, 69, 165–175.

12. Mahmoued, M. M., Salama, M. E., &Galal, H. (2008). Rapid diagnosis of subclinical Staphylococcus aureus mastitis in dairy buffaloes using PCR technique. Assiut Veterinary Medical Journal, 54(116), 78–89.

13. Neelesh, S., Maiti, S. K., & Sharma, K. K. (2007). Prevalence, etiology and antibiogram of microorganisms associated with sub-clinical mastitis in buffaloes in Durg, Chhattisgarh State (India). International Journal of Dairy Science, 2(2), 145–151.Oliveira, M. V. V., Mota, R. A., Oliveira, A. A. F., Meirelles, F. S., & Silva, F. F. (2004). Use of the Modified Whiteside and California Mastitis Test on the diagnosis of buffalo subclinical mastitis related to microbiological exam. Ciência Animal, 14(1), 39–45.