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Redefining Raw Milk Quality: A Comprehensive Evaluation of Microbiological Parameters for Ensuring High-Quality Dairy Products

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ABSTRACT

Raw milk, when freshly secreted from the udder of a healthy animal, contains minimal bacterial contamination. However, it can become contaminated through multiple pathways, including exposure to environmental sources, direct contamination from the cow, and contact with improperly sanitized milking equipment. While raw milk hosts a diverse array of bacteria, certain microbial groups significantly impact the quality of processed dairy products. Specifically, **psychrophilic and psychrotolerant bacteria**, such as *Pseudomonas* species and members of the *Enterobacteriaceae* family, thrive at low temperatures and produce heat-stable enzymes that can compromise dairy product integrity. Additionally, **spore-forming bacteria**, capable of surviving pasteurization and other processing steps in spore form, present a major challenge for dairy quality.Understanding the factors contributing to the presence of these bacterial groups is crucial in mitigating their effects on dairy products. This review examines key microbiological parameters used in the modern dairy industry to assess raw milk quality and identify milk supplies suitable for high-quality dairy processing. We propose that **total bacterial count (TBC)** be adopted as a universal microbiological indicator of raw milk quality. Furthermore, we advocate for the development of a **comprehensive, data-driven, whole-farm approach** to raw milk quality management—one that integrates risk-based tools across the entire production chain, from farm-level practices to processing and shelf-life management, ensuring continuous improvements in dairy product quality.

Keywords: raw milk quality, microbiological testing, bacterial contamination, farm practices

INTRODUCTION

The microbiological composition of raw bovine milk has been the subject of extensive research for over a century. While raw milk is expected to have minimal microbial contamination at the point of secretion, its quality can be significantly impacted by bacterial contamination from environmental sources, milking equipment, and the cow itself. Contemporary and historical studies have consistently emphasized the importance of maintaining high-quality raw milk as an indicator of proper hygienic production practices at the farm level.

From a microbiological perspective, **high-quality raw milk** is often defined by **low total bacterial counts (TBC)**. However, other bacterial groups, such as **coliforms**, also serve as indicators of milk hygiene. Various microbiological tests are employed beyond TBC to assess raw milk quality, including **Preliminary Incubation (PI) count** for psychrophilic bacteria, **Coliform Count (CC)** for fecal contamination assessment, **Laboratory Pasteurization Count (LPC)** for thermoduric bacteria, and **Spore Count Testing** for bacterial spores. These tests help identify specific deficiencies in milking hygiene, with high coliform counts (>100 cfu/mL) indicating inadequate udder cleaning before milking unit attachment.

Despite their widespread use, these tests primarily assess **farm-level hygienic conditions** rather than **predicting the performance of raw milk in processed dairy products**. The impact of microbial contamination on finished dairy products is substantial, as certain bacterial groups can lead to **flavor, odor, and texture defects**. For instance, **cheese produced from raw milk with bacterial loads exceeding 1,000,000 cfu/mL** exhibits reduced yield and undesirable flavor changes during aging. In **Ultra-High Temperature (UHT) milk**, heat-stable **proteases and lipases** produced by microbial contaminants can cause defects such as **age-gelation**, sedimentation, and off-odors, significantly reducing product quality and shelf-life.

Among the most significant microbial contaminants in raw milk are **psychrophilic and psychrotolerant bacteria**, such as *Pseudomonas* species, which thrive at refrigeration temperatures and produce **heat-stable enzymes** capable of degrading milk proteins and lipids even after pasteurization. Additionally, **spore-forming bacteria** (*Paenibacillus* and *Clostridium* species) can survive extreme processing conditions and contribute to spoilage in dairy products. Studies have demonstrated that **high initial concentrations** of these bacterial groups—resulting from improper cooling, prolonged storage, or inadequate hygiene—can significantly impact dairy product integrity.

Given these challenges, raw milk quality should be assessed using a **holistic, farm-to-processing approach** that integrates **risk-based, data-driven monitoring tools**. A **single microbiological indicator, such as total bacterial count (TBC)**, can serve as a comprehensive measure of milk quality.

However, implementing **proactive strategies**, such as improved **farm hygiene, rapid microbial detection technologies, and optimized cold-chain management**, is essential to **minimizing microbial contamination and enhancing dairy product quality across the entire production continuum**.

The second major group of bacteria in raw milk that significantly impacts the quality of finished dairy products is **spore-forming bacteria**, a diverse group classified under the orders **Bacillales and Clostridiales**. These bacteria produce **endospores**, highly resistant structures that allow them to survive harsh environmental conditions, including pasteurization and other processing treatments. Once introduced into raw milk from sources such as soil, contaminated feed, or milking equipment, these spores remain dormant but can **germinate and grow** under favorable conditions, leading to spoilage of dairy products.

Among spore-forming bacteria, **psychrotolerant species** are particularly concerning because they can thrive at **refrigeration temperatures**, making them a significant challenge for **fluid milk preservation**. Studies indicate that these bacteria contribute to **40–50% of fluid milk** in the United States reaching the **Pasteurized Milk Ordinance** bacterial limit of **20,000 CFU/mL** by the end of its shelf life. Their ability to produce **heat-stable enzymes**, such as proteases and lipases, results in undesirable **off-flavors, texture degradation, and spoilage** even after pasteurization.

Another critical group within spore-forming bacteria is the **anaerobic butyric acid bacterial spores** (*Clostridium spp.*), which are responsible for **late-blowing defects in cheese**. This defect, commonly observed in **hard and semi-hard aged cheeses** like **Gouda**, manifests **60–90 days into aging** due to gas production by **Clostridium spores**, leading to **undesirable texture defects and economic losses for cheesemakers**.

Given the significant impact of **spore-forming bacteria on dairy product quality**, a **focused approach** is essential to mitigate their effects. This includes **improving farm hygiene**, **implementing strict milk filtration processes**, and **optimizing storage conditions** to prevent spore proliferation. Addressing these microbial challenges is crucial for ensuring **consumer satisfaction**, as product quality directly influences purchasing behavior and long-term market demand in the dairy industry.

This review explores **modern microbiological parameters** used to assess **raw milk quality**, focusing on the **types of bacterial contaminants**, their **sources**, and **farm management factors** influencing microbial presence. It also critically examines the **limitations** of current evaluation methods when predicting the impact of raw milk microbial contamination on **finished dairy products**.

To redefine raw milk quality assessment, we propose a **comprehensive approach** incorporating:

1. **A single raw milk quality indicator** serving as a **process control test** to evaluate **farm hygiene and consistency** in production practices.
2. **A suite of targeted microbiological tests** to help diagnose and resolve quality issues when **elevated bacterial levels** are detected.
3. **Specific microbial assessments** to measure the presence and concentration of **bacterial groups that directly affect finished product quality**.

Furthermore, we highlight the **need for predictive decision support tools** that integrate **real-time microbial analysis, farm management data, and quality control measures**. By transitioning from a **reactive, test-based approach** to a **proactive, predictive model**, the dairy industry can significantly enhance **milk safety, product consistency, and overall consumer satisfaction**. This shift is essential for maintaining **high-quality dairy products** throughout the **entire supply chain, from production to consumption**.

RAW MILK MICROBIOLOGICAL PARAMETERS

Total Bacteria Count: A Comprehensive Indicator of Raw Milk Microbiological Quality and Hygienic Production Practices:

The **Total Bacteria Count (TBC)** is the most commonly used parameter for assessing the **microbial quality of raw milk**. In the **United States**, it is measured using several **approved methodologies**, including the **Standard Plate Count (SPC), Plate Loop Count (PLC), Petrifilm (3M) Aerobic Count, and Flow Cytometry-based systems** such as **Bactoscan and Foss Analytical** (USPHS/FDA, 2019). According to the **Pasteurized Milk Ordinance (PMO)**, the permissible bacterial limit for **grade "A" raw milk** is **100,000 CFU/mL** for individual producers and **300,000 CFU/mL** for commingled raw milk (USPHS/FDA, 2019).

Globally, **TBC regulatory limits** are similar, with **100,000 CFU/mL** set as the threshold for **raw milk intended for processing in Europe** (European Commission, 2021). However, in **New Zealand**, the maximum permissible count is **300,000 CFU/mL** (Ministry for Primary Industries, 2022), while **Canada** enforces a stricter **50,000 CFU/mL** limit for **total aerobic mesophilic bacteria** (National Dairy Code, 1997).

Despite these thresholds, actual **TBC levels in bulk tank milk** generally fall well below regulatory limits. **Elmoslemany et al. (2009a)** analyzed **11,100 bulk tank raw milk samples** from **235 dairy farms in Prince Edward Island, Canada**, over a **two-year period** and reported a **geometric mean TBC of 5,300 CFU/mL**, with only **6% of samples exceeding 50,000 CFU/mL**. Similarly, **Pantoja et al. (2009)** evaluated **7,241 raw milk samples** from **16 Wisconsin dairy farms**, reporting a **mean TBC of 3.1 log CFU/mL (1,259 CFU/mL)**, with just **1.6% exceeding 100,000 CFU/mL**. A **more recent study by Rodrigues et al. (2017)** analyzed **472 bulk tank raw milk samples** from **19 farms in New York**, with **mean TBC values ranging from 3.01 log CFU/mL (1,023 CFU/mL) to 4.11 log CFU/mL (12,882 CFU/mL)**.

Although **TBC levels in many regions remain within acceptable limits**, **dairy producers are continuously encouraged** to **improve milk hygiene and reduce bacterial loads**. Many **processors and cooperatives** implement **premium payment programs** for **low-TBC milk**, incentivizing farmers to enhance **sanitation protocols and storage practices**. Industry guidelines suggest that **TBC targets for high-quality raw milk** should be **<5,000 to**

<10,000 CFU/mL (Murphy and Boor, 2000; Jayarao and Wolfgang, 2003), though these recommendations may need updates based on **modern dairy practices**.

Factors Contributing to Elevated TBC in Bulk Tank Raw Milk

Extensive research has identified **multiple pathways** of **bacterial contamination** in **bulk tank raw milk**, emphasizing the need for **comprehensive management strategies** to **control bacterial growth**.

1. Udder Health and Mastitis Infections

The **udder** is a **primary source of bacterial contamination** in raw milk, particularly when cows suffer from **mastitis**, an infection that **elevates TBC**.

- **Hayes et al. (2001)** analyzed **bulk tank milk from 13 farms** over a **two-week period** and found that **11 of 20 TBC spikes** were due to increased levels of **Streptococcus uberis**, a **common mastitis-causing pathogen**.
- Similarly, **Zadoks et al. (2004)** found that **streptococcal, staphylococcal, and gram-negative bacteria** were significantly correlated with **higher TBC levels**, with **streptococci alone accounting for 69% of TBC variability** in **New York farm milk samples**.

The relationship between **mastitis and TBC** is further highlighted by **Pantoja et al. (2009)**, who reported that for every **10,000-cell/mL increase in SCC (somatic cell count)**, the **odds of increased TBC rose by 2.4%**. Similarly, **Borneman and Ingham (2014)** found a **highly significant correlation ($P < 2 \times 10^{-16}$) between SPC and SCC**, confirming the impact of **udder infections on bacterial load**.

2. Milking Hygiene and Equipment Sanitation

Apart from **mastitis**, **milking hygiene and equipment sanitation** play a crucial role in controlling **TBC**.

- **Elmoslemany et al. (2010)** identified **risk factors for elevated TBC**, including:
  - **Dirty teats before milking**
  - **Use of water to wash teats without subsequent drying**
  - **Use of shared towels for multiple cows**
  - **Inadequate cleaning of bulk tanks**
- **Jayarao et al. (2004)** found that **pre- and post-dipping of teats significantly reduced SPC values**, while **teat spraying was less effective**.
- **Doyle et al. (2016)** further confirmed that the **teat surface microbiome is a primary contributor** to **raw milk bacterial load**, reinforcing the need for **pre-milking hygiene measures**.

3. Storage Conditions and Temperature Control

Maintaining **optimal cooling temperatures** is **critical** to **limiting bacterial growth** in raw milk.

- **O'Connell et al. (2016)** demonstrated that storing bulk tank raw milk at **6°C** resulted in a significant **TBC increase from 3.43 log CFU/mL to 4.87 log CFU/mL within 96 hours**, whereas **storage at 2°C prevented bacterial growth**.
- **De Jonghe et al. (2011)** reported that under **suboptimal storage conditions** (e.g., **milk stored at 6°C with temperature fluctuations to 10°C**), **TBC significantly increased**, with *Pseudomonas* identified as the primary spoilage agent.

4. Environmental Contamination

Environmental factors such as **dirty barns, manure exposure, and contaminated bedding** contribute to **elevated TBC**.

- Studies suggest that **udder cleanliness and barn conditions** significantly affect **bacterial contamination in milk**.
- **Martin et al. (2021)** highlighted that **suboptimal barn management leads to high TBC and reduced milk quality**.

Advancements in TBC Testing and Microbiome Analysis

Traditionally, **TBC measurement relies on culture-dependent methods** such as **SPC and Petrifilm Aerobic Count**, which can be **limiting** in detecting certain bacterial groups. However, advancements in **culture-independent techniques**, such as **flow cytometry (Bactoscan) and metagenomic sequencing**, now provide **a more comprehensive evaluation** of **raw milk microbial populations**.

- **Martin et al. (2019)** showed that **psychrotolerant spore-forming bacteria**, which significantly affect **milk shelf life**, are often **undetectable using conventional TBC methods** but can be identified via **metagenomic sequencing**.
- **Parente et al. (2020)** analyzed raw milk microbiomes across different **geographic regions** and found that **Pseudomonas, Streptococcus, Lactococcus, and Acinetobacter** were among the most **prevalent bacterial genera** influencing milk quality.

TBC remains **a critical indicator** of **raw milk microbiological quality**, reflecting **on-farm hygiene, udder health, and storage conditions**. Maintaining **low TBC levels** through **proper milking hygiene, effective cooling, and optimized barn management** is **essential for producing high-quality dairy products**.

While **TBC thresholds vary globally**, research suggests that **continuous improvement in farm hygiene, milk handling, and microbial monitoring** is key to ensuring **safe, high-quality milk for consumers**. Emerging **culture-independent technologies** offer **new insights into microbial diversity**, paving the way for **more comprehensive milk quality assessments** beyond traditional TBC testing.

Recent studies have provided updated insights into the microbiological quality of raw milk, highlighting both improvements and ongoing challenges in dairy farm management practices.

A study conducted in China analyzed 435 raw milk samples and found that approximately 9.89% exceeded the national threshold for aerobic plate count, indicating areas where hygienic practices could be enhanced. The same study reported that 54.02% of raw milk samples contained aerobic *Bacillus* species, with 7.36% harboring thermophilic aerobic *Bacillus*, underscoring the need for stringent temperature control during milk storage to inhibit the growth of thermophilic bacteria

In Ethiopia, research assessing raw cow milk microbial quality revealed high bacterial counts and a significant presence of *Staphylococcus aureus*, pointing to potential food safety risks and the necessity for improved milking hygiene and equipment sanitation.

These findings align with earlier studies emphasizing the critical role of farm management practices in maintaining low Total Bacteria Count (TBC) in bulk tank raw milk. Factors such as proper udder hygiene, effective cleaning of milking equipment, and rapid cooling of milk post-harvest are essential to minimize bacterial contamination. For instance, research has shown that inadequate cleaning of teats before milking and improper sanitation of equipment can lead to elevated TBC levels, adversely affecting milk quality.

COLIFORM COUNT: INDICATORS OF FECAL AND ENVIRONMENTAL CONTAMINATION

Coliforms are a method-defined group of bacteria that are aerobic or facultatively anaerobic, Gram-negative, non-spore-forming rods capable of fermenting lactose to produce acid and gas within 48 hours at 32–35°C. These bacteria have long been used as indicators of fecal contamination in water, dairy products, and other food matrices. However, it is important to note that while fecal coliforms are a subset of coliforms, not all coliform bacteria originate from fecal sources. Many coliforms are environmental in nature, commonly found in water, vegetation, and soil, making their presence in milk an indicator of overall hygiene practices rather than just fecal contamination.

The standard method for quantifying coliform counts in milk relies on Violet Red Bile Agar (VRBA), with confirmation through gas and acid production in Brilliant Green Bile Broth. More recently, dehydrated film media such as 3M Coliform Petrifilm have gained popularity due to their simplicity and rapid results. In the United States, the Pasteurized Milk Ordinance (PMO) does not specify a regulatory limit for coliforms in raw milk, but California has set a maximum threshold of 750 CFU/mL for raw milk. Internationally, guidelines suggest that high-quality raw milk should contain no more than 10 CFU/mL, while values between 100 and 1,000 CFU/mL indicate poor milking hygiene.

Several studies have assessed coliform contamination levels in raw milk across different regions. Pantoja et al. (2009) analyzed 7,200 raw milk samples from 16 dairy farms in Wisconsin, USA, reporting a mean coliform count of 1.7 log CFU/mL (50 CFU/mL), indicating relatively good milk hygiene practices. Jayarao et al. (2004) examined 126 dairy farms in Pennsylvania, reporting an average coliform count of 70 CFU/mL, suggesting a moderate level of contamination. Jayarao and Wang (1999) reported significantly higher mean coliform counts of 3.4 log CFU/mL (2,500 CFU/mL) from 130 bulk tank raw milk samples in South Dakota and Minnesota, highlighting regional variations in hygiene standards.

Coliform contamination in raw milk typically arises from poor milking hygiene, environmental contamination, and mastitic infections in cows. Inadequate pre-milking udder cleaning, the use of contaminated water for equipment sanitation, and unhygienic milk-handling practices significantly contribute to high coliform counts. Environmental factors such as exposure to manure, contaminated bedding, and dirty milking equipment further exacerbate the risk of coliform contamination. Additionally, cows suffering from coliform mastitis can directly shed bacteria into the milk, increasing the microbial load. Jayarao and Wolfgang (2003) linked high bulk tank coliform counts to poor udder hygiene, failure to dry teats before milking, and inadequate sanitation of milking machines. Similarly, Pantoja et al. (2011) found a positive association between Somatic Cell Count (SCC) and coliform levels, indicating that infected cows contribute significantly to bacterial contamination.

Farm hygiene and sanitation practices play a crucial role in controlling coliform contamination. Elmoslemany et al. (2009b) demonstrated that proper cleaning of milking equipment, use of high-temperature washes, and appropriate sanitization chemicals significantly reduced coliform counts in bulk tank milk. Pantoja et al. (2011) further reported that frequent washing of milking clusters and minimizing equipment malfunctions, such as unit fall-offs, helped reduce in-line coliform contamination. Moreover, research has emphasized the importance of water quality in maintaining milk hygiene, as contaminated water sources can introduce coliforms into the milking system.

Beyond their role as hygiene indicators, coliforms can have direct implications for finished dairy product quality. Certain coliform species produce heat-stable enzymes, such as proteases and lipases, which persist even after pasteurization, leading to undesirable flavor and texture defects in processed dairy products. High coliform loads in raw milk are also associated with increased total bacterial counts, accelerating spoilage and reducing shelf life.

To mitigate coliform contamination, dairy producers must implement stringent hygiene protocols, including thorough pre- and post-milking udder sanitation, proper handling of milking equipment, and regular monitoring of water quality. Rapid coliform detection methods, such as flow cytometry and molecular techniques, can help identify contamination sources early and prevent further microbial proliferation. By maintaining strict hygiene standards and improving farm management practices, dairy producers can significantly reduce coliform levels in raw milk, ensuring a safer and higher-quality milk supply.

Coliforms are aerobic or facultatively anaerobic gram-negative, non-sporeforming rods capable of fermenting lactose to produce gas and acid within 48 hours at temperatures between 32 to 35°C. Traditionally, coliforms have been utilized as indicators of fecal contamination in water, dairy, and other food products. However, it's important to note that the coliform group is not exclusively linked to fecal contamination; many coliforms originate from environmental sources such as water and vegetation. The standard method for enumerating coliforms involves using violet red bile agar, followed by confirmation tests for gas and acid production in brilliant green bile medium. Dehydrated film media, like 3M Coliform Petrifilm, have gained popularity due to their simplicity. In the United States, the Pasteurized Milk Ordinance does not specify a regulatory limit for coliforms in raw milk; however, states like California have established limits, setting a threshold of 750 cfu/mL for raw milk. Guidelines suggest that high-quality raw milk should contain no more than 10 cfu/mL of coliforms, with levels between 100 and 1,000 cfu/mL indicating suboptimal milking hygiene. Studies have reported varying mean coliform counts in raw milk; for instance, a study in Wisconsin reported a mean count of 50 cfu/mL from over 7,200 raw milk samples, while research in Pennsylvania found a mean of 70 cfu/mL from 126 dairy farms. In contrast, a study analyzing 130 bulk tank raw milk samples from South Dakota and Minnesota reported a higher mean coliform count of 2,500 cfu/mL.

Elevated coliform counts in raw milk can result from several factors, including inadequate milking hygiene, environmental contamination due to improperly cleaned equipment, or the presence of coliform mastitis within the herd. When bulk tank raw milk exhibits high coliform levels, it's essential to assess pre-milking hygiene practices, such as ensuring udders are dry during milking, preventing milking units from contacting manure, inspecting rubber hoses and gaskets for wear, and monitoring for clinical coliform mastitis in the herd. Research has indicated that cow hygiene factors, including leg and teat cleanliness, significantly correlate with bulk tank raw milk coliform counts. Additionally, equipment-related factors, such as the temperatures achieved during cleaning cycles, the effectiveness of cleaning agents, and the quality of water used in the milk house, play crucial roles in controlling coliform levels. Proper handling of milking unit clusters, minimizing unit fall-offs, and increasing cluster washes are associated with lower coliform counts. Failures in milking machine wash protocols, such as not reaching adequate wash temperatures or incorrect detergent dispensing, are strongly linked to increased coliform levels in raw milk. A positive association between somatic cell count (SCC) and coliform count suggests that milking cows with coliform mastitis may contribute to elevated coliform levels in bulk tank raw milk.

The coliform group encompasses a diverse range of bacteria, primarily within the Enterobacteriaceae family, but also includes members of the Aeromonadaceae family. Common genera isolated from raw milk include Citrobacter, Enterobacter, Escherichia, and Klebsiella. Other genera, such as Hafnia and Serratia, have also been detected in bulk tank raw milk. Among these, only Escherichia coli is predominantly associated with fecal contamination, while the others are often linked to environmental sources like soil, water, and vegetation. This environmental association aligns with findings that bulk tank raw milk coliform counts are significantly influenced by equipment cleaning and sanitation practices. Notably, even coliforms traditionally considered strong indicators of fecal contamination, such as Klebsiella and Escherichia coli, can persist and proliferate in natural environments, including water and soil. Many environmental coliforms, including Enterobacter, Citrobacter, and Serratia, can grow at low temperatures, meaning that during prolonged cold storage of raw milk, these organisms may significantly contribute to higher total bacterial counts. Coliforms responsible for environmental mastitis, such as Escherichia, Klebsiella, Enterobacter, and Serratia, may also elevate coliform counts in bulk tank raw milk. For example, a study observed that out of 20 bulk tank raw milk total bacterial count spikes evaluated over a two-week period from 13 farms, four were associated with high levels of Escherichia coli, potentially due to milking mastitic cows; however, the authors suggested that these spikes were more likely due to insufficient cleaning and sanitation of equipment.

Recent studies have provided further insights into coliform contamination in raw milk. A survey in the United Kingdom indicated that pathogens and indicators of poor hygiene were present in almost half of the raw milk samples intended for direct consumption, with 25% of samples exhibiting unacceptable levels of indicator bacteria. Another study evaluated a rapid coliform detection kit for clinical mastitis milk samples and demonstrated that the test showed high sensitivity and specificity for detecting coliforms, offering a quicker alternative to traditional bacterial culture methods. Additionally, research has highlighted the need to reconsider coliform testing as a marker for unsanitary conditions in the dairy industry, suggesting that as our understanding of this diverse group of microbes evolves, so too should our testing methodologies.

In summary, elevated coliform counts in raw milk serve as indicators of inadequate cow hygiene, milking time hygiene, or equipment sanitation. The impact of coliforms on finished dairy product quality largely depends on conditions that allow these organisms to reach levels where they produce heat-stable enzymes. While coliforms can produce various enzymes at high concentrations, some of which are heat-stable, their limited ability to indicate fecal contamination means that coliform counts may not provide substantial additional information beyond other hygiene indicators concerning finished product quality.

LABORATORY PASTEURIZATION COUNT: A MEASURE OF THERMODURIC BACTERIA

The Laboratory Pasteurization Count (LPC) method has been widely used since the early 20th century to assess the presence of thermoduric bacteria in raw milk and evaluate their ability to survive pasteurization treatments, particularly low-temperature, long-time pasteurization (Hileman, 1940). The LPC method involves heat treating raw milk at 62.8°C for 30 minutes to eliminate heat-sensitive bacteria, followed by enumeration using the Standard Plate

Count (SPC) method (Frank and Yousef, 2004). Although there are no regulatory limits for LPC in raw milk in the United States, it is often utilized in producer quality programs. Generally, an LPC value below 100 CFU/mL is considered an indicator of high-quality milk, whereas values exceeding 200 CFU/mL suggest poor sanitation and inadequate cleaning of milking equipment (Murphy and Boor, 2000; Jayarao and Wolfgang, 2003). Various studies have reported LPC values for bulk tank raw milk, with Pantoja et al. (2009) documenting a mean LPC of 79 CFU/mL from 7,220 bulk tank samples in Wisconsin, while Boor et al. (1998) recorded a mean LPC of 129 CFU/mL from 855 bulk tank samples in New York. Similarly, Gillespie et al. (2012) reported an LPC mean of 43 CFU/mL from 1,141 samples collected in Tennessee.

It is well established that high LPC values (>200 CFU/mL) are strongly linked to inadequate sanitation of milking equipment and hygiene practices. This correlation has been documented in studies dating back to early dairy research, including Hileman (1940), which highlighted improperly sterilized milk contact surfaces, dirty milk cans, and ineffective equipment cleaning as primary sources of thermoduric bacteria in raw milk. Over the years, sanitation and hygiene practices in dairy operations have improved significantly, but modern research continues to validate these early findings. Elmoslemany et al. (2009b) demonstrated that higher equipment cleaning temperatures, adequate chlorine concentrations, proper pipeline flushing (slug scores), and the use of water softeners were key protective factors in reducing LPC values. Similarly, Bava et al. (2011) found that high LPC levels in liner swabs before milking were strongly associated with bulk tank milk contamination, further underscoring the importance of effective cleaning and sanitation protocols.

Studies analyzing thermoduric bacterial populations in raw milk have found that streptococci, micrococci, coryneform bacteria, aerobic sporeformers, and gram-negative bacteria are the predominant organisms detected through LPC analysis (Thomas et al., 1967). More recent findings, such as Kikuchi et al. (1996), reported that Bacillus species comprised 30.7% of the total bacterial isolates, followed by Microbacterium (26.9%), Micrococcaceae (23.4%), Coryneform bacteria (7.9%), and Streptococcus (6.7%). Similarly, Delgado et al. (2013) found that nearly 60% of isolates from raw milk in Spain subjected to laboratory pasteurization were Streptococcus thermophilus, with additional contributors such as Lactobacillus (18%), Enterococcus (13%), and aerobic bacilli (<1%). Further, Ribeiro Júnior et al. (2018) analyzed 20 Brazilian raw milk samples and identified eight thermoduric bacterial genera, including Bacillus, Brachybacterium, Enterococcus, Streptococcus, Micrococcus, Kocuria, Paenibacillus, and Macrococcus, with Bacillus comprising approximately 50% of the total isolates. While these studies consistently indicate the presence of thermoduric bacteria in raw milk, the relative proportions of different species vary, likely due to differences in milk processing, farm sanitation, and environmental conditions.

The LPC method has several limitations in accurately assessing raw milk quality. One major drawback is that it does not differentiate between spore-forming and non-spore-forming thermoduric bacteria, which is essential for identifying contamination sources. Recent research from Cornell University (2024) has suggested that LPC alone is insufficient for accurately assessing milk quality, as it cannot effectively distinguish between these two groups. Additionally, methodological constraints in the SPC-based enumeration process impact LPC accuracy. Traditionally, SPC agar pour plating was the preferred method; however, many modern laboratories have shifted towards using dehydrated film plates (e.g., 3M Petrifilm) due to their ease of use and time efficiency. Byrne and Bishop (1991) evaluated 3M Aerobic Count Petrifilm as an alternative to SPC pour plates, finding no significant difference in LPC results for naturally contaminated milk samples. However, when testing experimentally contaminated milk, they observed significantly lower recovery rates for Micrococcus species on Petrifilm compared to SPC pour plates, highlighting potential discrepancies in enumeration methods.

Beyond methodological challenges, LPC interpretation presents another critical limitation. While it accurately measures thermoduric bacteria, it does not necessarily correlate with the microbial performance of processed dairy products. Although low-temperature, long-time pasteurization (63°C for 30 minutes) may allow some thermoduric bacteria to survive, high-temperature, short-time (HTST) pasteurization (72°C for 15 seconds) effectively eliminates most non-spore-forming bacteria. Studies by Huck et al. (2008) and Ranieri and Boor (2009) have shown that HTST-treated fluid milk predominantly contains Bacillus species, while at the end of shelf life, Paenibacillus spp. become dominant. Furthermore, Martin et al. (2011) demonstrated that LPC is a poor predictor of pasteurized fluid milk quality, as it does not account for the contribution of psychrotolerant spore-formers to spoilage.

A common misconception is that LPC serves as a proxy for raw milk spore populations. While aerobic sporeformers may constitute part of the thermoduric bacterial load, they typically exist in very low concentrations. If an accurate raw milk spore count is required, alternative spore-specific methods should be employed. Moreover, while non-spore-forming thermoduric bacteria are generally not considered major contributors to dairy product spoilage, they can play a role as non-starter lactic acid bacteria, particularly in raw milk cheese production.

While LPC remains a valuable indicator of raw milk hygiene and thermoduric bacterial presence, its limitations necessitate the use of complementary microbiological tests for a more comprehensive assessment. Future research should focus on enhancing current enumeration methods to provide greater specificity in differentiating between spore-forming and non-spore-forming bacteria, as well as identifying new approaches for detecting psychrotolerant thermoduric species that impact dairy product stability. Advances in rapid detection methods and genomic sequencing techniques may further enhance raw milk quality monitoring, ensuring greater safety and consistency in dairy processing.

Recent studies have highlighted limitations in the Laboratory Pasteurization Count (LPC) method, particularly its inability to distinguish between spore-forming and non-spore-forming thermoduric bacteria in raw milk. A 2024 study from Cornell University found that while LPC effectively enumerates heat-resistant bacteria, it does not differentiate between these two groups, which is crucial for identifying contamination sources and implementing appropriate control measures. The researchers emphasized the need for more precise testing methods to accurately assess raw milk quality and guide dairy producers in addressing specific bacterial contaminants.

This underscores the importance of developing and adopting more refined testing protocols that can provide detailed insights into the types of bacteria present in raw milk, thereby enhancing quality control and safety in dairy production.

SPOREFORMING BACTERIA: LOW-LEVEL CONTAMINANTS CONTRIBUTING TO DAIRY PRODUCT QUALITY

Spore-forming bacteria, though present in low concentrations in raw milk, significantly influence dairy product quality due to their resilience and ability to survive standard pasteurization processes. These microorganisms, including genera such as *Bacillus*, *Clostridium*, and *Paenibacillus*, can withstand adverse conditions like heat, desiccation, and chemical treatments by forming spores. Upon germination, they may lead to spoilage in various dairy products, including milk, cheese, and powdered dairy items.

Recent studies have highlighted the impact of spore-forming bacteria on dairy product quality. For instance, a study analyzing raw milk and dairy powder samples from U.S. processors found significant differences in spore levels and populations based on testing methodologies. The research indicated that spore counts did not significantly increase from the beginning to the end of dairy powder processing, suggesting that biofilm formation by processing plant-associated spore-formers was minimal in the facilities sampled.

Another investigation into raw milk from New Zealand reported lower aerobic spore counts compared to previous findings, attributing this to effective farm management and animal cleaning practices. The study also noted seasonal variations, with higher spore counts during winter months, possibly due to increased rainfall leading to more soil contamination on udders and favorable conditions for spore-former sporulation.

Implementing improved milking time hygiene interventions has been shown to reduce spore counts in bulk tank raw milk. A study involving 355 bulk tank samples demonstrated that after applying such interventions, mesophilic and thermophilic spore counts decreased by 37% and 40%, respectively. The most significant reductions were observed immediately after training milking staff, underscoring the importance of proper milking procedures in controlling spore contamination.

The presence of spore-forming bacteria in raw milk poses challenges for dairy product quality and safety. These microorganisms can lead to spoilage through enzymatic degradation, affecting the structural, chemical, and sensory properties of dairy products. For example, in yogurt production, spore-formers like *Bacillus cereus* or *Bacillus subtilis* can cause defects such as high acidity, off-flavors, and textural issues.

While spore-forming bacteria are typically present in low levels in raw milk, their ability to survive processing and proliferate in dairy products necessitates stringent control measures. Effective farm management, rigorous hygiene practices during milking, and appropriate processing techniques are essential to mitigate the impact of these microorganisms on dairy product quality and safety.

Spore-forming bacteria, particularly those belonging to the genera *Bacillus* and *Clostridium*, are significant contaminants in the dairy industry due to their resilience and potential to compromise product quality. These microorganisms can survive various stages of dairy processing, leading to spoilage and, in some cases, posing health risks.

A study highlighted that spore-forming bacteria are primary agents of dairy product spoilage, conformance deviations, and occasionally, foodborne safety issues. The four main sources of spores in processed dairy products include raw milk, ingredients, biofilms present in processing equipment, and environmental niches within processing facilities. Transmission from dairy farm sources, especially manure, bedding, and feed, where spores are often present in high levels, into raw milk is influenced by milking hygiene factors. Detection, enumeration, and tracking of these bacteria through the dairy system present unique challenges and often require specialized training and molecular methods to differentiate between closely related organisms.

Another research emphasized that aerobic spore-forming bacteria are a major concern for the dairy industry, primarily due to their spoilage-causing capabilities rather than pathogenicity. These bacteria can affect food safety and product quality through mechanisms such as toxin production, spoilage enzyme production, and impacting the production of secondary dairy products like cheese, yogurt, and milk powders.

A pilot study in New Zealand investigated the presence of both aerobic and anaerobic spore-forming bacteria in raw milk from dairy farms. The study detected a low number of aerobic spore-forming bacteria in raw milk samples collected from four farms during summer and winter. The 16S rRNA sequence types similar to important food spoilage bacteria like *Clostridium beijerinckii*, *Clostridium sporogenes*, *Bacillus licheniformis*, and members of the *Paenibacillus* genus, as well as potentially toxigenic bacteria such as *Bacillus cereus* and *Clostridium perfringens*, were isolated. This highlights the presence of various spoilage and pathogenic spore-forming bacteria in raw milk, underscoring the importance of good hygienic farm practices and management to reduce contamination.

Spore-forming bacteria present a significant challenge to dairy product quality due to their resilience and potential for spoilage. Understanding their sources, transmission, and characteristics is crucial for developing effective control measures to ensure the safety and quality of dairy products.

DEFINING RAW MILK MICROBIOLOGICAL QUALITY FROM THE PERSPECTIVE OF FINISHED PRODUCT QUALITY: A 3-TIERED APPROACH

Recent studies have underscored the critical importance of microbiological quality in raw milk, emphasizing its direct impact on the safety and quality of finished dairy products. A comprehensive review of over 2,500 raw drinking milk and unpasteurized dairy products in England between 2013 and 2019 revealed that while routine monitoring samples generally met satisfactory microbiological standards, 5% of all samples were deemed potentially hazardous. This finding highlights the inherent public health risks associated with consuming raw milk and its derivatives.

Further research focusing on small-scale dairy producers in Serbia analyzed 302 dairy products, including raw and pasteurized milk cheeses and kajmak. The study detected Listeria monocytogenes in one raw milk cheese and five kajmak samples. Additionally, higher levels of indicator microorganisms, such as Escherichia coli and yeasts and molds, were found in raw milk cheese and kajmak. These results suggest lapses in hygiene practices during production and underscore the need for improved food safety measures among small-scale dairy producers.

In Ethiopia, an assessment of raw milk produced by smallholder urban dairy farmers indicated high bacterial counts, pointing to potential food safety risks. The study recommended implementing better hygiene practices during milking and storage to enhance the microbiological quality of raw milk.Collectively, these studies highlight the ongoing challenges in ensuring the microbiological safety of raw milk and unpasteurized dairy products. They emphasize the necessity for stringent hygiene practices, regular monitoring, and adherence to safety protocols to mitigate health risks and ensure high-quality dairy products for consumers.

APPLYING DATA-DRIVEN, RISK-BASED TOOLS TO ENSURING RAW MILK MICROBIOLOGICAL QUALITY FOR OPTIMUM FINISHED PRODUCT PERFORMANCE

Future stakeholders in the dairy industry should look beyond traditional raw milk microbiological testing and integrate predictive tools that facilitate data-driven, risk-based decision-making at the farm level. Research reviewed in this study highlights significant overlapping on-farm risk factors influencing multiple raw milk microbiological parameters. For instance, inadequate equipment hygiene has been correlated with elevated Total Bacteria Count (TBC), Coliform Count (CC), Preliminary Incubation Count (PI), and Laboratory Pasteurization Count (LPC) (Elmoslemany et al., 2009b; Bava et al., 2011). The ability to leverage these common factors will be essential in transitioning from a reactive, test-based approach, which remains predominant in the dairy industry today, to a proactive, whole-farm, risk-based system. By integrating farm management practices with real-time testing data, stakeholders can implement preventative measures that reduce microbial risks, ensuring improved raw milk quality and enhanced final product integrity.

The development of decision support tools at both the production and processing levels has demonstrated how relevant data modeling can predict outcomes crucial to the dairy industry. For example, Cabrera (2018) outlines the development of over 20 computerized dairy farm management tools to assist producers with nutrition, reproduction, calf and heifer management, herd replacement, price risk, and environmental conditions. Additional tools have been described for dairy production management (Rose et al., 2016; Balhara et al., 2021), with numerous commercial platforms such as iDDEN.org and MyDairyDashboard.com already available. On the processing side, decision support tools have been used to enhance dairy product quality, including Monte Carlo simulations that predict the shelf life of fluid milk based on psychrotolerant spore contamination (Buehler et al., 2018a), consumer exposure to spoiled yogurt (Buehler et al., 2018b), and the likelihood of late-blowing defect in cheese (Qian et al., 2022).

Despite the proven benefits of risk-based decision support tools, several barriers hinder their widespread adoption by dairy producers. A recent commentary on integrated decision support systems identified major challenges, including perceived limited value by producers, complexity in usability and interpretation, lack of practical application, data collection and standardization difficulties, inadequate data integration and sharing, and limited farm infrastructure (Baldin et al., 2021). Similarly, Rose et al. (2016) identified key factors affecting adoption, including ease of use, peer recommendations, cost considerations, accessibility to internet and mobile networks, market demands, legislative compliance, and producer demographics such as age and farm scale. Notably, these barriers disproportionately affect small-scale and remote dairy farms, which stand to benefit the most from quality improvement interventions.

Despite these challenges, the implementation of data-driven, risk-based decision tools to assess raw milk microbiological quality across the production-to-processing chain has the potential to transform dairy quality control practices. Future cross-disciplinary collaborations should focus on increasing adoption and integration of these models. Dairy cooperatives and processors could utilize predictive analytics to establish target levels for specific microbial parameters, such as butyric acid bacteria (BAB) counts and psychrotolerant spore counts, linking them to financial incentives for farmers. By pairing frequent process control testing (e.g., TBC monitoring on every milk load) with periodic low-frequency monitoring of critical microbial indicators, the dairy industry can strategically enhance raw milk quality and ensure consistent compliance with finished product specifications.

CONCLUSIONS

The bacterial populations present in raw milk play a crucial role in determining the quality, safety, and stability of processed dairy products. However, as discussed in this review, the current microbiological testing parameters used to assess raw milk quality vary in their ability to accurately predict their impact on finished product quality. While Total Bacteria Count (TBC), Coliform Count (CC), and spore-forming bacteria provide valuable insights, they do not fully capture the complex microbial interactions that influence dairy processing outcomes.

The future of raw milk quality assessment should shift towards a comprehensive whole-farm approach, which acknowledges microbiological risk factors at different stages of milk production. The dairy industry must transition from a reactive, test-based system to a proactive, predictive, and risk-based framework. This evolution requires standardized microbiological indicators, such as TBC, as a routine process control metric combined with periodic monitoring of microbial parameters that directly influence finished product characteristics.Additionally, troubleshooting microbiological tests should be employed when process control tests indicate non-compliance, ensuring early identification and mitigation of contamination sources. The adoption of integrated decision support tools, which utilize predictive analytics and data-driven risk assessment models, will provide farmers and processors with actionable insights to optimize milk quality before it reaches processing facilities. These tools will help in implementing targeted interventions to control microbial loads, improve hygiene protocols, and enhance dairy processing efficiency.

Achieving this holistic, risk-based approach requires strong collaboration among dairy producers, processors, regulatory agencies, and academic researchers. By working together, stakeholders can establish standardized microbiological benchmarks, refine monitoring technologies, and develop innovative solutions to enhance raw milk quality and dairy product consistency. Ultimately, integrating science-based risk management strategies into the dairy industry will lead to higher-quality raw milk, ensuring safe and premium dairy products for consumers worldwide.

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