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A comprehensive review of microbiological hazards in the dairy supply chain

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Abstract
Milk and its derived products are vital components of the human diet, providing essential nutrients. However, their rich composition and neutral pH make them highly sceptible to microbial contamination and proliferation, posing significant risks to public health and economic stability. Effective microbiological quality control throughout the dairy production chain, from farm to consumer, is therefore paramount. This abstract outlines the critical aspects of ensuring the microbiological safety and quality of milk and milk products. It encompasses the sources of microbial contamination, including the animal, environment, processing equipment, and human handling. Key spoilage microorganisms and pathogens commonly associated with milk and milk products, such as *Escherichia coli*, *Salmonella spp.*, *Listeria monocytogenes*, *Staphylococcus aureus*, and various psychrotrophic bacteria, are highlighted. Furthermore, this abstract discusses the diverse range of microbiological testing methods employed for quality assessment, including traditional culture-based techniques, rapid methods like PCR and ELISA, and emerging technologies. The importance of Hazard Analysis and Critical Control Points (HACCP) and Good Manufacturing Practices (GMP) in preventing and controlling microbial contamination is emphasized. Ultimately, robust microbiological quality control measures are essential to guarantee the safety, extend the shelf life, and maintain the nutritional value of milk and milk products for consumers.

Keywords: Microbiological testing, culture-based methods, rapid methods enumeration identification, quality assessment

Introduction

Milk is a biologically complex fluid, primarily produced by mammals for neonatal nourishment. It is rich in nutrients such as lactose, fats, proteins, vitamins, and minerals, which not only make it a staple of human nutrition but also an excellent medium for microbial growth (Goff, 2021). Because of its composition and water activity, milk is highly perishable and susceptible to microbial spoilage and contamination from the point of extraction to final consumption.

Microbiological contamination of milk can occur from multiple sources, including the animal itself, the milking environment, equipment, handlers, and during processing and storage (Tamine & Robinson, 2017). Pathogenic microorganisms such as *Salmonella spp.*, *Listeria monocytogenes*, *Escherichia coli O157:H7*, and *Staphylococcus aureus* can cause foodborne illnesses of serious public health concern if proper control measures are not implemented (Oliver *et al*., 2005) [10]. Additionally, spoilage organisms like *Pseudomonas*, *Lactobacillus*, and *Clostridium* species can lead to sensory degradation, resulting in economic losses and reduced consumer acceptance. Ensuring the microbiological safety and quality of milk is therefore essential. Quality control measures involve routine microbial testing, hygienic production practices, pasteurization, refrigeration, and systematic monitoring using Hazard Analysis and Critical Control Points (HACCP) and Good Hygienic Practices (GHP) (FAO/WHO, 2020). [6] These controls not only help in detecting contamination but also in preventing potential outbreaks and ensuring compliance with national and international food safety standards.

The application of modern microbiological techniques, including rapid detection assays such as polymerase chain reaction (PCR), enzyme-linked immunosorbent assays (ELISA), and biosensors, have significantly enhanced the speed and accuracy of microbial detection in dairy products (Zhao *et al*., 2014) [14]. However, classical culture-based methods remain the gold standard for regulatory purposes due to their reliability and cost-effectiveness. Given the global dependence on milk and dairy products, a robust microbiological quality control system is vital to safeguard public health, ensure consumer confidence, and uphold the integrity of the dairy supply chain.

Sources of Microbial Contamination

Milk, as a natural secretion, is sterile when synthesized in the alveoli of a healthy udder. However, during and after the milking process, it becomes highly susceptible to microbial contamination. This contamination may originate from various intrinsic and extrinsic sources, including the animal itself, the environment, equipment, water, handlers, and processing steps. These sources can introduce a wide range of microorganisms including spoilage organisms, pathogens, and indicator bacteria, thereby affecting milk safety, shelf life, and consumer health (Oliver, Jayarao, & Almeida, 2005; Quigley *et al*., 2013) [12].

1. Animal-Related Contamination

a. Udder Infections and Mastitis

One of the primary biological sources of microbial contamination is mastitis, an inflammation of the mammary gland. Subclinical and clinical mastitis can result in the excretion of pathogenic bacteria such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli* into milk (Bradley, 2002). These bacteria not only compromise milk quality but also pose zoonotic risks.

b. Fecal Contamination

Contamination can also occur via fecal material from the animal's skin, tail, or bedding, which may harbor enteric pathogens like *Salmonella spp.* and *Listeria monocytogenes* (Slavik *et al*., 1994) [14]. These pathogens can be transferred into the milk during milking if hygiene is inadequate.

2. Environmental Contamination

a. Airborne and Dust Particles

Dust and air in barns or milking parlors can carry microorganisms such as *Bacillus*, *Clostridium*, and yeasts. These airborne particles settle on open containers or milk surfaces during milking or transfer operations, increasing the microbial load (Griffiths, 1997).

b. Soil and Bedding Material

Soil particles and organic bedding are common reservoirs for spore-forming bacteria like *Clostridium tyrobutyricum*, which can cause defects in cheeses (Klijn, Nieuwenhof, Hoolwerf, van der Waals, & Weerkamp, 1995). Milking in dirty environments enhances the likelihood of soil-borne contamination.

3. Milking Equipment and Containers

Improperly cleaned and sanitized milking equipment—such as teat cups, pipelines, and bulk tanks—are a significant source of microbial contamination. Biofilm formation inside these systems can harbor spoilage organisms and pathogens that are resistant to standard cleaning procedures (Flint, Bremer, Brooks, & Lindsay, 1997). Residual milk, water, and organic material provide ideal conditions for microbial colonization.

4. Water Used for Cleaning

Water used for udder preparation, equipment rinsing, or dilution must be of potable quality. Non-potable water often contains coliforms and *Pseudomonas spp.*, which can compromise milk safety. Contaminated water is especially problematic in rural and low-resource areas where water treatment is minimal (Rysanek, Zouharova, & Babak, 2009).

5. Human Handling

Human handlers can introduce microorganisms via skin, clothing, or respiratory droplets, particularly if proper hygiene (handwashing, gloves, protective clothing) is not practiced. *Staphylococcus aureus*, a common skin bacterium, is frequently transmitted through this route (Tamine & Robinson, 2017). Inadequate training and personal hygiene of dairy workers are major contributors to this risk.

6. Post-Milking and Processing Contamination

Even after pasteurization, contamination can occur due to faulty packaging, poor equipment sanitation, or cross-contact with raw materials. *Listeria monocytogenes* is particularly known for its post-pasteurization contamination potential, especially in soft cheeses and ready-to-eat dairy products (Massa *et al*., 2007) [8]. Furthermore, cold-loving (psychrotrophic) organisms like *Pseudomonas spp.* can grow during refrigeration, leading to spoilage without necessarily causing illness.

Common Microorganisms in Milk

Milk, owing to its rich nutrient composition and near-neutral pH, provides an ideal environment for the growth of a diverse array of microorganisms. These include both beneficial and harmful microbes—ranging from spoilage bacteria to pathogenic organisms and fermentative species. The microbial population in milk varies depending on multiple factors including hygiene practices, temperature control, animal health, and handling conditions (Quigley *et al*., 2013; [11] Tamine & Robinson, 2017). Understanding these microorganisms is crucial for ensuring milk safety, extending shelf life, and producing high-quality dairy products.

1. Psychrotrophic Bacteria

Psychrotrophs are cold-tolerant bacteria capable of growing at refrigeration temperatures (4–7°C). Even though their initial numbers may be low, they can multiply rapidly during milk storage if pasteurization is delayed or post-pasteurization contamination occurs.

- **Examples:** *Pseudomonas spp.*, *Acinetobacter spp.*, *Flavobacterium spp.*

- **Significance:** These organisms produce heat-stable enzymes like proteases and lipases that cause spoilage in milk and dairy products (Rajmohan, Dodd, & Waites, 2002). *Pseudomonas fluorescens*, in particular, is notorious for causing off-flavors and degradation of milk proteins.

2. Thermoduric and Spore-Forming Bacteria

Thermoduric bacteria survive pasteurization temperatures and may persist in dairy products. Many are spore-formers, capable of resisting harsh environmental conditions.

- **Examples:** *Bacillus cereus*, *Clostridium tyrobutyricum*, *Geobacillus stearothermophilus*

- **Significance:** These organisms can cause spoilage in UHT (ultra-high temperature) milk and late blowing in cheeses. Some species like *B. cereus* are also opportunistic pathogens (Scheldeman *et al*., 2005) [13].

3. Coliforms and Indicator Organisms

Coliform bacteria are used as indicators of poor hygiene or post-pasteurization contamination. Though not all are harmful, their presence suggests the potential for pathogenic contamination.

- **Examples:** *Escherichia coli*, *Enterobacter spp.*, *Klebsiella spp.*

- **Significance:** *E. coli* is considered a hygiene indicator; however, certain strains like *E. coli O157:H7* can cause severe illness. Their presence in pasteurized milk is unacceptable under most regulatory frameworks (Oliver *et al*., 2005) [9].

4. Lactic Acid Bacteria (LAB)

LAB are naturally present in raw milk and are essential in the production of fermented dairy products. They contribute positively by producing lactic acid, antimicrobial compounds, and enhancing flavor profiles.

- **Examples:** *Lactococcus lactis*, *Lactobacillus spp.*, *Leuconostoc spp.*, *Streptococcus thermophilus*

- **Significance:** LAB are generally recognized as safe (GRAS) and are critical in dairy fermentation. However, uncontrolled growth in raw milk can result in acidification and curdling (Tamine & Robinson, 2017).

5. Pathogenic Bacteria

These bacteria can cause foodborne illnesses and are a serious concern in unpasteurized or improperly handled milk.

- **Examples**

Listeria monocytogenes**:** causes listeriosis, especially in immunocompromised individuals

Salmonella spp: responsible for salmonellosis

Staphylococcus aureus: produces enterotoxins leading to food poisoning

Mycobacterium bovis**:** cause of bovine tuberculosis

- **Significance:** Pathogens can survive in raw milk and even in processed products if post-pasteurization contamination occurs. Controlling these microbes is a key objective of dairy microbiological quality assurance systems (Farber & Peterkin, 1991) [7].

Microbiological Quality Standards of Milk and Milk Products

Ensuring the microbiological safety and quality of milk is fundamental to protecting public health and maintaining consumer trust in dairy products. Regulatory agencies and international bodies have established microbiological quality standards to limit the presence of harmful and spoilage microorganisms in milk and its derivatives. These standards serve as benchmarks for assessing raw material hygiene, processing efficacy, and post-production contamination control.

Microbiological standards vary by product type (e.g., raw milk, pasteurized milk, fermented milk, cheese), regional legislation, and intended use (direct consumption vs. further processing). Compliance with these standards is a legal requirement in most countries and a critical component of Hazard Analysis and Critical Control Point (HACCP) systems (ICMSF, 2005; Codex Alimentarius, 2018) [2].

1. Raw Milk Standards

Raw milk, being a highly perishable and microbiologically unstable product, is subject to stringent quality checks before acceptance for processing. Most standards focus on the total bacterial count (TBC), somatic cell count (SCC), and the presence of specific pathogens.

- **Total Plate Count (TPC):** Typically limited to < 100,000 CFU/mL in high-quality raw milk in many countries.

- **Somatic Cell Count (SCC):** Used as an indicator of udder health; limits are usually < 400,000 cells/mL for cow milk (EU Regulation No. 853/2004) [4].

- **Coliforms:** Indicator of fecal contamination; should be absent or minimal in high-grade milk.

- **Pathogens:** *Salmonella spp.*, *Listeria monocytogenes*, and *E. coli O157:H7* should be absent in 25 mL of raw milk.

Example

The European Union regulation (EU Regulation No. 853/2004) [3] and the U.S. Pasteurized Milk Ordinance (PMO) both stipulate TPC ≤ 100,000 CFU/mL and SCC ≤ 400,000 cells/mL for cow's milk.

2. Pasteurized Milk Standards

Pasteurized milk must meet stricter microbial limits since thermal processing should destroy most vegetative microorganisms.

- **Total Plate Count:** Should not exceed 20,000 CFU/mL (U.S. PMO) or 30,000 CFU/mL (Codex standard).

- **Coliform Count:** Typically, < 10 CFU/mL.

- **Pathogens:** *Listeria monocytogenes*, *Salmonella spp.*, and *E. coli* must be absent in 25 mL.

Pasteurization does not eliminate spores or recontamination risks; hence, cold chain maintenance and hygiene are essential.

3. Fermented Milk Products (e.g., Yogurt, Cheese)

Fermented milk contains lactic acid bacteria, so standards focus more on pathogen absence and acceptable levels of spoilage organisms.

- **Yeasts and Molds:** Should not exceed 10^2–10^3 CFU/g depending on product type and shelf life.

- **Coliforms:** Ideally absent or below 10 CFU/g.

- **Pathogens:** *Listeria monocytogenes* must be absent in 25 g; *Salmonella spp.* must also be absent in 25 g (Codex, 2018).

Some semi-hard cheeses may allow non-pathogenic spore-formers due to maturation processes.

4. UHT and Sterilized Milk

Ultra-high temperature (UHT) treated milk is expected to be commercially sterile, meaning:

- No growth in 30–35°C incubation for 15 days, or
- No growth at 55°C for 7 days (used for testing sterility).
- **Spores:** Though technically present, should not germinate under proper storage conditions.

5. Ice Cream and Frozen Dairy Desserts

These are tested after manufacturing but before freezing:

- **Total Plate Count:** Usually < 100,000 CFU/mL.

- **Coliforms:** < 100 CFU/g; ideally absent.

- **Pathogens:** *Salmonella spp.* and *L. monocytogenes* must be absent in 25 g.

6. International and National Standards

Key bodies setting microbiological standards include:

- **Codex Alimentarius Commission (CAC):** International food standards (Codex, 2018)
- **Food and Drug Administration (FDA), USA:** PMO and CFR Title 21
- **European Food Safety Authority (EFSA):** EU hygiene package
- **Food Safety and Standards Authority of India (FSSAI):** BIS/FSSAI milk quality norms
- **International Dairy Federation (IDF):** Scientific guidelines and harmonization

Quality Control Techniques for Milk and Milk Products
Quality control (QC) techniques are essential for ensuring the safety, shelf-life, and nutritional and sensory quality of milk and its derivatives. These techniques encompass both microbiological and physicochemical assessments performed at multiple stages—from raw milk reception to final product distribution. Modern QC involves a combination of traditional microbiological tests, rapid methods, and automation-based analytical tools to detect contaminants and ensure compliance with standards (Chatterjee *et al*., 2006; [1] Jay, 2000).

1. Microbiological Quality Control Techniques
Microbiological analysis is central to dairy quality control because of milk's high susceptibility to spoilage and pathogenic contamination.

1.1 Plate Count Techniques
- **Standard Plate Count (SPC):** Estimates total viable bacterial load.
- **Coliform Count:** Indicator of fecal contamination and hygiene.
- **Psychrotrophic and Thermoduric Counts:** Assess spoilage potential and pasteurization resistance.

1.2 Pathogen Detection
- **Enrichment and Selective Media:** For detecting pathogens like *Salmonella spp.*, *Listeria monocytogenes*, and *E. coli O157:H7*.
- **ISO and BAM Protocol: s**Internationally standardized methods.
- **Example**: ISO 11290-1 for *Listeria monocytogenes* detection.

1.3 Rapid and Automated Methods
- **ATP Bioluminescence:** Indicates microbial contamination by detecting cellular ATP.
- **Enzyme-linked Immunosorbent Assay (ELISA):** Detects specific pathogens or toxins (e.g., *Staphylococcus aureus* enterotoxins)

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- **PCR and qPCR**: Molecular methods for rapid, sensitive detection of microbial DNA.
- **Flow Cytometry:** Rapid enumeration of live/dead cells.

2. Physicochemical Quality Control Techniques
These tests monitor the chemical composition, adulterants, and thermal processing efficacy of milk.

2.1 Basic Tests
- **Fat content:** Gerber method or infrared analysis.
- **SNF (Solids-Not-Fat):** Lactometer reading with temperature correction.
- **Freezing Point:** Used to detect water adulteration.
- **pH and Acidity:** Indicators of freshness and microbial activity.

2.2 Adulteration Tests
- Tests for added water, urea, starch, detergents, and neutralizers.
- Detected using colorimetric kits or standard BIS/FSSAI protocols.

2.3 Heat Treatment Verification
- **Phosphatase Test:** Detects residual alkaline phosphatase to verify proper pasteurization.
- **Peroxidase Test**: Indicates insufficient pasteurization if positive.

2.4 Sensory Evaluation Techniques
Sensory analysis assesses consumer acceptability and detects early spoilage.

- **Organoleptic Evaluation:** Appearance, color, odor, taste, and texture.
- **Trained Panel Testing:** Used for grading milk and products like butter, cheese, and yogurt.
- **Flavour Profile Analysis (FPA)**: Quantifies intensity of taste and off-flavors.

2.5 Instrumental Techniques
Modern dairy plants use advanced tools for rapid and accurate testing.

1. **Lactoscan / Milkoscan**: Automated analyzers for fat, SNF, protein, lactose, and density.
2. **Gas Chromatography–Mass Spectrometry (GC-MS):** Identifies volatile compounds in spoilage or flavor profiling.
3. **High-Performance Liquid Chromatography (HPLC):** Detects mycotoxins (e.g., aflatoxin M1), vitamins, and antibiotics.

2.6 Hygienic Monitoring
- **Surface and Air Sampling**: Swabs and air plates for assessing microbial load in the environment.
- **Clean-in-Place (CIP) Validation**: Checking sanitation efficiency using microbiological swabs and chemical residue detection.

Good Hygienic Practices (GHP) and HACCP in Dairy

The dairy industry is highly susceptible to microbial contamination due to the nutrient-rich composition of milk and the complexity of its processing and distribution chain. To ensure milk safety, quality, and compliance with international food safety standards, dairy operations must implement Good Hygienic Practices (GHP) and Hazard Analysis and Critical Control Point (HACCP) systems. These two frameworks are complementary pillars of food safety management that work together to minimize contamination risks, improve consumer confidence, and ensure regulatory compliance (FAO/WHO, 2006; [5] Codex Alimentarius, 2020).

1. Good Hygienic Practices (GHP)

GHP refers to the basic hygiene conditions and activities necessary to maintain a sanitary environment throughout the food chain—from farm to factory. In the dairy sector, GHP covers raw milk production, handling, transportation, processing, and storage.

Key Elements of GHP in Dairy

1. Animal Health and Milking Hygiene
- Regular veterinary checks, mastitis control, and clean udder practices reduce microbial load in raw milk.
- Use of clean milking equipment, proper hand hygiene, and single-use udder towels.

2. Clean Water Supply
- Water used in cleaning, cooling, and dilution must meet potable standards.

3. Sanitation of Equipment and Surfaces
- Clean-in-place (CIP) systems, effective sanitizers, and validated cleaning schedules.

4. Personnel Hygiene
- Training in hygiene, use of protective clothing, handwashing protocols.

5. Facility Hygiene and Pest Control
- Proper design and maintenance of buildings, effective pest prevention systems.

6. Temperature Control
- Rapid chilling of raw milk (to ≤ 4°C) after milking to suppress bacterial growth.

2. Hazard Analysis and Critical Control Point (HACCP)

HACCP is a science-based, systematic approach to identifying, evaluating, and controlling food safety hazards. It focuses on preventive measures rather than relying solely on end-product testing.

The Seven Principles of HACCP

1. Conduct a hazard analysis
- Identify biological (e.g., *Listeria monocytogenes*), chemical (e.g., aflatoxins), and physical (e.g., metal shavings) hazards.

2. Determine Critical Control Points (CCPs)
- Steps where control can be applied to prevent or reduce hazards (e.g., pasteurization).

3. Establish critical limits
- E.g., milk pasteurization must reach 72°C for 15 seconds.

4. Establish monitoring procedures
- Continuous temperature checks, automated logging systems.

5. Establish corrective actions
- If pasteurization temperature is not achieved, product must be held back or reprocessed.

6. Establish verification procedures
- Audits, microbial testing, and validation of equipment and methods.

7. Establish documentation and record-keeping
- Critical for traceability, inspections, and accountability.

3. Integration of GHP and HACCP in Dairy Processing

1. **GHP lays the foundation:** Without GHP, HACCP cannot function effectively. Poor hygiene undermines hazard control efforts.

2. **HACCP builds on GHP:** By identifying CCPs, HACCP adds a targeted risk management layer to hygiene practices.

3. **Together, they**
- Enhance product safety
- Reduce recalls and foodborne outbreaks
- Promote international trade (especially for export dairy producers)
- Ensure compliance with regulatory frameworks like the EU Hygiene Package and FSSAI Food Safety Regulations (India)

4. Benefits of GHP and HACCP in the Dairy Industry
- Reduced microbial contamination and spoilage
- Compliance with export/import regulations
- Enhanced brand trust and consumer confidence
- Economic benefits from fewer recalls and rejections

Regulatory Framework and Guidelines for Microbiological Quality Control in Dairy

Regulatory frameworks and guidelines for the dairy sector are critical for safeguarding public health, promoting fair trade practices, and ensuring food quality and safety from farm to consumer. These frameworks, established by international bodies, national agencies, and scientific organizations, define the permissible microbiological limits, inspection protocols, quality assurance systems, and enforcement mechanisms to be followed by dairy producers and processors.

1. International Regulatory Bodies and Guidelines

1.1 Codex Alimentarius Commission (CAC)

Jointly established by the FAO and WHO, Codex develops harmonized international food standards.
It provides:
- **General Principles of Food Hygiene (CXC 1-1969):** including HACCP guidelines

- **Milk and Milk Products Standards (CXS 206-1999):** covering microbiological limits, compositional criteria, and labeling

- **Microbiological Criteria (CAC/GL 21-1997):** for assessing food safety.

1.2 International Dairy Federation (IDF)
- Provides technical guidelines for sampling, microbial testing, hygiene, and food safety in milk production and processing.
- Supports the harmonization of global dairy microbiological standards.

2. National Food Safety Authorities

2.1 United States – FDA and USDA
- The U.S. Food and Drug Administration (FDA) oversees milk safety under the Grade A Pasteurized Milk Ordinance (PMO).
- The PMO sets standards for:
- Raw and pasteurized milk microbiological limits
- Facility sanitation
- Personnel hygiene
- Pasteurization effectiveness

2.2 European Union – EFSA and EC Regulations
The European Food Safety Authority (EFSA) advises the European Commission on food safety.

Key regulations include
- **Regulation (EC) No. 852/2004:** General food hygiene

- **Regulation (EC) No. 853/2004:** Specific hygiene rules for animal-origin foods

- **Regulation (EC) No. 2073/2005**: Microbiological criteria for foodstuffs, including milk and cheese

2.3 India FSSAI (Food Safety and Standards Authority of India)
- FSSAI sets national food safety standards under the Food Safety and Standards Act, 2006.
- Key dairy-related regulations:
- FSS (Food Products Standards and Food Additives) Regulations, 2011
- FSS (Contaminants, Toxins and Residues) Regulations, 2011
- Microbiological limits for milk and milk products under FSSAI Manual of Methods of Analysis of Foods – Milk and Milk Products

3. Quality Management and Certification Programs
- **ISO 22000:** International standard for food safety management systems, incorporating HACCP principles.

- **GMP/GHP Certification:** Often mandatory for export markets, ensuring that hygiene and processing protocols meet international norms.

- **BRCGS (British Retail Consortium Global Standard) and IFS (International Featured Standards):** Common in private labeling and international trade.

4. Implementation and Compliance Mechanisms
- **Inspection and Auditing:** Conducted by government or third-party agencies to assess compliance with hygiene and microbiological standards.

- **Sampling and Testing:** Random sampling of dairy products to detect microbial contamination and chemical adulterants.

- **Penalties and Enforcement:** Legal actions for violations include fines, recalls, or shutdowns.

Conclusion
Microbiological quality control in milk and dairy products is essential for consumer safety and regulatory compliance. Continuous monitoring, adherence to hygienic practices, and implementation of preventive systems like HACCP significantly reduce the risk of contamination and outbreaks.

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