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Listeria monocytogenes IN DAIRY PRODUCTS OCCURRENCE, MONITORING AND SURVEILLANCE

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ABSTRACT. Cross-contamination with foodborne microorganisms is a challenge at every stage of food preparation. *Listeria monocytogenes* poses serious and persistent problems for the food industry because of its ability to withstand a broad range of temperatures and pH levels and thrive under high salt concentrations. These factors significantly increase the risks to consumers. Although *Listeria* is uncommon in the general population, the bacterium is frequently isolated from food and environmental sources. The prevalence of systemic listeriosis is notably higher among vulnerable groups, such as the elderly, pregnant women, and individuals with weakened immune systems. Among the species within the *Listeria* genus, *L. monocytogenes* is the most significant in food contexts because of its capacity for proliferation and its adaptability to changing environments. Advancements in detection technologies have enabled the identification of more outbreaks with fewer cases per incident. To monitor and validate the efficacy of control measures, robust environmental monitoring programmes are essential. These programmes include establishing protocols for sampling and detection, determining sampling frequency, selecting sampling zones, and implementing corrective actions. This study aims to review the specialist literature on the management, surveillance, and prevalence of *L. monocytogenes* in dairy products.

Keywords: food safety; health priority; microbial contamination; risk assessment.

INTRODUCTION

The Food and Agriculture Organization of the United Nations emphasises the urgent need to increase food production by 60% by 2050 to meet the demands of a growing population and address the effects of climate change. However, one-third of the food produced globally is wasted annually, causing significant economic and ecological impacts. Extending shelf life and reducing food spoilage are therefore essential priorities (Ribeiro *et al.*, 2023).

Food safety remains a major public health issue, with millions of illnesses reported each year due to contaminated food. According to the World Health Organization, approximately 600 million people develop food-borne diseases annually, resulting in 420,000 deaths (Talari *et al.*, 2024). Children under 5 years of age are particularly vulnerable, and the mortality rate is disproportionately high among this group. Effective food safety management is therefore crucial to mitigating risks throughout the supply chain (Gonzales-Barron *et al.*, 2023).

Food contamination is often caused by pathogens such as *Staphylococcus aureus*, *Salmonella*, and *Listeria monocytogenes*, among others, which account for 40% of annual deaths due to food poisoning. Biofilms, which are communities of microorganisms resistant to cleaning methods, also contribute significantly to food contamination. Estimates suggest that microbial contamination leads to food losses ranging from 33% to 50% of global production (Bevilacqua *et al.*, 2023; Bodie *et al.*, 2023; Erol *et al.*, 2024).

In Europe, approximately 23 million people fall ill each year due to contaminated food, with 5,000 associated deaths reported annually. Preventing contamination is crucial given the chemical and structural diversity of food, which complicates microbiological safety control. Awareness of bacterial characteristics and behaviour is therefore fundamental to developing effective food safety strategies. For instance, in 2019, 25,866 cases of foodborne illness were reported, involving pathogens such as *Salmonella* and *Listeria*. Recent analyses have identified the prevalence of these agents in various food products, underscoring the importance of comparing antibiotic resistance across different regions (Bland *et al.*, 2022; Bolten *et al.*, 2023; Farber *et al.*, 2020).

The European dairy industry, valued at USD 164.73 billion, faces *Listeria* risks, particularly in ready-to-eat (RTE) products. Regulations differ between countries, with Europe setting specific limits and the United States adopting a stricter approach (EU Regulation, 2005; US Regulation, 2017). Microbial risk assessment involves identifying hazards and management strategies (Bland *et al.*, 2022; Bolten *et al.*, 2023).

Disease outbreaks in the dairy industry, including those caused by *Listeria*, remain a significant challenge, requiring constant updates to control strategies. Although the number of outbreaks is relatively low, their impact can be severe, often necessitating hospitalisation. Vulnerable groups, such as pregnant women and individuals with compromised immune systems, are particularly at risk (Gupta and Adhikari, 2022; Brown *et al.*, 2024). Contamination control is essential in the dairy industry because raw milk provides a favourable environment for the growth of pathogens.

Modern technologies, such as hazard analysis and critical control points (HACCP), facilitate the identification of risks during processing. Quantitative microbial risk assessment plays a vital role in minimising health risks (Kammoun *et al.*, 2022; Rudke *et al.*, 2024). The Indian dairy industry faces a pressing need for effective traceability systems to address issues such as milk adulteration and contamination. Research has highlighted the evolution of detection techniques and their potential to improve transparency and safety (Malik *et al.*, 2023).

Omics technologies, such as genomics, enhance our understanding of microbial diversity and can improve risk assessment. Currently, dairy products account for 12% of all foodborne diseases. Contamination can occur early in the supply chain, with risks heightened by improper handling and incorrect storage (Joshi *et al.*, 2022; Osek *et al.*, 2022c).

Modern methods of pathogen detection enable improved monitoring of contamination. Combining advanced technologies with risk analysis can support the development of more effective strategies to ensure food safety. Overall, the food sector must adapt to emerging food safety challenges by integrating new technologies and assessment methods to safeguard public health (Ribeiro *et al.*, 2023). This paper provides a brief review of the literature on the incidence, prevalence, detection, and identification of *L. monocytogenes* in the dairy industry.

Listeria monocytogenes: An ongoing challenge for dairy industry processors

Listeria monocytogenes has been recognised as a pathogen since 1929 but gained significant attention in the 1980s following outbreaks of listeriosis in North America and Europe. The genus *Listeria* comprises 28 species, of which only *L. monocytogenes* and *L. ivanovii* are pathogenic to humans. This Gram-positive, facultatively anaerobic bacterium can survive under extreme conditions, contaminating a variety of foods including fish, meat, and unpasteurised dairy products. Listeriosis typically causes flu-like symptoms but is particularly serious for vulnerable individuals, with a fatality rate of 20% to 30%. A major challenge in controlling this bacterium is its ability to form biofilms, which confer resistance to disinfectants. Biofilm formation depends on factors such as surface type and environmental conditions, including temperature (Angelidis *et al.*, 2023). Additionally, *L. monocytogenes* is an intracellular pathogen that rapidly adapts to environmental stress. Preventing listeriosis requires stringent hygiene management in the food industry, and a deeper understanding of its persistence mechanisms and control strategies is essential to safeguarding public health (Basak *et al.*, 2024).

Listeria monocytogenes is a foodborne pathogen that poses a significant challenge to food safety because of its ability to survive under extreme conditions. This bacterium can grow at refrigeration temperatures and tolerate environments with high salt concentrations and variable pH levels, enabling its persistence in food processing environments. Its ability to form biofilms resistant to sanitising agents further complicates control and eradication efforts (Bashiry *et al.*, 2022).

The most commonly contaminated foods include RTE products such as fish, dairy, meat, and fruit. Contamination can occur at any stage of production, from farms to distributors, with soil serving as the main reservoir of *Listeria*. Animals and vegetables are primary sources of contamination, while at the distribution level, equipment and personnel can contribute to its spread (Bolten *et al.*, 2024; Dincer, 2024; Lindsay *et al.*, 2023).

To minimise the risk of listeriosis outbreaks, continuous food monitoring is essential in accordance with national and international regulations. The recall of contaminated products significantly disrupts the supply chain and places a strain on health systems. In the United States, a zero-tolerance policy mandates the recall of any contaminated RTE product, whereas some European countries and Canada permit low concentrations (Brandelli *et al.*, 2023).

Listeria is an opportunistic pathogen, particularly affecting vulnerable groups such as pregnant women, the elderly, and individuals with compromised immune systems. The severe form of the infection, invasive listeriosis, can result in abortions, septicaemia, and meningitis, with a high mortality rate. Globally, approximately 23,150 cases and 5,463 deaths are reported annually. Of the five known *Listeria* species, *L. monocytogenes* is the only one pathogenic to humans, with serotype 4b most commonly involved in outbreaks (Cheng *et al.*, 2022; Lakicevic *et al.*, 2022).

This bacterium can survive in diverse conditions, ranging from wet to cold environments, and it is frequently isolated from dairy and poultry products. Although resistant to heat, *L. monocytogenes* can be destroyed at 70°C for 2 minutes. This poses a significant challenge in the American and European regions, where listeriosis is more prevalent due to dietary habits (Lotoux *et al.*, 2022; Osek *et al.*, 2022a).

Antimicrobial resistance is another significant issue arising from the overuse of antibiotics in animal feed production. This has led to the emergence of resistant strains, which can be transmitted from animals to humans. The first multidrug-resistant strain was identified in 1988, and the phenomenon has since become a global concern. The evolution of antibiotic resistance is linked to their excessive use in agriculture, where they are applied both in animal husbandry and for prophylaxis (Karssa *et al.*, 2024; Osek *et al.*, 2022b).

Preventing listeriosis requires strict hygiene measures in food production and the responsible use of antibiotics. Although the disease is rare, its high mortality rate underscores the importance of effective control measures (Aleksic *et al.*, 2023, 2024).

The prevalence of *L. monocytogenes* in cheeses varies, with some strains exhibiting greater heat resistance, raising concerns about the complete inactivation of pathogens. Careful monitoring of food processing facilities and the implementation of effective sanitation procedures are essential to prevent contamination (Fusco *et al.*, 2022).

In addition, *L. monocytogenes* can enter a viable but non-cultivable state under stress conditions, complicating detection through traditional methods. This state may facilitate the asymptomatic transport of the bacterium across different ecosystems. The "One Health" approach highlights the interconnectedness of human, animal, and ecosystem health, including the asymptomatic carriage observed in various mammalian species.

Recent studies have identified a significant prevalence of asymptomatic carriers, emphasising the need for more sensitive detection methods, such as polymerase chain reaction (PCR) amplification. *Listeria monocytogenes* demonstrates an extraordinary ability to adapt to various environments, necessitating systematic risk assessments of viable but non-cultivable bacteria in the future (Aladhadh, 2023).

Controlling *Listeria* contamination and the responsible use of antimicrobials are essential to safeguarding public health. A rigorous approach to food safety, particularly for RTE products, is crucial to preventing listeriosis.

Listeriosis: disease and pathways of transmission

Listeriosis is an infection caused by the bacterium *L. monocytogenes*, which is transmitted through the consumption of contaminated food. There are two forms of listeriosis: invasive and non-invasive. The invasive form is more serious, particularly affecting immunocompromised individuals, pregnant women, and the elderly, and can result in severe complications such as sepsis and meningitis. The non-invasive form, febrile gastroenteritis, typically occurs in healthy individuals and causes mild symptoms such as fever and diarrhoea (Ribeiro *et al.*, 2023).

Human transmission of the bacterium was first recognised in the 1980s during outbreaks in the United States and Switzerland, with sources of contamination primarily identified in dairy products, meat, and vegetables. Listeriosis is classified as a zoonotic disease, with an infectious dose ranging from 10^4 to 10^7 bacteria for vulnerable individuals and higher thresholds for healthy individuals. Although the incidence of listeriosis is low, the hospitalisation and mortality rates are alarmingly high. For example, approximately 1,600 people in the United States are estimated contract the disease annually; of these patients, approximately 260 die, and the hospitalisation rate is 94% (Bashiry *et al.*, 2022; Wei *et al.*, 2024).

Listeria can contaminate various foods, and proper handling and storage are essential to prevent infection. Regulations enforce strict standards for food products, particularly those intended for vulnerable populations, and rapid detection of the bacterium in food is crucial. Major outbreaks have been linked to the consumption of cheese, ice cream, and delicatessen products. *Listeria* contamination has prompted numerous food recalls, emphasising the importance of minimising exposure to this pathogen in the food chain. The incidence of listeriosis ranges from 0.4 to 1.8 cases per 100,000 inhabitants, with mortality rates reaching up to 30%, particularly among at-risk groups. Most cases of invasive listeriosis are sporadic and linked to the consumption of RTE foods such as smoked fish and soft cheeses. *Listeria monocytogenes* exhibits a remarkable ability to survive in food products, even under adverse conditions (Osek, 2022b).

The incubation period of listeriosis ranges from 1 week to 70 days, complicating efforts to identify the source of contamination. A high bacterial concentration is typically required to cause disease, and the infection can vary from mild to severe. Symptoms range from fever and muscle aches to serious complications, such as central nervous system infections. Listeriosis can lead to severe conditions, including septicaemia, meningitis, and encephalitis. The infection manifests with symptoms that often overlap with those of other bacterial infections. In 2020, the European Union reported 1,876 cases of invasive listeriosis, with a hospitalisation rate of 97.1% (Silva *et al.*, 2024).

Prevalence, detection and Control of *L. monocytogenes*

Over the years, authorities and food manufacturers have made considerable efforts to control *L. monocytogenes*, a ubiquitous bacterium in the environment that causes listeriosis, a serious foodborne infection. This bacterium has been implicated in notable outbreaks, such as the 2015 ice cream-related incident. Controlling *Listeria* in food production facilities is complex and requires a thorough understanding of contamination pathways, which include raw materials, equipment, employee activities, and environmental sources such as water and soil (Hawaz *et al.*, 2023).

Several factors contribute to the prevalence of *Listeria* in food environments, including the type of food, processing methods, effectiveness of cleaning protocols, and equipment design. Research suggests that certain strains of the bacterium can persist for months or years, even after sanitation, often due to contamination in hard-to-reach areas such as cracks in equipment or infrastructure. This persistence is partly because the decline in bacterial cell numbers is slower than their reproduction rate (Martin *et al.*, 2022; Sibanda and Buys, 2022).

While the persistence of *L. monocytogenes* is often linked to biofilm formation, the exact relationship remains debated. Biofilms are clusters of microbial cells that adhere to surfaces and develop a protective protein matrix. The biofilm formation process involves several steps, from the initial attachment of cells to a solid surface to the development and maturation of the biofilm. *Listeria* can adhere to various materials, including stainless steel and rubber. Bacteria within biofilms exhibit increased resistance to environmental factors and antimicrobial substances, enabling their survival in production environments (Monteith *et al.*, 2023; Schoder *et al.*, 2023; Unger *et al.*, 2023).

Studies have shown that common disinfectants, such as sodium hypochlorite and hydrogen peroxide, are not always effective in completely eliminating *Listeria* biofilms. Additionally, low concentrations of these agents may favour the development of resistant strains. However, no consistent link has been established between the persistence of the bacterium and its ability to form biofilms, suggesting that other mechanisms may contribute to its persistence (Finn *et al.*, 2023).

Environmental monitoring programmes are crucial for identifying sources of contamination. These involve sampling four types of areas within production facilities, each associated with a different level of contamination risk. Close monitoring of these areas helps prevent the contamination of food with *L. monocytogenes*, which is a significant issue, particularly for dairy products. Certain types of cheese, such as gorgonzola and camembert, are especially vulnerable.

HACCP-certified farms typically demonstrate better sanitation training, higher milk quality, and healthier dairy cows than do non-certified farms. However, these farmers often face challenges in managing their facilities and maintaining daily records. Because of the effort required, lack of recognition, and difficulties in obtaining government assistance, non-certified farmers tend to avoid HACCP implementation. Developing a standardised record chart and manual is therefore necessary to address these challenges (Chon *et al.*, 2021).

Research has shown a correlation between food pH and *Listeria* survival. For example, cheeses with a higher pH support greater bacterial growth than do those with a lower pH. Studies indicate that some cheeses, such as Mascarpone and Ricotta, are at higher risk of contamination, while others, such as Gouda, demonstrate greater resistance (Iulietto *et al.*, 2024; Obaidait, 2024; Sebastianski *et al.*, 2022).

In addition, *L. monocytogenes* exhibits resistance to several antibiotics, which is a significant public health concern. Strains resistant to first-line antibiotics have been identified in various countries, including Romania, where penicillin-resistant strains have been detected. This resistance complicates infection treatment and underscores the importance of monitoring and control in food production (Duma *et al.*, 2024).

Outbreaks of listeriosis are frequently caused by persistent strains, impacting both public health and the economy due to the costs associated with product recalls and financial losses. The mechanisms underlying *Listeria* persistence remain partially unclear. Increased tolerance to disinfectants and adaptation to stress in food environments are believed to contribute to this issue. Although modern technologies, such as genome sequencing, have not yet identified definitive genetic markers for persistence, a clearer definition of the term 'persistence' could aid in standardising studies and improving our understanding of this phenomenon (Obaidat and AlShehabat, 2023; Tola, 2024).

Listeria monocytogenes can colonise food processing facilities, with some strains persisting for years. This persistence is attributed to either unique strains or survival in inaccessible areas. Bacteria can also develop resistance to disinfectants, which facilitates biofilm formation.

Advanced methods, such as whole-genome sequencing (WGS), could enhance risk assessment and listeriosis prevention but require adequate infrastructure (Ramadan *et al.*, 2023; Sibanda *et al.*, 2023).

Outbreaks of infection can occur even in pasteurised cheeses, with soft cheeses being particularly vulnerable (Basak *et al.*, 2024). Factors such as humidity, pH, and ripening temperature significantly influence the inhibition of *Listeria* growth. Rigorous microbial risk assessments are especially critical on small farms. *Table 1* summarises the detection sites of *L. monocytogenes* in dairy plants (Hu *et al.*, 2022; Ribeiro *et al.*, 2023).

In March 2015, an outbreak of listeriosis linked to ice cream underscored the risks, resulting in nine cases and two deaths. The presence of multiple *Listeria* serotypes complicated the investigation. Even low levels of contamination can pose serious risks, particularly to individuals with compromised immune systems.

Listeria monocytogenes is prevalent in agricultural and processing environments, with foodborne transmission being a common occurrence. Inefficient cleaning practices and poor equipment design contribute to its persistence. Nearly 83% of listeriosis cases in the United States have been associated with processed meats and cheeses (Pospo *et al.*, 2023).

Although the presence of *L. monocytogenes* in raw milk is generally low due to strict hygiene measures, the bacterium can survive in refrigerated conditions. Temperature fluctuations during transport favour bacterial proliferation, and solid-liquid interface conditions can further facilitate growth (Wang *et al.*, 2024).

The Codex Alimentarius Commission has established criteria for RTE products that can support the growth of *Listeria monocytogenes*, facilitating improved risk assessment. Advances in detection methods, such as WGS, have enhanced the identification of listeriosis outbreaks, underscoring the need to reassess the risks associated with low doses.

In recent decades, the detection of *Listeria* spp., particularly *Listeria monocytogenes*, in food has become a public health priority because of the risks posed by this bacterium. Various detection methods have been developed, each with specific advantages and disadvantages (*Figure 1*).

Conventional methods, considered the gold standard, include bacterial culture techniques. These methods are recommended by organisations such as the Food and Drug Administration and the International Organization for Standardization.

Table 1 – *Listeria monocytogenes* detection in dairy plants (Ribeiro *et al.*, 2023)

Category	**Sites of isolation**
Liquid and drain-related areas	• Drain water, drain biofilms, floor drains • Processing-room drains, brine-room floor • Cooling-chamber drains, floors and platforms of processing rooms, plastic crates, gloves, brine
Milk and dairy products	• Milk from farm bulk tanks, raw milk in storage • Raw milk, food, floors, steps, drains • Bulk-tank milk, milk filters
Food and contact surfaces	• Brine, food and non-food contact surfaces, farm bulk tanks • Processing-room floor, raw milk, cooling tanks, processing room drains • Drains, shoes, floors
Equipment and machinery	• Conveyor belts, floors, food soil, packaging benches, conveyor belts • Processing-plant area

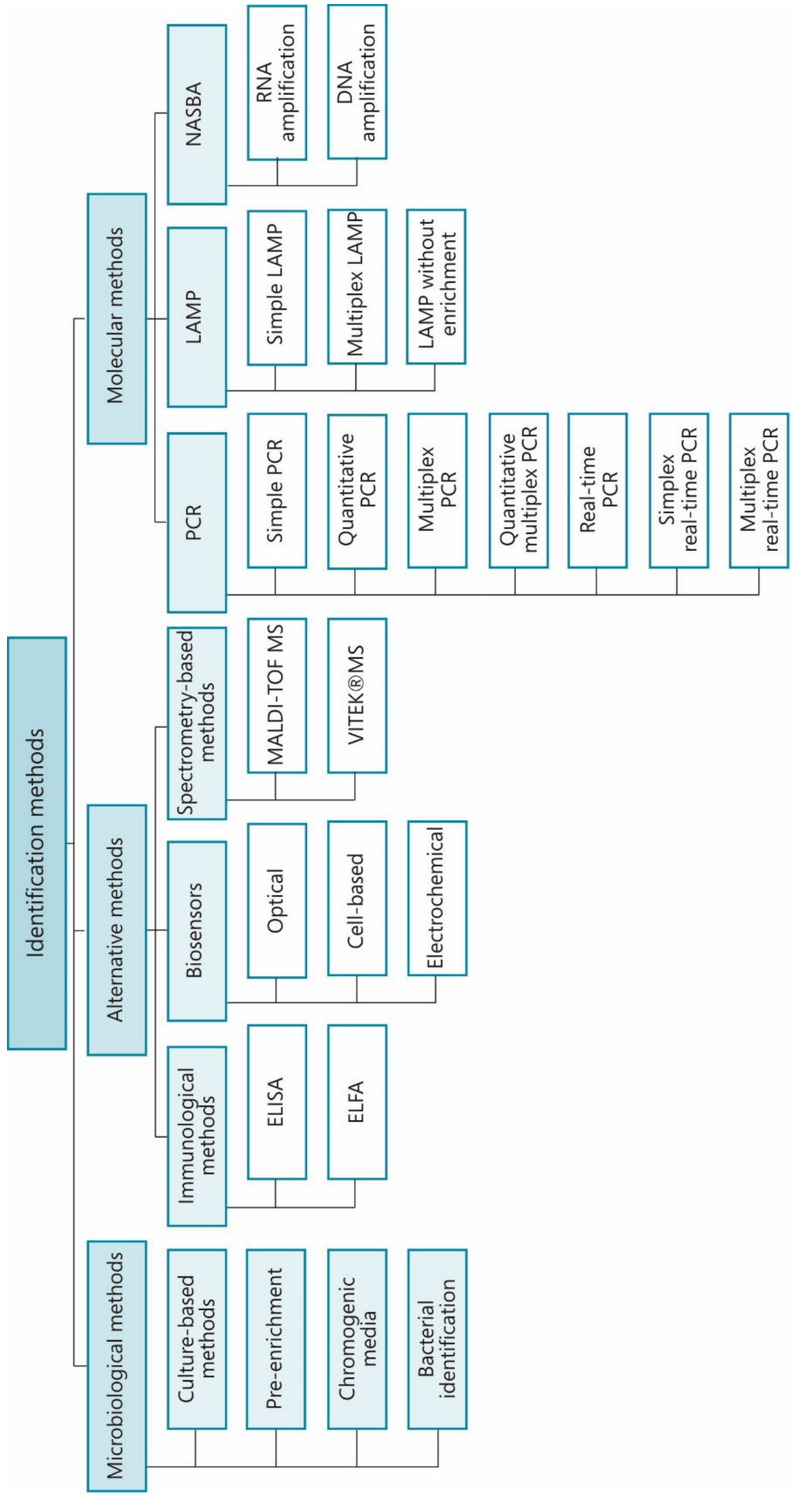

Figure 1 – Methods of detection and identification of the species *Listeria monocytogenes*

The screening process typically involves sample enrichment followed by plating on selective media, such as differential agar. While this approach allows for qualitative identification of *Listeria*, it is time-consuming, often requiring more than 7 days to produce results (Dincer, 2024; Lakicevic *et al.*, 2023).

Alternative methods have been developed to reduce the analysis time. These include methods based on DNA amplification, such as PCR. Such methods provide rapid and accurate results, with PCR used to detect virulence genes of *L. monocytogenes*. However, PCR cannot assess the viability of the microorganism. Techniques such as real-time nucleic acid sequence-based amplification (NASBA) and loop-mediated isothermal amplification (LAMP) amplify RNA and DNA at constant temperatures, making them useful for rapid detection. NASBA identifies viable microorganisms by amplifying messenger RNA, while LAMP uses a DNA polymerase with strand displacement activity and four to six primers specifically designed for six to eight distinct sites on the target DNA (Hou *et al.*, 2024; Silva and Evelyn, 2023; Tonti *et al.*, 2024).

Immunological techniques, such as enzyme-linked immunosorbent assay, are based on antibody–antigen interactions and are used for the rapid identification of bacteria. These methods include lateral flow assays and immunomagnetic capture, which enable efficient detection under laboratory conditions. For instance, recent tests have employed gold nanoparticles to enhance detection sensitivity (Aladhadh, 2023; Kalinin *et al.*, 2023).

Biosensors are a recent innovation in the field of sensing, incorporating bioreceptors that convert biological interactions into measurable signals. Biosensor technologies, such as optical and electrochemical systems, facilitate the rapid and efficient detection of *L. monocytogenes*, with direct applicability in the food industry (Mehrannia *et al.*, 2023; Péter *et al.*, 2022).

Hyperspectral imaging offers a rapid and non-destructive method for microbial identification. Additionally, the analysis of WGS data is becoming increasingly important for epidemiological monitoring and traceability. Organisations such as the Food and Drug Administration and the Centers for Disease Control and Prevention utilise WGS to link clinical isolates with food isolates, aiding in outbreak investigations (Quintela *et al.*, 2022).

Although technological advances are promising, challenges persist in integrating rapid methods into routine testing. There is also a critical need for affordable and reliable approaches for the rapid detection of contamination, particularly in food production facilities. Innovations in biosensors and microfluidic technologies could significantly enhance the efficiency of *L. monocytogenes* monitoring (Aladhadh, 2023; Li *et al.*, 2024).

CONCLUSIONS

Dairy products, raw milk, and soft cheeses are among the most vulnerable to *Listeria monocytogenes* contamination, particularly because of high pH values and low-temperature storage conditions. Raw milk poses a high risk of listeriosis, especially under inadequate pasteurisation conditions and poor hygiene practices. Listeriosis is a severe disease with high mortality rates, particularly among vulnerable populations. Preventing infection and implementing effective food safety management practices are crucial to mitigating the risks associated with *L. monocytogenes*.

The persistence of *Listeria monocytogenes* in food environments has significant economic and public health impacts, necessitating rigorous monitoring and control measures. While various methods, such as molecular techniques and biosensors, have been developed to detect this bacterium, traditional methods, such as culture on selective media, remain the gold standard. Emerging technologies, such as genomic sequencing and biosensors, offer promising perspectives for monitoring and preventing foodborne outbreaks, but they require sophisticated equipment and skilled personnel. Therefore, collaboration between researchers and the food industry is essential to develop more effective methods for controlling and preventing *L. monocytogenes*.

To enhance food safety and minimise the economic burden of *L. monocytogenes* contamination, further research is critical. Future studies should prioritise developing more effective methods for controlling and preventing the persistence of this bacterium in food environments, thereby protecting public health.

In conclusion, the detection of *L. monocytogenes* remains a complex challenge. Although traditional methods are effective, advances in molecular technologies and biosensors present promising solutions for reducing risks to public health. Continued collaboration between researchers and the food industry is vital to improving food safety and preventing outbreaks of infection.

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