# Microbial Profiling of Fresh and Spoiled Milk Products from Local Dairies in Rajnandgaon, Chhattisgarh: Implications for Dairy Safety and Quality Control

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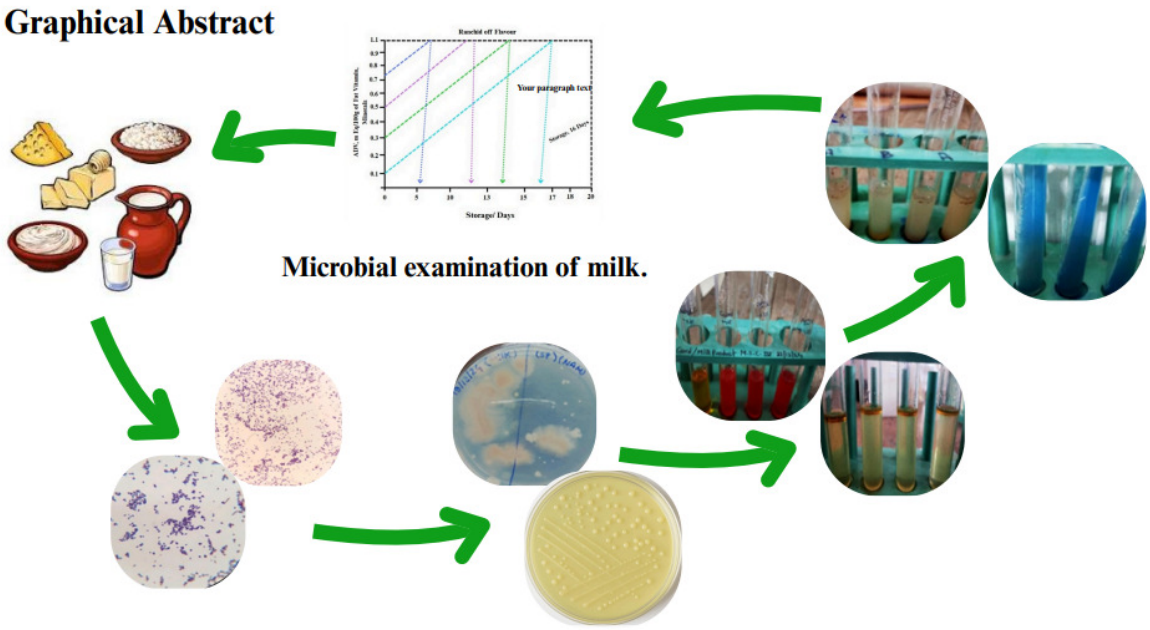
## Abstract

Milk and milk products are essential dietary components due to their rich nutritional profile, yet they are highly prone to microbial contamination, leading to spoilage and potential health hazards. This study investigates the microbial composition of fresh and spoiled milk and milk products collected from local dairies in the Rajnandgaon district of Chhattisgarh. Samples were subjected to microbial examination to identify and quantify contaminating microorganisms. The results revealed a significant variation in microbial load between fresh and spoiled samples, with the latter exhibiting higher levels of spoilage-associated bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas spp.* Factors contributing to contamination were linked to inadequate hygiene during milking, improper storage, and suboptimal processing practices. This study underscores the urgent need for implementing stringent quality control measures and adopting best practices in dairy management to ensure the safety and quality of milk and its derivatives in the region.

**Key words** *E. coli,* Milk, microorganisms, *Pseudomonas* spp., Rajnandgaon, Spoilage.




## Introduction

Milk and milk products have long been considered a cornerstone of human nutrition, offering an abundant source of essential nutrients such as proteins, vitamins, minerals, and bioactive compounds (Kumar, S., *et. al.,* 2025). Globally, dairy consumption forms an integral part of daily diets, and India, in particular, holds the distinction of being the world's largest producer and consumer of milk (Wang, Y., & Li, S. (2008). Within India, the state of Chhattisgarh has witnessed a steady growth in local dairy farming and milk-based enterprises, with districts like Rajnandgaon emerging as pivotal hubs for milk production and distribution (Sahoo, K., & Jena, L. K. (2022). However, alongside increased milk consumption comes the pressing concern of dairy safety, largely influenced by microbial contamination, poor handling practices, and inadequate quality control mechanisms (Ntuli, V., Sibanda *et. al.,* 2023). Microbial contamination is an inherent challenge in dairy production, primarily because milk is an excellent growth medium for a wide variety of microorganisms (Pal, M., *et. al.,* 2016). From the point of milking to its final consumption, milk can be contaminated by pathogenic and spoilage microbes through numerous vectors unclean equipment, handlers, water supply, storage conditions, and environmental exposure (Girma, K., *et. al.,* 2014).

The result is not only a reduction in shelf life and organoleptic quality of milk but also significant public health risks, especially due to pathogens such as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella spp.*, and *Staphylococcus aureus* (Fusco, V., *et. al.,* 2020). These microorganisms are often associated with foodborne illnesses and have been implicated in outbreaks linked to the consumption of contaminated milk and dairy products (Oliver, S. P., Jayarao, B. M., & Almeida, R. A., 2005). Spoilage microbes, though often non-pathogenic, play an equally important role in determining milk quality (Quintieri, L., *et. al.,* 2021). Species such as *Pseudomonas*, *Lactobacillus*, and certain yeasts and molds can cause undesirable changes in taste, texture, and appearance (Rawat, S., 2015). The economic impact of such spoilage is profound, particularly for small-scale and local dairies where profit margins are slim, and wastage due to spoilage can cripple operations (Abdullah, M., 2004). Therefore, microbial profiling that is, the identification and characterization of microbial communities present in fresh and spoiled milk is critical for understanding contamination patterns, improving safety protocols, and enhancing overall quality control (Chatterjee, D., Jha, S. K., & Maiti, S., 2018).

In the context of Rajnandgaon, a district characterized by a mix of traditional dairy farming and emerging small-scale commercial dairies, the need for systematic microbial profiling is particularly urgent (Lemma D, H., Mengistu, A., Kuma, T., & Kuma, B. 2018). Most local dairies operate with minimal mechanization and limited access to quality assurance tools, making them susceptible to microbial risks (Bhatt, P., *et. al.,* 2024). Moreover, climatic conditions in the region hot summers, monsoon humidity, and limited cold chain infrastructure further exacerbate microbial proliferation (Fusco, V., *et. al.,* 2020). Despite these risks, there is a paucity of scientific data regarding the microbial diversity present in locally produced milk and dairy products in this region (Bihola, A., *et. al.,* 2025). This research seeks to address this gap by conducting a comprehensive microbial profiling of fresh and spoiled milk samples collected from local dairies across Rajnandgaon (Fusco, V., & Quero, G. M., 2014). Using culture-dependent techniques complemented by molecular methods, this study aims to isolate, identify, and quantify both beneficial and harmful microorganisms in these dairy products (Karanth, S., *et. al.,* 2023). Fresh milk samples will be analyzed to assess their microbial baseline and adherence to food safety standards, while spoiled samples will be studied to understand spoilage pathways and dominant microbial taxa responsible for product degradation (Bokulich, N. A., *et. al.,* 2015).

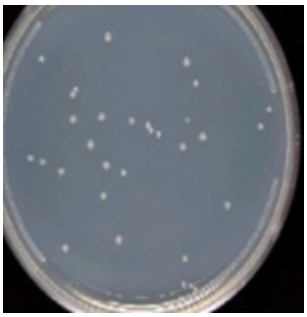
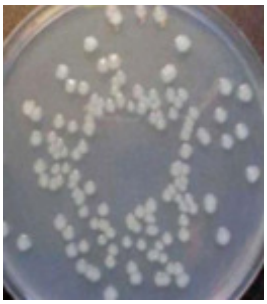
***Table No. 01:*** **Fungus isolate of samples**

| SN | Samples | Colonies | Isolate | Culture characteristics of on SDA and lactophenol cotton blue | Fungus |
|---|---|---|---|---|---|
| **1** | Milk (Fresh) | 1 | M1 | Creamy white colonies transversally and longitudinally septate breaker conidia in acroprtal manner. | *Candida species* |
| **2** | Milk (Spoilage) | 1 | M2 | Black colonies, conidiophores arising from a foot cell, basipetal conidia on phalides. | *Aspergillus species* |
| **3** | Curd (Fresh) | 1 | C1 | Greenish colonies, conidia in long chain on repeatedly branched conidiophoresrs resembling brush like head. | *Penicillium species* |
| **4** | Curd (Spoilage) | 1 | C2 | Creamy white colonies, transversally and longitudinally septate breaker conidia in acroprtal manner. | *Rhizopus Species* |

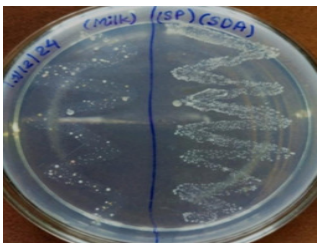
***Table No. 02:*** **Culture characteristics and Grams stain of bacterial isolates.**

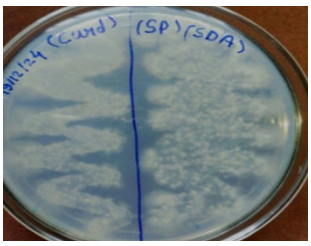
| SN | Samples | Colonies | Isolate | Culture characteristics | Grams stain |
|---|---|---|---|---|---|
| **1** | Milk (Fresh) | 1 | M1 | Circular convex opaque creamy white colonies | Gram Positive (Cocci ) |
| **2** | Milk (Spoilage) | 1 | M2 | Large opaque irregular white colonies | Gram Negative (Rod) |
| **3** | Curd (Fresh) | 1 | C1 | Circular convex opaque creamy white colonies | Gram Positive (Cocci) |
| **4** | Curd (Spoilage) | 1 | C2 | Large thick greyish white colonies | Gram Negative (Rod) |

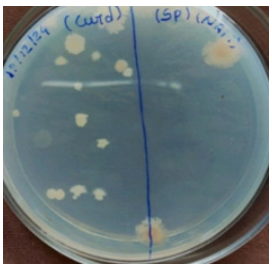
***Figure No. 01:*** **Culture of Milk Derivatives**

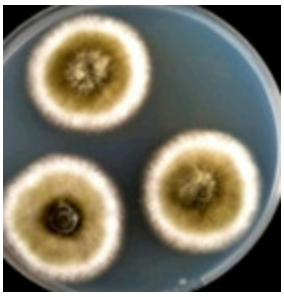
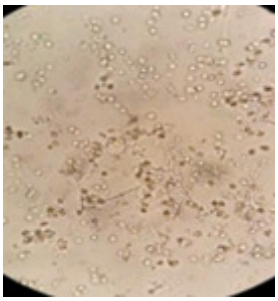
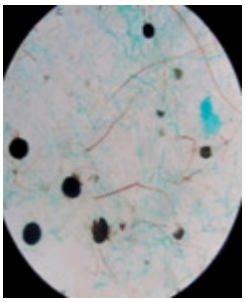
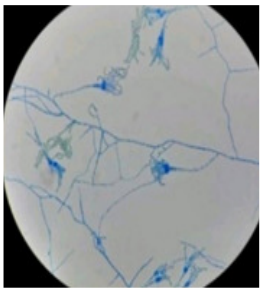
**Milk and Milk Product Culture Product**







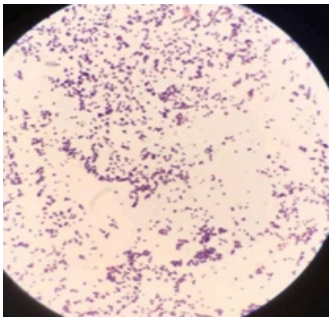

**SDA Culture** | **NAM Culture**

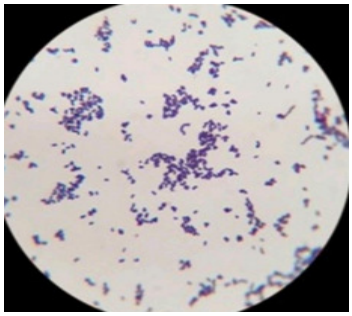
**Fungus Culture** | **Fungus Staining**

*Figure 02:* **Culture of Milk Derivatives and IMVIC Biochemical Test.**

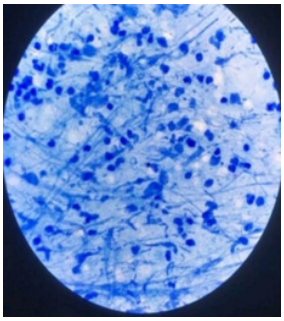
**Culture of Milk Derivatives**

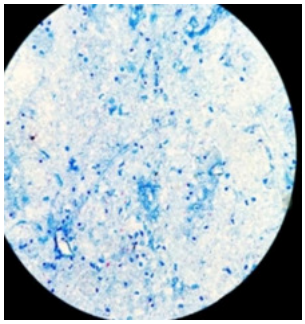


**Milk Gram Staining Negative**



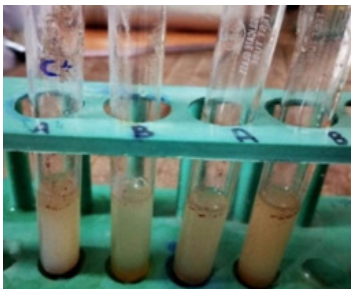
**Curd Gram Staining Positive**



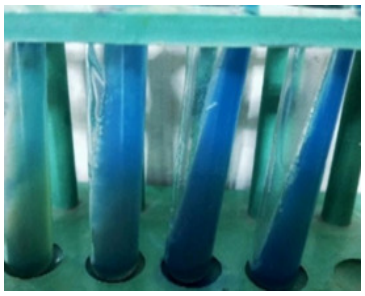


**Milk and Curd acid fast staining (Negative and Non acidic)**

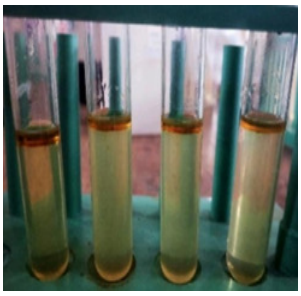
**IMVIC Biochemical Test**



**VP Test**



**Citrate Utilization Test**



**Indole test**

**MR Test**

# Material and Methods

## Collection of samples

Some fresh and spoiled milk and milk products samples are collected from local dairy of Rajnandgaon district in sterilized containers (Mukhopadhyay, M., *et. al.,* 2024, April)..

**Inoculation of samples**

Samples were inoculated on Nutrient Agar Medium and sabouroud Dextrose Agar Medium by pour plate and spread plate method (Acharya, T., & Hare, J., 2022).

**Incubation**

After inoculation plates were incubated in incubator at 37degree for bacteria for 24 hours and 25 degree for fungus for 48 hours respectively (Ayyadurai, N., *et. al.,* 2006).

**Culture characteristics**

**Bacteria**

Identification of isolates was done by culture characteristics include on Nutrient Agar Medium. Culture characteristics include colour, shape, size, margin and elevation (Patra, J. K., *et. al.,* 2020).

**Fungus**

Identification of isolates was done by culture characteristics on sabouroud Dextrose Agar Medium culture characteristics include colour, shape, size, margin and hypha or conidia (Devi, K. S., Misra, *et. al.,* 2018).

**Microscopic Examination**

**Bacteria**

Grams stain and simple staining were used for the identification of bacterial isolates. Simple staining determine shape and size of bacterial cells while Grams staining divide bacteria into the two major groups that is Gram Positive and Gram negative . Gram stain also gave detail about shape of the cell (Becerra, S. C., *et. al.,* 2016).

**Fungus**

Fungus were stained by lacto phenol cotton blue which stain hyphae cytoplasm , on the basis or arrangement of the hyphae fungus were identified (Li, D. W., Yang, C. S., & Harrington, F., 2007).

**Biochemical Examination**

Various biochemical test are performed for the identification of isolates grow on Nutrient Agar Medium (Holding, A. J., & Collee, J. G., 1971).

**Indole Productions Test**

Indole test is used to determine the ability of an organism to split amino acids tryptophan to form the compound indole (Barik, S., 2020). Tryptophan is hydrolyzed by tryptophanase to produce three possible end products – one of which is indole (Isenberg, H. D., & Sundheim, L. H., 1958). Indole Productions is detected by kovacs or Ehrlich's reagent which contains 4 ( p)-dimethylamino benzaldehyde, this react with indole to produce a red coloured compound (Barry, A. L., Bernsohn, *et. al.,* 1970).

**Methyl red test (MR)**

Methyl red test is performing in methyl red media (Cook, R. P., 1930). The methyl red test detects production of acid during metabolism using mixed acid fermentation pathway using pyruvate as a substrate (Salwan, R., Rana, A., & Sharma, V., 2023). The pH indicator methyl red is added to one tube and a red colour appears at pH lower than 4.2, indicating positive test (mixed acid fermentation) the solution remaining yellow pH 6.2 or above indicating negative result used butenedol fermentation (Levine, M., 1916)..

**Voges - Proskauer (VP)**

The Vogue's Proskauer test is used to determine if an organism produces acetylmethyl carbinol from glucose fermentation (PRADHAN, M., 2023). If present, acetylmethyl carbinol is converted to diacetyl in the presence of alpha-napthol, strong alkali, and atmospheric oxygen (Barritt, M. M., 1936). The alpha naphthol was not part of the original procedure but was found to act as a color intensive by Barritt and must be added first (MacWilliams, M. P., 2009).

**Citrate Utilization Test**

This Test uses simmons citrate Agar to determine ability of a microorganisms to citrate as a sole source of carbon (Reddy, M. S., Vedamuthu, *et. al.,* 1972). The Agar contains citrate and ammonium ions and bromothymol blue as an indicator (Tindall, B. J., *et. al.,* 2007). The citrate

Agar is green before incubation and turns blue as a positive test indicator meaning citrate is utilized (Dimri, A. G., *et. al.,* 2020).

**Oxidase Test**

The oxidase test is a test used in microbiology to determine if a bacterium produce certain cytochrome C oxidase detect all gram Negative bacteria expect lactose fermenter which are negative (Becker, K., Skov, R. L., & von Eiff, C., 2015). The reagents is dark blue to maroon when oxidised, and colourless when reduced (Clark, W. M., Cohen, B., & Gibbs, H. D., 1925).

**The Catalase Test**

This test is performing to identification of *Enterococci*and *staphylococcusaureus*. Catalase is an enzyme produced by microorganisms (Watt, B. E., Proudfoot, A. T., & Vale, J. A., 2004). It breaks down hydrogen peroxide in hydrogen and oxygen because hydrogen peroxide is toxic for cells (Mann, P. J. G., & Quastel, J. H., 1946).

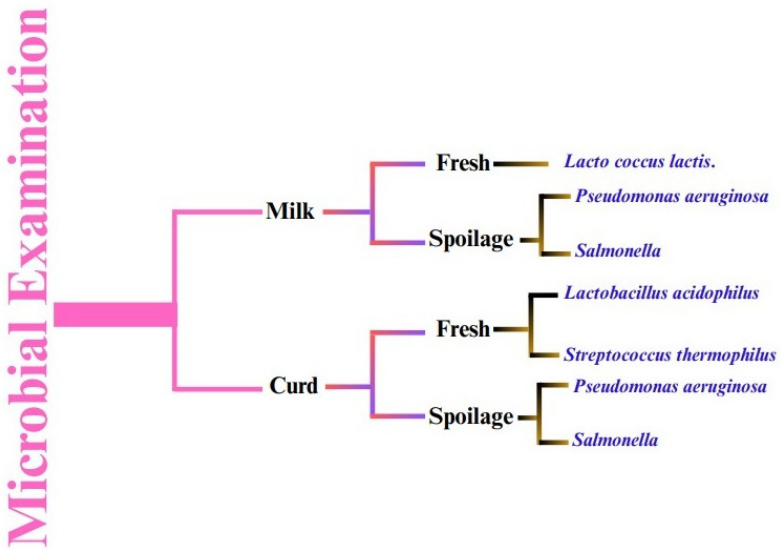
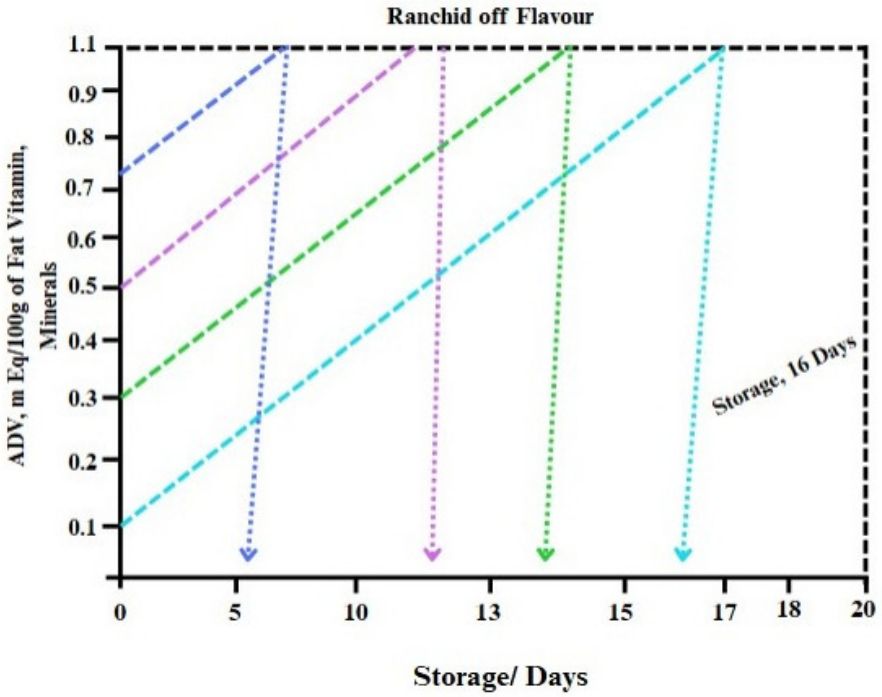



***Figure No.03:*** Cladogram of identify species.

**Result and Discussion**

Fresh Milk yielded *Candida* species, forming creamy white colonies with acropetal conidia, indicating the presence of opportunistic yeasts, commonly found in raw milk, possibly introduced via unhygienic handling or storage. Spoiled Milk was found to be contaminated with *Aspergillus* species, forming black colonies with conidiophores arising from foot cells, a common spoilage and toxigenic mold in milk. Its presence reflects poor storage or prolonged exposure to ambient conditions. Fresh Curd showed the presence of *Penicillium* species, forming greenish colonies with characteristic brush-like conidiophores. While some *Penicillium* strains are used in dairy fermentations, wild types can contribute to spoilage if curd is stored improperly. Spoiled Curd contained *Rhizopus* species, producing creamy colonies with typical septate hyphae. *Rhizopus* is a fast-growing spoilage mold, suggesting contamination during post-production handling or inadequate refrigeration. Nutrient Agar Medium (NAM) supported bacterial growth in all dairy samples, with gram staining and colony morphology aiding identification [From, Table 02 and Figure 02]. Fresh Milk and Fresh Curd samples showed circular, convex, creamy white colonies and were Gram-positive cocci, possibly *Lactococcus* or *Streptococcus* species, known for their role in fermentation and probiotic effects. Spoiled Milk and Curd both showed Gram-negative rods with irregular large opaque colonies, suggestive of Enterobacteriaceae, such as *Escherichia coli* or *Pseudomonas* spp. These spoilage and pathogenic bacteria often indicate fecal contamination or poor sanitation during handling and storage. This differentiation between cocci in fresh samples and rods in spoiled ones indicates a microbial shift from benign to potentially harmful flora as spoilage progresses. Biochemical assays were conducted to further differentiate Gram-negative isolates [From, Table 02 and Figure 03]. Indole Test; Positive indicating ability to degrade tryptophan, typical of *E. coli*. MR Test; Positive confirming mixed acid fermentation. VP Test; Negative consistent with absence of butylene glycol fermentation [From, Table 01 and Figure 01]. Citrate Utilization; Positive suggesting versatility in carbon source metabolism. These results strongly point toward *Escherichia coli* contamination in spoiled milk, a red flag for public health due to its association with gastrointestinal infections [From, Figure 01 and Graph 01].

Spoiled curd isolates showed similar profiles, reinforcing the risk posed by improper curd handling and storage. Acid-fast staining of all bacterial isolates was negative, indicating absence of *Mycobacterium* spp., such as *M. bovis*, a zoonotic concern in dairy [From, Figure 01 and Graph 01]. Catalase Test; Positive suggesting presence of catalase-producing Gram-positive cocci like *Staphylococcus aureus*, particularly in curd samples [From, Figure 02, 03 and Graph 01]. Oxidase Test; Positive in Gram-negative rods, confirming *Pseudomonas* or *E. coli* groups, consistent with other IMVIC findings. The study reveals a clear pattern of microbial progression from beneficial or benign flora in fresh samples to spoilage and pathogenic organisms in spoiled ones [From, Table 01, 02; Figure 01, 03 and Graph 01]. The detection of *Candida*, *Aspergillus*, and *Rhizopus* in spoiled products underscores the critical need for improved hygiene and cold chain maintenance in Rajnandgaon dairies. Spoilage organisms were dominant in curd and milk with poor storage history, especially under high ambient temperatures and humidity typical of monsoon conditions in the region. The high bacterial load in spoiled samples, particularly Gram-negative rods with enteric biochemical profiles, suggests contamination routes through water, handlers, or equipment [From, Table 02 and Figure 01].




***Graph 01:*** Milk Quality as per days.

**Conclusion**

This study highlights a significant microbial shift in dairy products from beneficial or benign organisms in fresh samples to spoilage and pathogenic microorganisms in spoiled ones primarily due to inadequate hygiene and storage practices. Fresh milk and curd predominantly harbored lactic acid bacteria such as *Lactococcus* and *Streptococcus*, known for their probiotic and fermentative roles. In contrast, spoiled samples were dominated by opportunistic fungi (*Candida*, *Aspergillus*, *Penicillium*, and *Rhizopus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas* spp.), indicating microbial spoilage and potential public health risks. The presence of *E. coli*, confirmed through biochemical profiling, points toward fecal contamination, likely introduced through unclean handling, equipment, or water sources. Additionally, catalase-positive *Staphylococcus* spp. detected in curd samples suggest another route of contamination. These findings underscore the urgent need for improved sanitation, rigorous cold chain maintenance, and awareness among dairy handlers in Rajnandgaon, especially during the monsoon season when ambient conditions favor microbial growth. Enhanced monitoring and stricter hygiene protocols are essential to ensure the microbial safety and quality of dairy products in the region.

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