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Assessment of microbial contamination and quality parameters in packaged cow milk: A comparative study across different brands

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Abstract: *Ensuring microbiological safety and quality of packaged cow milk is a critical concern for both the dairy industry and public health. This study investigated the sources and extent of microbial contamination in packaged cow milk, alongside evaluating key quality parameters. By using physical and chemical methods, bacterial contaminants in pasteurized milk samples were identified and quantified. The present study indicated that contamination could be found even in branded packaged cow milk which claims to be pasteurized. The findings emphasized the need for stringent hygienic practices, improved processing technologies, and continuous assessment to ensure the safety and quality of packaged cow milk. This study advocates for the development of better industry standards and practices, thereby reducing health risks associated with microbial contamination and ensuring high-quality milk products for consumers.*

Keywords: *Microbial contamination, Packaged cow milk, Pasteurization, Quality parameters, Food safety, Hygienic practices.*

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Introduction:

Milk is an important part of our diet due to its rich nutritional value, yet its perishable nature makes it prone to microbial contamination (Hanna et al., 2019). Despite undergoing pasteurization, packaged cow milk can still contain harmful pathogens such as *Escherichia coli, Salmonella* spp., *and Listeria monocytogenes*, as well as fungal spores that survive the process. These contaminants pose health risks and negatively impact the sensory and nutritional properties of milk (Silva., 2010).

Milk has long been considered as one of nature's most complete foods and remains a dietary staple for over 6 billion people worldwide. In India, bovine milk constitutes a significant portion of the nation's dairy production, contributing greatly to the nutritional needs of its vast population. Over the past three decades, India's milk production has seen substantial growth, aligning with global trends that have witnessed a 60% increase from 522 million tons in 1987 to 828 million tons in 2017 to meet rising demand. According to projections by the OECD and FAO, per capita consumption of milk and dairy products is expected to remain high, particularly in developing regions like North Africa, the Middle East, and Asia, including India.

The emergence of pathogens, along with the overall effects of climate change on microbiological and chemical hazards, makes it a necessity for continuous research and innovation in dairy processing and safety methods. By deeply understanding the origins and mechanisms of contamination, the dairy industry can devise effective strategies to enhance food safety standards, thereby ensuring the provision of high-quality dairy products to consumers.

The collection and processing of milk in India introduce various contaminants, such as pesticide residues, metals, mycotoxins, and hormones, often through cow feed or veterinary drugs. Although pasteurization significantly reduces many diseases, it does not entirely eliminate the risk of infections from pasteurized milk (Micaela et al., 2022). This is due to the presence of high levels of microorganisms in raw milk that survive pasteurization or contamination occurring post-pasteurization. The persistence of pathogens in pasteurized milk without altering its taste or appearance underscores the need for improved detection and control measures. This introduction sets the stage for a detailed exploration of microbial hazards in milk within the Indian context, highlighting the importance of stringent safety protocols to ensure public health. This research aimed to explore the prevalence and sources of microbial contamination in pasteurized milk, utilizing various physico-chemical parameters like pH levels to detect contaminants and assess quality (Yadav., 2014). It is essential to guarantee the microbiological safety and quality of milk, particularly for vulnerable populations such as children, the elderly, and individuals with compromised immune systems. The presence of aflatoxin M1, a potent mycotoxin resulting from contaminated feed, highlights the need for stricter regulations and systematic assessment (Iqbal et al., 2015)

Materials and Methods:

For the analysis of milk quality, selected samples were tested on different parameters. This testing was specifically done on brand labeled pasteurized milk which was made possible by the use of proper equipment, chemicals, hygienic environment and careful procedures. The brands on which testing was done were Amul, Aashirvad, Raj and Sudha. The quality of milk was tested by following (Melese & Addisu., 2015; James et al., 2020).

Testing of pH was done with pH strips for all the four brands. Acidity of milk was determined by titrimetric method. Standard plate count technique helped in the culture of various bacteria found in milk. Culture plates containing medium of milk agar were streaked with milk samples of four brands and incubated for 24- 48 hours. Differential plate counting of specific gram negative and positive bacterial colonies on the medium of Mac Conkey was done for the finding of microbes in milk. A catalase test was performed to detect the presence of bacteria such as *Staphylococcus, Micrococcus, Enterococcus* spp. when H_2O_2 was added to sample. The Indole ring test detected the presence of bacteria such as *E.coli, Enterobacter* spp.*, Lactobacillus,* spp. and *Salmonella* on the basis of ring formation on sample by adding Kovac reagent. Urease test detected the presence of bacteria such as *E.coli and Proteus* spp. which was identified via the color change of urea agar after streaking it with sample. The Methyl red test detected the presence of *E.coli, Enterobacter* spp.*, Klebsiella* spp. and *Yersinia* spp. by addition of sample in glucose phosphate broth. The results were seen by the addition of methyl red indicator. The triple sugar iron test identified the presence of different sugars in milk which was done by streaking sample on TSI agar giving different colors for different sugars. The citrate utilization test was conducted to detect the presence of citrate in milk sample, indicated by a color change on Simmon's citrate agar slants inoculated with the sample. Additionally, the methylene blue reduction test was performed to assess the microbial load and overall quality of the milk sample. The Fehling solution method helped in detection of lactose in milk by addition of Fehling solution to the sample. Results were found by the formation of brown precipitate after water bath treatment.

Results and Discussion:

The pH analysis of different milk brands revealed that AMUL had a slightly alkaline pH of 8, whereas AASHIRVAD, RAJ, and SUDHA maintained a neutral pH of 7 as shown in Table 1. The slightly higher pH in AMUL possibly indicates difference in processing, storage conditions, or microbial activity compared to the other brands. These variations in pH may influence the microbial growth patterns and overall quality of the milk samples. The pH range of milk as approved by FSSAI standards is 6.7-6.9, which alters with the age of sample or the presence of adulterants in it.

Table 1. The pH value of different brands of milk samples

MILK BRANDS	pH VALUE
AMUL	8
AASHIRVAD	7
RAJ	7
SUDHA	7

A Standard Plate Count (SPC) of bacterial colonies on skimmed milk agar plate allowed the population density of microorganism in liquid milk. The standard plate count analysis showed variations in microbial load across the milk samples (Table 2). AMUL and AASHIRVAD had similar colony counts of 131×10^3 CFU/ml and 130×10^3 CFU/ml, indicating moderate microbial presence. SUDHA recorded the highest microbial load at 239×10^3 CFU/ml, suggesting higher contamination or microbial activity. RAJ displayed compact colonies, indicating dense microbial growth that could not be fully quantified under standard conditions. These findings highlighted differences in microbial quality and potential variations in hygiene practices during the production and storage of the milk samples.

Table 2. Standard plate count measured in the unit CFU/ml

MILK BRANDS	NO. OF COLONY (*10ˆ3 CFU/ml)
AMUL	131
AASHIRVAD	130
RAJ	COMPACT COLONIES
SUDHA	239

The different types of Gram positive and Gram negative bacterial colonies were isolated on agar plates on the basis of lactose fermentation. Analysis of isolated colonies on agar plates identified both Gram-positive and Gram-negative bacteria in the milk samples as shown in Table 3. AMUL showed the presence of *Enterococcus* spp. as the Gram-positive species, alongside a diverse range of Gram-negative bacteria, including *E. coli, Salmonella* spp.*, Shigella* spp.*, Proteus* spp., and *Klebsiella* spp. In AASHIRVAD, *Enterococcus* spp. was detected as the Gram-positive species, with *Salmonella* spp.*, E. coli,* and *Shigella* spp. among the Gram-negative bacteria. RAJ contained *Staphylococcus* spp. (Gram-positive) and *E. coli* (Gram-negative), while SUDHA exhibited *E. coli* and *Shigella* spp. as Gram-negative species, with no Gram-positive bacteria identified. These findings highlight significant bacterial diversity, including potential pathogens, across the milk samples.

Table 3. The different species of bacteria identified in the milk samples on the basis of isolated colonies prepared on agar plates

MILK BRANDS	GRAM +ve	GRAM –ve
AMUL	*Enterococcus* spp.	*E.coli, Salmonella* spp.*, Shigella* spp.*, Proteus* spp.*, Klebsiella* spp.
AASHIRVAD	*Enterococcus* spp.	*Salmonella* spp.*, E.coli, Shigella* spp.
RAJ	*Staphylococcus* spp.	*E.coli*
SUDHA	–	*E.coli, Shigella* spp.

Biochemical tests demonstrated distinct bacterial profiles among the samples as shown in Table 4. AMUL showed the most extensive metabolic activity, testing positive for Fehling's, catalase, indole, urease, methyl red, citrate utilization, and hydrogen sulfide production in the TSI test, indicating versatile bacteria such as *Klebsiella* or *Proteus* species. AASHIRVAD displayed a similar profile but lacked urease and citrate activity, suggesting the presence of *Escherichia coli*. RAJ exhibited limited metabolic activity, with negative results for catalase, urease, and citrate tests, and a yellow TSI slant and butt, indicative of fermentative bacteria like *Enterobacter*. SUDHA showed a profile resembling AASHIRVAD but with less metabolic diversity, aligning with *E. coli*. These findings underscore the metabolic variability of bacterial species across the samples.

Table 4. The results of biochemical tests confirming the presence of specific bacterial species

BIOCHEMICAL TEST	AMUL	AASHIRVAD	RAJ	SUDHA
FEHLING'S TEST	+ve	+ve	+ve	+ve
CATALASE TEST	+ve	+ve	-ve	-ve
INDOLE TEST	+ve	+ve	+ve	+ve
UREASE TEST	+ve	-ve	-ve	-ve
METHYL RED TEST	+ve	+ve	+ve	+ve
CITRATE UTILIZATION TEST	+ve	+ve	-ve	-ve
MBRT TEST	Decolorized	Decolorized	No change	No change
TSI TEST	Blackening	Red slant, yellow butt	Yellow slant, yellow butt	Red slant, yellow butt

MacConkey agar is a selective and differential medium widely used for the isolation and identification of Gram-negative bacteria, particularly those belonging to the *Enterobacteriaceae* family, which are commonly found in contaminated milk.

The MacConkey plate count analysis highlighted varying levels of Gram-negative bacterial contamination across the milk samples (Table 5). AASHIRVAD recorded the highest bacterial load with 201×10^3 CFU/ml, followed by AMUL with 161×10^3 CFU/ml and SUDHA with 123×10^3 CFU/ml. In contrast, RAJ showed the lowest contamination level, with only 10×10^3 CFU/ml, indicating better microbial quality. These results suggested differences in hygienic practices and microbial contamination during the processing and storage of the milk from different brands.

Table 5. MacConkey plate count determining the colonies of bacteria

MILK BRANDS	MAC CONKEY PLATE COUNT (*10^3 c.f.u./ml)
AMUL	161
AASHIRVA D	201
RAJ	10
SUDHA	123

Discussion:

Cow milk is one of the commonly consumed beverages globally, but it has also been listed as a possible avenue of chemical and microbial contaminants that are directly and indirectly caused by different sources. Even though the most common way to treat milk is through the process of pasteurization, it can only perform partially when it comes to destroying these contaminants (Surajit et al., 2015). The process has been rather popular, yet it does not guarantee complete milk clearing of harmful microorganisms and chemical deposits that may still remain in it (Vincenzina et al., 2020), that questions the effectiveness of this process to ensure milk safety and quality, and, therefore, the topicality of alternative methods of its treatment.

Although cow milk is very popular across the world, it often contains microbial and chemical-related contamination because it undergoes contamination at several points. Conventional pasteurizing is what is typically used by the industry, but it fails to eliminate these contaminants fully; it can produce negative effects as well (Ryser et al., 2016). Studies have revealed that milk is often contaminated with species like Micrococcus, Staphylococcus and Streptococcus with Staphylococcus aureus being a larger cause of mastitis in dairy cows (Silva et al., 2010). It is known to cause gastrointestinal diseases because it contains pathogens that can cause diseases when consumed, leading to such consequences as fever, nausea, and diarrhea (Alqeer et al., 2022; Yadav et al., 2014). The level of microbial contamination in milk of various brands also differed significantly, and this fact can be explained by the fact that various production processes, environmental factors, and packaging were used. Among the brands which tested, the microbial presence was greatly present in Amul and Aashirvaad brands with the standard plate count showing the same in the result. The samples used captured up to 201x 103 CFU/mL limit exceeding the maximum bacteria count limit reported earlier as 100,000 CFU/mL. These observations have been supported by historical data, as the bacterial determines had been 93,000 CFU/mL in 2010 and 94,000 CFU/mL in previous cases.

These facts indicate the increasing bacterial resistance to traditional schemes of sterilization. The presence of significant microbial contamination was identified by use of microbiological tests (Methylene Blue Reduction Test and standard plate counts) making it very important to employ adequate practices of handling milk. Precautions like boiling and storing under 8oC are advised in order to decrease the microbial danger and extend the shelf life of milk.

The new pasteurization technologies have shown promise in effectively decreasing the microbial contamination. So-called supercritical and high-pressure carbon dioxide methods have also been effective to deactivate pathogenic forms of E. coli and L. monocytogenes, as has UV-C treatment and Ozone exposure, in controlled settings. However, there exist a few ways (including YAG laser treatment) that only reduce bacteria but do not change the physicochemical properties of milk much (Totan et al., 2020). Despite the novel technologies that provide potential substitutes and assist in filling the research gaps that currently exist, the technical requirements and cost of operation restrict the extensive application of the technologies among consumers and in dairy industry. It requires research through the further study to come with inexpensive, feasible, and adaptable solutions to enhance safety and quality of milk.

Conclusion:

This study showed the presence of harmful bacterial species in packaged cow milk which can have harmful impact on humans, especially on infants and old people who are the major consumers of these packaged cow milk. The harmful bacterial species *Escherichia coli, Salmonella* spp., *Staphylococcus* spp., *Listeria* spp., *Yersinia* spp., *Clostridium* spp. were present which can pose health risk to the consumer. Pasteurization is not successful in eradicating or inactivating spores from the milk as spores can resist high and low temperatures, desiccation, radiation, ionization, disinfectant agents and ultraviolet light. The goal of this research is to raise awareness about the importance of ensuring the safe production, distribution, and processing of milk. By providing consumers with detailed information about the potential risks of consuming contaminated milk, the research seeks to empower them to make informed decisions. When consumers are aware of the dangers associated with unsafe milk, they are more likely to demand higher safety standards from producers, suppliers, and processors. This increased consumer advocacy can drive improvements in the milk supply chain, ultimately leading to safer, higher-quality milk for all. This study aimed to assist the dairy industry in improving food safety protocols and ensuring that consumers receive high-quality milk.

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