



## Hygiene indicators in a milk processing plant – a review

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### ABSTRACT

This review paper focuses on the current microbiological parameters employed to evaluate the hygiene of raw milk and dairy products within processing plants. Key indicators of raw milk hygiene and overall quality include the total bacterial count (TBC) and somatic cell count (SCC). As the primary raw material in dairies for producing drinking milk and various dairy products, milk offers an ideal environment for the proliferation and spread of numerous microorganisms. In the dairy industry worldwide, contamination after pasteurization is commonly detected using indicator groups such as coliform bacteria, enterobacteria, total gram-negative bacteria, *Pseudomonas*, and gram-positive spore-forming bacteria. Enterobacteriaceae serve as hygiene indicators because they encompass a broad range of bacteria, including harmful species like *Salmonella* and *Klebsiella pneumoniae*. However, the standard coliform test depends on lactose fermentation, and since not all harmful enterobacteria—such as *Salmonella*, *Shigella*, or *Yersinia*—can ferment lactose, they may go undetected. Contamination of final dairy products can cause outbreaks of foodborne illnesses and incur additional expenses due to product recalls. Therefore, it is advisable to monitor hygiene indicators throughout milk production and processing to evaluate the effectiveness of the production process and decrease the necessity for repeated testing of final products.

## 1. Introduction

Milk, being the primary raw material in dairy plants for producing drinking milk and various dairy products, provides an ideal environment for the growth and proliferation of diverse microorganisms. The multiplication of these microorganisms can lead to food contamination and subsequently cause foodborne illnesses in consumers (Lakticova *et al.*, 2020). Data from the United States Foodborne Disease Surveillance System indicate that, between 2009 and 2015, dairy products ranked as the second

most frequent source of outbreaks, following fish (Dewey-Mattia, 2018). Prompt and systematic monitoring of food quality, particularly milk quality, is indispensable for ensuring hygiene, safety, and public health throughout the entire food supply chain. The microbiological data obtained through such surveillance plays a pivotal role in validating food safety management systems and mitigating the incidence of product recalls and associated economic losses (Kadyan *et al.*, 2020; Poghosian *et al.*, 2019).

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Milk contains all the essential amino acids needed by the human body (Ahmed *et al.*, 2019), but in its raw form, it also carries a variety of microorganisms. Some of these can be harmful and cause foodborne illnesses, while others, though not dangerous, can lead to spoilage (Souad *et al.*, 2021; Merwan Ahmedsham *et al.*, 2018). To make milk safer and extend its shelf life, it undergoes heat treatment—pasteurization. This process kills or deactivates 99.99% of both harmful and harmless microorganisms (Golić *et al.*, 2019), including all vegetative forms of bacteria, psychotropic microorganisms, yeasts, molds, and certain unwanted enzymes, all while maintaining the milk's nutritional quality. Despite pasteurization, milk can still become contaminated. This can happen due to contact with unclean processing equipment, improper handling by workers, contaminated packaging, or if the pasteurization process itself is not performed correctly (Postoli and Shehu, 2018).

Maintaining proper hygiene is essential in producing safe and high-quality milk and dairy products, as it helps reduce microbial contamination. One way to assess cleanliness and safety is through the use of indicator organisms. These are specific microbes whose presence signals the overall sanitary condition of the food or processing environment. They can reveal contamination that may have occurred after cleaning procedures, during handling, or during storage. Additionally, these indicators can suggest the possible presence of harmful pathogens that could pose a risk to public health. In the global dairy industry, common groups of indicator bacteria used to detect post-pasteurization contamination include coliforms, Enterobacteria, total Gram-negative bacteria, *Pseudomonas*, and Gram-positive spore-forming bacteria (Hervet *et al.*, 2016). These groups help monitor cleanliness and ensure that safety standards are being met.

This review will discuss the current microbiological parameters used to assess the hygiene of raw milk and dairy products in the processing plant.

## 2. Milk production hygiene

Advanced milking automation is increasingly replacing manual methods on leading dairy farms. Nonetheless, proper hygiene training remains essential for all personnel, as contamination primarily stems from milking equipment and human contact. The total bacterial count (TBC) and somatic cell count (SCC) are key indicators of raw milk

hygiene and overall quality (Rodrigues *et al.*, 2017). These are directly influenced by factors such as barn cleanliness, milking techniques, udder hygiene before, during, and after milking, equipment sanitation, and prompt cooling of milk post-milking. High TBC and SCC levels—common in milk from many dairy farms in Serbia—signal breakdowns in hygiene practices before and during milking. Literature data indicate that enhancing corrective and preventive hygiene measures related to farm conditions, milking procedures, and immediate milk cooling after milking leads to significant and continuous improvements in milk quality. This is reflected in improved TBC and SCC results. At the beginning of a study in Serbia, only 19.7% of milk samples met first-class quality standards, whereas by the end, this number had increased to 50.0%, clearly demonstrating the effectiveness of the implemented measures (Mihajlović *et al.*, 2022). These tests (TBC and SCC) mostly reflect on-farm hygiene rather than the final quality of dairy products.

However, two main groups of bacteria in raw milk can affect product quality: psychophilic and psychrotolerant bacteria (like *Pseudomonas*), spore-forming bacteria (like *Paenibacillus* and *Clostridium*). If raw milk is poorly refrigerated or stored too long, these bacteria can multiply and produce enzymes that survive pasteurization. These enzymes can continue breaking down milk components, causing off-flavors, aroma issues, and texture problems in dairy products. For example, high bacterial counts in raw milk can reduce cheese yield and cause defects during aging. In UHT products, leftover enzymes can lead to age gelation and spoilage during long-term storage, with signs like thickening, sediment, and bad odors (Martin *et al.*, 2023; Murphy *et al.*, 2016).

Spore-forming bacteria are another major group of microorganisms in raw milk that significantly impact the quality of finished dairy products. These bacteria, from the Bacillales and Clostridiales orders, form endospores that originate in the farm environment, make their way into raw milk, survive processing, and later grow, causing spoilage (Gopal *et al.*, 2015). Psychrotolerant spore-formers, which can grow at refrigeration temperatures, are a particular concern for liquid milk spoilage. Research shows they are responsible for 40–50% of the cases where milk in the U.S. reaches the 20,000 CFU/mL limit set by the Pasteurized Milk Ordinance during its shelf life. These bacteria are considered the main contributors to the limited shelf life of liquid milk (Alles *et al.*, 2018; Reichler *et al.*, 2018).

Addressing these key microbial contaminants in raw milk is essential for improving the quality of dairy products. It is a critical focus for the entire dairy supply chain, because consumer dissatisfaction with product quality directly impacts purchasing behavior and long-term consumption.

### 3. Hygiene indicators in dairy products

In developing countries, over 20% of milk production is lost due to early spoilage and microbial contamination occurring at different points throughout the production process (Fox et al., 2017). Some of this loss may result from dairy products spoiling too early, often because of poor sanitation or contamination after pasteurization during processing. Bacteria such as coliforms, Enterobacteriaceae, and other Gram-negative organisms are common indicators of these hygiene issues in dairy products around the world (Hervert et al., 2016). The use of Enterobacteriaceae as an indicator of hygiene covers a wide range of bacteria, including harmful species, such as *Salmonella* and *Klebsiella pneumoniae* (Hervert et al., 2017). The detection of these bacteria in milk suggests that safety protocols may have been followed during processing, but that subsequent handling of the milk was likely poor. Common sources of contamination with these bacteria include human or animal feces, water, equipment, and poor personal hygiene (Yilma and Faye, 2006). When equipment and facilities are not properly designed for cleanliness, they can have hidden areas where bacteria like *Salmonella* can survive and thrive, even after cleaning. These bacteria do not always appear in predictable patterns—they can come from many different sources. They can also stick to surfaces and form protective layers (called biofilms), making them harder to remove and increasing the risk that the food will be contaminated (Hervert et al., 2017). Additionally, pests like rodents and insects can carry *Salmonella* and spread it further.

The choice of which hygiene indicator to use depends on the objective. For routine monitoring during production, it is not very important whether Enterobacteriaceae or coliform counts are used. However, coliforms are easier to cultivate and identify, making them more practical when the goals are to determine which species of bacteria are present and to assess their potential risks to consumers. On the other hand, Enterobacteriaceae are more suitable for assessing overall food hygiene and safety (Souad et al., 2021).

Dairy products, like yogurt, can carry Enterobacteriaceae. That is why it is essential to follow strict hygiene and manufacturing practices at every step of the production process. Just because common foodborne pathogens are not found in yogurt does not necessarily mean it is safe, as other harmful bacteria from the Enterobacteriaceae family could still be present. Monitoring for Enterobacteriaceae helps assess whether the preventive hygiene measures in place are effective. This kind of testing offers valuable information about the cleanliness, quality, and safety of yogurt products and helps ensure that sanitation procedures in the production facility are working properly (Knezevic et al., 2021). That study found that 21.29% of yogurt samples contained more than 10 CFU/g of Enterobacteriaceae, indicating problems such as poor hygiene, processing problems, or post-production contamination (Knezevic et al., 2021).

Coliforms are Gram-negative, rod-shaped bacteria, able to survive with or without oxygen. They can ferment lactose to produce gas and acid (Feng et al., 2002). For a long time, coliform bacteria counts have been used to check the hygiene of dairy products. For example, studies in the U.S. from 2001 to 2010 found that 7.6–26.6% of liquid milk samples were contaminated with coliforms after production (Martin et al., 2012). In a study conducted in Cameroon (2012–2013), most yogurt samples had high levels of coliforms—over  $10^2$  CFU/mL. From these samples, 21 different species of Enterobacteriaceae were found among the 72 bacterial isolates originally identified as coliforms. The standard test for coliform bacteria relies on lactose fermentation, but not all harmful Enterobacteriaceae—such as *Salmonella*, *Shigella*, or *Yersinia*—can ferment lactose, so they would not be detected. Replacing lactose with glucose in the test would help detect a wider range of these bacteria, including harmful ones. Because Enterobacteriaceae are more resistant to harsh conditions than coliform bacteria, they can serve as more reliable indicators of hygiene and sanitation in food production (Knezevic et al., 2021).

Research shows that raw milk typically contains low levels of coliform bacteria and/or *E. coli*. However, their presence in raw milk does not necessarily predict their levels in final cheese products. This is because the cheesemaking and ripening processes can greatly influence the growth and survival of these bacteria. Various factors—such as the type of starter cultures, salting methods, aging conditions, and ripening duration—affect the final composition, texture, and bacterial content of the cheese.

Since there are hundreds of different raw milk cheeses with differing characteristics, it is difficult to generalize the number of indicator bacteria present in all of them. Usually, an initial increase in bacterial numbers occurs during early ripening, followed by a decline as the cheese matures. This early increase may be due to bacteria multiplying or being trapped in the curd during production (Metz *et al.*, 2020). Research shows that *E. coli* and other indicator bacteria are commonly found in raw milk, typically at low levels—often below 100 CFU/g, and under 10 CFU/g in high-quality milk. During the early stages of cheese production, these bacteria can multiply, but their numbers generally decline significantly during aging and ripening, except in fresh, unripened cheeses. By the time the cheese is fully matured, indicator bacteria are usually either absent or present at very low levels—often below the limits (10–100 CFU/g) set by many countries. When high levels of indicator bacteria are found in cheese, the cause is usually poor-quality raw milk, unsanitary production conditions, or both. However, raw milk cheeses made from clean, high-quality milk and produced using good manufacturing practices typically do not have high levels of these bacteria (Metz *et al.*, 2020).

#### 4. Milk plant processing hygiene

Good hygiene practices (GHP) form the foundation of any effective food safety management system and ensure that the Hazard Analysis and Critical Control Points (HACCP) system operates effectively. GHP helps to prevent the introduction and spread of harmful microorganisms, minimize the risk of food contamination during processing, handling, and storage, ensure compliance with national and international food safety regulations, and maintain consumer trust by showing a commitment to producing safe and high-quality food (Postoli and Shehu, 2017).

Keeping food contact surfaces clean, a fundamental of GHP, is essential to prevent cross-contamination and maintain food safety. Inadequately cleaned surfaces can harbor microorganisms that reduce a product's shelf life, but also pathogens that pose serious health risks if transferred to food. The high incidence in Europe of outbreaks (around 39%) associated with cross-contamination highlights its

importance as a food safety risk (Whitehead and Verran, 2006). To mitigate these risks, food safety management systems like HACCP, supported by prerequisite programs (PRPs), emphasize the necessity of effective cleaning and sanitation protocols.

However, the variability in cleaning outcomes across the food industry highlights the importance of systematically evaluating and adjusting cleaning schedules to ensure effectiveness. Manufacturers have a legal and ethical responsibility to maintain cleanrooms by designing and following targeted cleaning programs, especially for surfaces that come into direct contact with food. Microbiological tests, such as surface swabbing and agar contact plates, are commonly employed to detect bacterial contamination on food contact surfaces. Quantitative analyses determine the presence of indicator microorganisms on surfaces to assess overall hygiene levels, with high counts signaling inadequate cleaning or sanitation. These indicators, such as total bacterial counts and Enterobacteriaceae counts, help assess the potential risk of contamination during food production. Qualitative analyses, on the other hand, focus on detecting specific pathogens—such as *Salmonella* spp. or *Listeria monocytogenes*—and reveal whether these dangerous microorganisms are present or absent in the tested area, rather than how many are present. Together, these two data provide a comprehensive picture of both the overall hygiene status and specific safety threats in the production environment (Postoli and Shehu, 2017).

#### 5. Conclusion

Relying solely on final product control to ensure food safety has its drawbacks. Contaminated food products can lead to outbreaks of foodborne illness and result in additional costs due to product recalls. Therefore, it is recommended to use hygiene indicators throughout the milk production and processing process, which help to assess how well the production process is functioning and reduce the need for repeated controls of finished products. By consistently applying and monitoring GHP, food businesses can reduce the risk of foodborne illnesses and product recalls, while also providing documented proof of their commitment to food safety and hygiene standards.

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