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Review Article

Listeria monocytogenes **in Food Production and Food Safety: A Review**

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Abstract

In the current world of global food distribution and marketing, the outbreak of a particular foodborne disease in a region may eventually occur in another part of the world at the same time. Foodborne diseases are estimated to cause over 600 million illnesses and 420,000 deaths each year. Listeriosis, a zoonotic bacterial disease caused by *Listeria* spp. has been reported in various parts of the world's disease outbreak and incidence. The highest number of diseases and outbreaks is caused by *Listeria monocytogenes* and is highly predominant among elderly persons, immunocompromised individuals, pregnant women, and infants. This bacterium possesses the ability to survive and multiply under refrigeration temperature, a wide range of pH (4.1 - 9.6), and high salt (≤ 10%). The disease can be minimal (non-invasive/febrile gastroenteritis) and the pathogen possesses the ability to cross the epithelium and infect sterile organs (invasive). Outside food contamination, resistance to antibiotics by *Listeria monocytogenes* poses major public health concerns as this bacterium has developed resistance to various antibiotics in use. In addition to food, *Listeria* species have been isolated from the soil, water, plants, fruits, and vegetables coupled with the long incubation period (11 - 70 days), which poses a challenge to infection source identification and trace back principle. Controlling *Listeria* in foods can be achieved by practicing a Hazard Analysis and Critical Control Point (HACCP) strategy in the food industry, improving hygiene measures, and avoiding the consumption of contaminated foods. Also, susceptible individuals should be educated on the risks associated with the consumption of contaminated foods.

Introduction

Foodborne diseases are a major cause of illness and death worldwide, affecting people of all ages and genders. They result in more than 600 million illnesses and over 420,000 deaths annually on a global scale [1]. These diseases have diverse causes and are prevalent in both developed and developing countries. Due to global food distribution and marketing, contaminated products can affect multiple countries at the same time. The discovery of one contaminated ingredient or product can lead to massive recalls of food products, causing substantial illness, diseases, economic losses, trade embargoes, and impacting the tourism industry. Despite improvements in food production, the incidence of foodborne diseases has increased over the past decades. There are over 250 different types of foodborne illnesses caused by various pathogens or toxins [2]. While most cases are mild and resolve on their own, severe instances

can lead to high mortality and morbidity, especially among vulnerable groups such as infants, young children, the elderly, and those with weakened immune systems [3].

Foodborne illnesses arise from consuming contaminated foods, which can be contaminated by pathogens (such as bacteria, viruses, and parasites), chemicals, or toxins at various stages of production and preparation. According to the WHO, approximately 550 million people fall ill and 230,000 die annually due to diarrheal diseases associated with consuming contaminated food with 220 million children and 96000 deaths per year [4]. Developing countries are particularly vulnerable due to factors such as inadequate access to potable water for drinking and food preparation, improper food transportation, preservation and storage practices, and lack of awareness regarding safe food handling practices. Additionally, many developing countries lack the resources to enforce food safety

regulations, conduct effective surveillance, and implement educational programs on food hygiene [5]. Despite causing considerable illness in developed countries, the greatest impact of foodborne diseases is felt in developing nations. These diseases pose a growing public health challenge in both developed and developing countries. One notable emerging foodborne illness is Listeriosis caused by *Listeria monocytogenes* primarily affects vulnerable populations such as pregnant women, newborns, the elderly, and individuals with weakened immune systems. Healthy individuals may experience no symptoms or mild gastrointestinal issues.

Listeria monocytogenes is one of the major causative agents that accounts for serious diseases in humans and animals through the consumption of contaminated foods. Identified as a human pathogen in 1929, its transmission route became evident in the 1980s following several outbreaks linked directly to food [6]. *L. monocytogenes* is a Gram-positive bacterium, anaerobic facultative, non-spore-forming, facultative intracellular, catalase positive, non-capsulated, L-Rhamnose positive, oxidase negative, motile at 10 °C to 25 °C and rodshaped bacteria capable to grow in temperatures between 0 and 45 **°**C [7]. This bacterium tolerates high salinity (up to 10%) and wide pH range (4.1 to 9.6) [8]. *Listeria monocytogenes* is a foodborne bacterium that can be found in natural environments such as water, soil, and decaying vegetation, and animal reservoirs such as cattle, sheep, and fowl. Humans may also act as reservoirs and asymptomatic fecal carriage has been reported in humans, particularly abattoir workers and laboratory workers exposed to *L. monocytogenes* culture [9]. Transmission of *L. monocytogenes* is principally via the fecaloral route through the consumption of contaminated food. Serotyping based on the somatic (O) and flagellar (H) antigens, revealed more than 13 serotypes of *L. monocytogenes* comprising 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7, with only four of them (1/2a, 1/2b, 1/2c and 4b) are responsible for the majority of global listeriosis cases [7,10].

Listeriosis

The incubation period ranges from 11 to 70 days after exposure to the contaminated food, with an average of 31 days [11]. Following ingestion, the bacteria that survive/resist the stomach acidity enter the small intestine, breach the epithelial barrier, and then disseminate to organs such as the liver, spleen, central nervous system, and fetus. The mortality rate among individuals infected with *Listeria* averages around 20- 30%, even with appropriate antimicrobial treatment [12]. Despite its relatively low incidence compared to diseases like salmonellosis and campylobacteriosis, listeriosis is recognized as a significant foodborne pathogen due to its persistent mortality rates over the years [13].

Most healthy individuals infected with listeriosis experience no symptoms or only mild febrile illness. Symptomatic infection is common in pregnant women, infants, the elderly, and those with weakened immune systems. High-risk individuals primarily include those with chronic debilitating conditions that compromise their immune system, such as cancer, diabetes, or alcoholism; HIV/AIDS; individuals

taking immunosuppressive medications and those over the age of 60 - 65, especially those with pre-existing medical conditions. Healthy children and immunocompetent adults have a low risk of severe *L. monocytogenes* infection. In severely immunocompromised patients, the fatality rate may reach as high as 75% [14]. Clinical manifestations of listeriosis can be categorized into invasive, where the bacteria spread beyond the gastrointestinal tract, and non-invasive forms.

Invasive listeriosis occurs when *L. monocytogenes* that initially infect the intestinal tissue subsequently invade the sterile system and organs of the body such as the pregnant uterus, the central nervous system, and the bloodstream. *L. monocytogenes* can evade the phagosome, and immune defense mechanism, and multiply within the cell. The possibility of *L. monocytogene*s to evade intestinal defense mechanisms and establish infection depends on the number of bacteria ingested, the susceptibility of the host, and the strain. This infection can be life-threatening, with hospital fatality rates ranging from 20% to 30% CDC reported *L. monocytogenes* as having the secondhighest case fatality rate (21%) and the highest hospitalization rate (90.5%) among foodborne pathogens it tracked in 2000 [15]. In adults, the syndromes of invasive Listeriosis include bacteremia, meningitis, encephalitis, and gastrointestinal symptoms accompanied by fever while pregnant women often experience an influenza-like illness with bacteremia, which can lead to infection of the fetal and fetal membranes and result in abortion, stillbirth, or premature birth.

Non-invasive listeriosis, also known as febrile listerial gastroenteritis, typically occurs during outbreaks where individuals experience symptoms such as chills, diarrhea, fever, headache, abdominal pain, nausea, vomiting, fatigue, joint and muscle pain, and myalgia shortly after being exposed to high doses of *L. monocytogenes*. Symptoms typically appear within 9 to 48 hours after exposure to the bacteria. Healthy individuals with robust immune systems are generally at lower risk of developing severe complications from non-invasive listeriosis. However, vulnerable populations are at greater risk of developing invasive forms of the infection.

Prevalence of *L. monocytogenes*

In 1983, Fleming, et al. documented the first instance of *L. monocytogenes* in 2% of pasteurized milk samples in Massachusetts [16]. Earlier, *L. monocytogenes* had been identified as the causative agent of mastitis in dairy cows, leading to contamination of the milk they produce [17]. Since then, numerous studies have investigated and revealed the presence of *L. monocytogenes* in milk, fresh produce, meat, fish, dairy products, vegetables, food processing environments, ready-to-eat meats, etc. as shown in Table 1. 100% occurrence of *L. monocytogenes* was found in spiked soft cheeses [18]. The detection of *L. monocytogenes* in both frozen and pasteurized samples demonstrates its ability to survive under refrigeration, highlighting the complexity of producing L*isteria*-free foods, especially under suboptimal processing and handling conditions.

In 2004, a study in the Czech Republic reported a 2.6%

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contamination rate of *L. monocytogenes* in milk samples, attributed to soil contamination before pasteurization [19]. Although pasteurization reduces the risk of infection, it does not eliminate it; a Finnish report indicated that milk could be contaminated in stages following pasteurization [20]. A study in Iran found that the prevalence of *Listeria* species in raw milk, ice cream, cream, and porridge was 5.49%, 19.04%, 11.11%, and 4%, respectively and no *Listeria* was detected in yogurt, butter, kashk, or cheese. Also, Sayevand, et al. identified *L. innocua* and *L. monocytogenes* as the dominant species, with prevalences of 5.44% and 1.36%, respectively [21].

Most of the research on *L. monocytogenes* has focused on milk and dairy products, meat and meat products, and readyto-eat foods as shown in Table 1. However, fewer studies have examined environmental and processing equipment, which are also critical vectors for *L. monocytogene*s transmission and should not be neglected. It was discovered that *L. monocytogenes*

can persist in food processing environments (e.g., meat and dairy facilities), particularly in cool, damp areas such as conveyors, floors, and drains, even with rigorous sanitation protocols [22]. Raw foods like milk and meat can be directly contaminated with the pathogen at the farm and during slaughter.

In a review on the burden of bacterial meningitis, about 10% of the causative agents of bacterial meningitis were unconfirmed in Africa and about 36% of the bacterial cause of meningitis in Angola were unconfirmed $[23,24]$. It can be inferred that these unconfirmed cases could potentially be a result of less monitored pathogens like *L. monocytogenes*. Out of 290 raw vegetable samples collected from raw produce arriving at frozen food manufacturing facilities, 96 and 17 samples were positive for *Listeria* spp. (33.1%) and *L. monocytogenes* (5.9%) respectively in which 82 samples had greater than 100 MPN of *Listeria* spp. per g and 14 samples had less than 100 MPN *Listeria* spp. per g [25]. Gombas, et al. examined 31,705 samples from retail markets across the USA and discovered an overall *L. monocytogenes* prevalence of 1.82%, among the product categories tested showing prevalence rates between 0.17% and 4.7% [26]. Bacteriological examination of 400 random samples of muscles, liver, spleen, and kidneys taken from 100 diseased chickens from different poultry farms and markets in El Gharbia Governorate showed 13.25% (53) were contaminated with *Listeria monocytogenes* [27]. Eight (7.4%) out of 108 fresh croaker samples collected from retail outlets in Lagos, Nigeria were positive for *L. monocytogenes* identified by colony morphology, sugar fermentation, PCR incorporating 16S rRNA, and hemolytic properties [28].

Outbreak of listeriosis

The outbreak of *Listeria* is relatively low when compared with pathogens such as *E. coli* and *Salmonella* in various regions of the world. In 2001, listeriosis was added to the list of nationally notifiable diseases by the CDC's National Center for Zoonotic, Vector-Borne, and Enteric Diseases. In a meta-analysis, it was found that approximately 23,150 cases of listeriosis occurred globally in 2010, leading to 5,463 deaths. The majority of reported listeriosis outbreaks were documented in Europe, Canada, the United States, Australia, and New Zealand [46]. Table 2 shows the listeria outbreak in the USA, Europe, South Africa, and Canada

Research indicates that the annual infection rate in the USA is about 1795-1860 cases per 100,000 people [47]. The mortality rate can be as high as 30% in some parts of the country. The number of reported cases in the United States dropped from 7.7 per million people in 1990 to 3.1 per million by 2003. The 2024 outbreak in the USA that resulted in 26 illnesses, 23 hospitalizations, and 2 deaths in 11 states was linked to the consumption of contaminated Quesco fresco and Cotija cheese $[48]$. In 2006, US public health officials noted that only one out of 884 foodborne pathogen outbreaks were due to *Listeria*; however, in the following year, the CDC recorded 122 Listeria outbreaks globally, which increased to 158 by 2008. Despite this, the incidence of *Listeria* infections showed a 42% decline compared to 1998 [49]. *Listeria*

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outbreaks are also occasionally connected to the consumption of fruits, vegetables, and ice cream as depicted in Table 2. For instance, a *Listeria* outbreak due to contaminated cantaloupe was reported in 2011. Low contamination levels have been found in ice cream samples, indicating a lower infectious dose. The health status of individuals significantly affects the likelihood of *L. monocytogenes* infection, with higher infection rates possibly in immunocompromised individuals. The most common serotypes of *Listeria* in foods and environments are 1/2a and 1/2b. However, serotype 4b is responsible for 50% of human *Listeria* outbreaks, while serotype 1/2a accounts for 27% of clinical listeriosis cases [50]. The 2015 *Listeria* outbreak in the USA linked with ice cream consumption was distinguished by three *Listeria* serotypes: 1/2b, 3b, and 1/2a.

In Europe, the incidence decreased from 4.5 to 3.4 per million between 1999 and 2003. ECDC reported 30 EU/EEA countries confirmed 2575, 2652, 1931, 2268, and 2770, listeriosis cases in 2018, 2019, 2020, 2021, and 2022 respectively [51]. In 2013, the European Food Safety Authority (EFSA) reported 1760 human cases of listeriosis, with 99.1% of these cases requiring hospitalization [52]. From 2010 to 2017, Germany and Denmark reported four significant *L. monocytogenes* outbreaks associated with fish and fishery products to EFSA. Germany reported one outbreak in 2010, and Denmark reported three outbreaks in 2010, 2014, and 2017, totaling 44 cases. In 2015, the Netherlands reported an outbreak with weak evidence connecting smoked salmon consumption to three human cases. The 2017 Danish outbreak, caused by *L. monocytogenes* ST8 (CC8), was part of a larger multi-country outbreak involving 12 cases in Denmark, France, and Germany, all linked to ready-to-eat cold-smoked salmon produced in Poland [52]. Between 2013 and 2017, 30 EU/EEA countries reported between 1,905 and 2,527 listeriosis cases annually to The European Surveillance System (TESSy). Germany, France, and Spain accounted for 26%, 17%, and 10%

of these cases, respectively. Severe *L. monocytogenes* infections were more prevalent in males (54%) and in individuals over 65 years old (65% of cases) across both genders. The vast majority of cases (98%) were of domestic origin. In England and Wales, Gillespie, et al. documented 48 listeriosis cases linked to the consumption of butter and hospital sandwiches [53]. In France, de Valk, et al. identified listeriosis in 42 individuals associated with eating pork rillettes and jellied pork tongue [54].

August 2023, the Government of Canada announced the outbreak of listeriosis linked to recalled plant-based refrigerated beverages resulting in 9 hospitalizations and 2 deaths in the 12 cases reported in Ontario, Quebec, and Scotia provinces as of July 17, 2024 [55]. In Japan, Makino, et al. documented 38 cases related to cheese consumption [42] (Table 2).

Qualitative and quantitative methods of detecting *L. monocytogenes*

L. monocytogenes occurs in very low numbers in the environment, food, and food processing facilities, and its growth can be suppressed by competing organisms. To counter this, inhibitory agents such as nalidixic acid and acriflavine are incorporated into their culture media. Standard culturing involves enriching the samples in one or two enrichment media such as Fraser broth, University of Vermont Media, *Listeria* enrichment medium, etc. The International Standard (ISO 11290) and United States Department of Agriculture (USDA) standards involve a two-step enrichment process using Fraser broth and University of Vermont Media (UVM), respectively. These standards take 4-5 days before inoculation into selective media. Alternatively, the One-broth *Listeria* method requires the use of one enrichment broth, less incubation time, and generates results within two days [69]. There are several methods to qualitatively and quantitatively detect *L. monocytogenes* in food, water, environment, and clinical samples.

Conventional method

Culture-based methods: In the laboratory, culture-based methods are usually utilized. These methods are timeconsuming and occur in two stages. To isolate *L. monocytogenes* from food samples, there is a need to enrich their growth to sufficient numbers and suppress the growth of other possible pathogens present in the food. The enrichment step dilutes inhibiting substances in the samples, provides nourishment for the bacteria to grow, hydrates the bacteria, and activates injured cells. This step is followed by inoculation onto selective media such as PLACAM, Chromogenic agar, ALOA agar, and Oxford agar, which contain different salts and antibiotics to suppress the growth of competing organisms and support the growth of the target bacteria under favorable temperature and duration of growth. The growth of *L. monocytogenes* on these selective media in a defined morphology and color indicates a positive result. This pathogen can further be isolated for confirmatory testing using appropriate morphological, biochemical, and serological tests.

Selective media enhances accuracy by inhibiting unwanted

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competing microflora and enabling the appearance of *L*. *monocytogenes* in predetermined color without the need for subculturing or biochemical tests to confirm the pathogen. However, some selective media such as PLACAM and Oxoid agar have limitations in differentiating between pathogenic and non-pathogenic *Listeria* spp. [70]. Chromogenic media are effective in isolating pathogenic virulence factors of *L. monocytogenes* after 24 hours [71]. Incubation in both the enrichment and agar plates occurs at 30 $^{\circ}$ C - 35 $^{\circ}$ C for 24 hours - 48 hours under aerobic conditions. *L. monocytogenes,* a non-hemolytic species, can be differentiated from hemolytic species based on the fermentation of xylose in Rapid L' mono agar [71]. *L. ivanovii*, a hemolytic species, ferments xylose with blue colonies and yellow halo zones on Rapid L' mono, while *L. monocytogenes* do not ferment xylose

Gran's staining and biochemicatests: These tests are used following the isolation of pure culture on selective media for the confirmation of *Listeria* spp. Gram staining is used to differentiate between Gram-positive (cell-walled bacteria) and Gram-negative bacteria (non-cell-walled bacteria). *Listeria* spp. are Gram-positive bacteria and are stained purple under a microscope. Biochemical tests such as catalase, oxidase, hemolytic, Voges-Paskeur, and coagulase are also used to identify bacterial species. While these tests are useful for identification, they can sometimes produce false-positive results.

Rapid diagnostic method

Several rapid diagnostic methods have been developed to detect *L. monocytogenes* in food, laboratories, food processing environments, etc. within a shorter time. They are faster than conventional culture, Gram staining, and biochemical test methods. These methods include immunoassays/antibodybased methods, bacteriophage-based detection methods, biosensors, gene/nucleic acid amplification methods, ribotyping, esterase typing, etc.

Biosensors: Biosensors are devices that convert biological responses into electrical signals, enabling the detection of target pathogens and biomolecules. They integrate biological components with physicochemical detectors to detect analytes such as proteins, cells, nucleic acids, and enzymes associated with pathogens and diseases. Initially developed in the 1960s for glucose detection, biosensors have evolved significantly through nanotechnological advancements, becoming portable, rapid, and capable of detecting multiple organisms simultaneously. These biosensors typically consist of three components: a detector element, a reading device, and a biologically sensitive element. They are used for detecting biomarkers in various samples such as food, body fluids, saliva, urine, stool, and environmental samples. The technology has been applied extensively in studying various pathogens, including *Ebola* virus, HIV, Hantavirus, *Escherichia coli, Neisseria meningitides*, *Zika* virus, *Pseudomonas aeruginosa*, and *Salmonella* [72,73]

In medical diagnostics, biosensors play a crucial role in detecting cancer/tumor biomarkers, allergic responses, and

cardiovascular disease markers. For instance, biosensors have been developed with high sensitivity, such as detecting *Mycobacterium tuberculosis* with a Limit of Detection (LOD) as low as 8.948×10^{-13} [74]. Specific biosensor technologies include voltammetric DNA biosensors for *L. monocytogenes* detection and quartz crystal microbalance-based methods for detecting *L. monocytogenes* in contaminated foodstuffs [75,76]. Additionally, e-Nose techniques have been employed to detect a range of pathogens including *E. coli*, *Enterococcus faecalis*, *Staphylococcus lentus*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella enterica*, *L. monocytogenes*, *Salmonella typhi,* and *Listeria innocua* [77].

Bacteriophages: Bacteriophages, also known as phages, are viruses that infect and replicate within bacteria and archaea, utilizing proteins to encapsulate DNA or RNA genomes. These viruses have found application as bio-receptors in the detection and control of pathogenic microorganisms. Phages possess the ability to bind to bacterial bio-receptors on the surface, injecting their genetic material into the host cell. Xu, et al. utilized wild-type T4 bacteriophage as bio-receptors in nanoelectronic devices, distinguishing between viable and dead bacteria cells in contaminated water samples [78]. The T4 phage showed specific and sensitive detection capabilities for both types of cells. In clinical settings, Park, et al. employed phage amplification techniques to detect *Mycobacterium tuberculosis* in patient sputum, demonstrating effective detection within 1hour - 3 hours of infection before applying a virucidal agent [79]. Oliveira, et al. developed a bacteriophage amplification technique using A511 listeriophage, capable of quantitatively detecting viable *L. monocytogenes* cells across different serotypes [80]. Ahmadi, et al. highlighted the efficacy of listeriophage A511 as a biocontrol agent, effectively reducing *L. monocytogenes* contamination on meat product surfaces [81]. Stambach, et al. integrated A511 bacteriophage with surface-enhanced Raman Spectroscopy and lateral flow immunochromatography for rapid *Listeria* detection, demonstrating a decrease in viable *L. monocytogenes* counts after incubation [82]. Studies have shown that phages have no adverse effects on humans or lower animals, addressing concerns about their use in food safety and biocontrol. For instance, studies on oral toxicity in rats exposed to Listeria phages P100 found no side effects [83]. Beyond their role in pathogen detection, bacteriophages serve as effective biocontrol by lysing pathogens, thereby reducing viable cell counts. These multifaceted applications underscore the potential of phages in enhancing food safety and clinical diagnostics.

Immunoassays: A range of immunoassay methods have been developed for the detection of *Listeria monocytogenes*, a highly invasive food pathogen in food. These methods or types of immunoassays include competitive fluorescence immunoassays, Enzyme-linked immunoassays, immunomagnetic assays, lateral flow immunoassays, and an immunochromatographic assay. These assays are specific and sensitive, with the potential for rapid and simple detection of *L. monocytogenes* in various food products and environments. These methods are based on the specific ability of the antibody to bind to an antigen. There are two types of antibodies in

immunoassays: monoclonal antibodies detect single epitopes, and polyclonal antibodies detect several epitopes on the antigens. In a study by Beauchamp, et al. a specific and highly conserved peptide of 11 amino acids from the p60 protein of *L. monocytogenes* was used to produce monoclonal anti-p60 antibodies, enabling the detection of as low as 1 CFU of *L. monocytogenes* in food samples [84]. This antibody increased the fluorescence signal of the eluate, indicating a positive result, and showed no cross-reactivity with non-pathogenic *Listeria* spp.

A collaborative study evaluated the efficiency of *Listeria*-TekTM, an enzyme-linked immunosorbent assay (ELISA), for detecting *L. monocytogenes* and other *Listeria* spp. in six different food samples (frankfurter, roast beef, Brie cheese, 2% milk, raw shrimps, and crab meat) inoculated with *Listeria* spp. [85]. *L. monocytogenes* was detected in 593 samples by ELISA compared to 574 by culture method, demonstrating the superiority of immunoassay over culture-based detection. In Brazil, a point-of-care test containing anti-Internalin A and B antibodies and a biotin-streptavidin system was used to detect pathogens in artificially contaminated foods [86]. This test, with a limit of detection (LOD) of 1000 CFU/ml or /25 g of contaminated food samples, showed 100% specificity when tested with pure cultures of various food pathogens used in the study.

Ueda, et al. developed an immunochromatographic assay based on gold-labeled monoclonal antibodies directed against *L. monocytogenes* antigens [87]. The assay demonstrated a positive reaction to all *L. monocytogenes* strains but negative reactions to other Gram-positive and Gram-negative bacteria, providing results within 30 minutes of the assay. Kim, et al. isolated five monoclonal antibodies (MAbs) purified from *L. monocytogenes* 4b, which showed specific reactions to *Listeria* and no crossreactivity with other bacteria, such as *E. coli* O157and *Salmonella enteritidis* tested in the experiment after 48 hours of culturing [88]. Couture, et al. developed a sandwich ELISA for detecting the p60 protein secreted by *L. monocytogenes*, addressing both rapidity and specificity. The ELISA detected 10³ CFU/ml of L. *monocytogenes* after 18 hours of incubation without crossreacting with *Listeria innocua, S. aureus, E. coli,* and *S. typhi* [89]*.*

PCR amplification: Understanding the ecology of *L. monocytogenes* in food, food facilities, and the environment is crucial for developing effective strategies to control and detect its presence. Norton, et al. applied commercial PCRbased detection strategies and molecular typing methods in the smoked fish industry to explore the ecology of *L*. *monocytogenes* in food and the food environment. Their application demonstrated that PCR-based screening reliably and efficiently detected the pathogen in various food samples, raw materials, and environmental samples [90].

Several studies have underscored the effectiveness of PCR in detecting *L. monocytogene*s in food. In a comparative study, Ennaji, et al. evaluated the efficiency of PCR versus the Official ISO procedure, ISO 11290-1, for *L. monocytogenes* isolation from different food samples [91]. PCR was able to detect approximately 15 CFU/25g of samples in less than 36 hours,

whereas culture-based methods detected lesser counts and required 7 days - 10 days. It also highlighted PCR as a rapid and sensitive method, emphasizing its ability to effectively eliminate false-positive results and detect all instances of *L. monocytogenes* in food samples [92].

Antibiotic resistance in *Listeria monocytogenes*

Currently, there is significant concern over the increasing prevalence of antibiotic-resistant bacteria, which poses a critical global issue. Historically, *L. monocytogenes* has generally been susceptible to most antibiotics used to treat Gram-positive bacterial infections. However, in recent years, there has been a noticeable rise in antibiotic resistance within this bacterial species isolated from various sources. Monitoring antibiotic resistance in food systems, environment, and clinical settings helps identify trends in resistance prevalence and allows for the planning and evaluation of strategies to prevent its spread. The challenge posed by pathogenic *L. monocytogenes* and other food-borne microorganisms extends beyond food contamination; it also includes the ability to resist commonly used antibiotics for infection treatment. Most *Listeria* spp. is sensitive to conventional antibiotics. However, exposure to pH, cold, and salt stresses can increase their resistance. The ability to form biofilms, efflux pump expression, hydrolysis, and mobile genetic elements are primary causes of antibiotic resistance in *Listeria*. The most common treatment for listeriosis is penicillin or ampicillin combined with aminoglycosides [12]. Other antibiotics like vancomycin, trimethoprim, sulfamethoxazole, and rifampicin have also been used to treat the infection successfully [93]. There are reports of *L. monocytogenes* resistance to penicillin, ampicillin, tetracycline, streptomycin, clindamycin, oxacillin, and vancomycin [94].

Two major efflux pumps, MdrL and Lde, are present in almost all *L. monocytogenes* serotypes. The MdrL pump detoxifies macrolides, cefotaxime, heavy metals, and EtBr, while the Lde pump detoxifies fluoroquinolone antibiotics and dyes such as EtBr and acridine orange [95]. Resistance to fluoroquinolones involves three plasmid-mediated mechanisms (PMQR) and chromosomal mutations that alter the amino acid sequences of topoisomerase II and IV subunits. In *Listeria* spp., resistance to macrolides like erythromycin is linked to the presence of the ermB and ermC genes, which encode methyltransferases that modify 23S rRNA. Five determinants of tetracycline resistance genes have been identified: $tet(K)$, $tet(L)$, $tet(M)$, $tet(S)$, and tet(T). The tet(M), tet(S), and tet(T) genes encode cytoplasmic proteins that protect ribosomes from antibiotics, while tet(L) and tet(K) encode membrane proteins that expel the antibiotic from the cell.

Byren, et al. demonstrated that 50% of *Listeria* spp. isolated from vegetables were resistant to penicillin G (PEG) and tetracycline (TET) [96]. The antibiotic susceptibility of 194 *L. monocytogenes* isolates recovered from common South African ready-to-eat foods against 22 antibiotics showed only two (1%) of the isolates did not exhibit phenotypic resistance against all the antibiotics screened, and 53.1%, 56.2%, 59.3%, 61.9%, 62.9%, and 62.9% were resistant to ceftriaxone, trimethoprim, streptomycin, sulfamethoxazole, vancomycin,

and oxytetracyclines respectively [97]. Thirty of the isolates (15.5%) were resistant to only one or two antibiotics, whereas 162 (83.5%) exhibited phenotypic multiple antibiotic resistance. A study on 163 *L. monocytogenes* isolates obtained from 100 samples of fresh poultry in North-Western Spain showed multiple antibiotic resistance indices between 4 and 11, accounting for 49.1% of *L. monocytogenes* isolated [44]. The antimicrobial sensitivity test of 53 *L. monocytogenes* obtained from 400 random samples of muscles, liver, spleen, and kidneys showed that 91.6%, 83.3%, 83.3%, 83.3%, 83.3%, 66%, 50%, and 50% were resistant to sulfamethoxazole-trimethoprim, ampicillin, gentamycin, vancomycin, chloramphenicol, ciprofloxacin, erythromycin, and tetracycline, respectively, while all the isolated strains were completely resistant to cephalothin [27].

Food regulations and safety standards relating to *L. monocytogenes*

Microbiological food safety standards provide a protective and regulatory guide that assures consumer protection against food safety hazards relating to food consumption. The World Health Organization (FAO/WHO) and the Food and Agriculture Organization held a joint regional conference on food safety for Africa aimed at identifying the agencies and bodies responsible for food safety in various countries in Africa.

Despite the biggest listeriosis outbreak recorded in South Africa in 2018, there is little awareness and monitoring relating to *L. monocytogenes* in other African countries and this has led to the availability of little information on the outbreak Only a few African countries have standards on *L. monocytogenes* in their food safety legislation. This standard varies in terms of allowable limits and types of food (raw and RTE food). Some countries adopt the absence of any pathogenic microbes such as *L. monocytogenes* and *Escherichia coli* in food (Nigeria, Egypt, Kenya) while some adopt the absence in five 25 g samples taken before the product left the establishment (Seychelles, Saint Helena, Tunisia), or less than 100 CFU/g before the end of the product shelf-life (Mauritania, Mauritius, Madagascar, Djibouti). With the daily increase in vulnerable groups (immunocompromised, infant, pregnant people), and consumption of high-risk raw and RTE foods, there is a great need for all African countries to develop strict safety standards and monitoring schemes. The implementation of such standards would provide a significant level of consumer protection against *L. monocytogenes* incidence and potential outbreaks. South Africa Dairy Standard Agency (DSA) has guidelines related to *L. monocytogenes* in raw milk, pasteurized milk, cream, and salted butter [98]. The guideline recommends the absence of *L. monocytogenes* in 25 g of raw milk for consumption and in other products.

In Europe, there exists a set of microbiological standards that foods with *L. monocytogenes* possible presence or contamination must comply with, Regulation (EC) No 2073/2005. This regulation possesses different criteria depending on the ability of the food product to support the growth of *L. monocytogenes*. This criterion has replaced the zero-tolerance requirement in Ready-To-Eat (RTE) foods unable to support the growth of

L. monocytogenes (foods with a pH of 4.4 and a water activity [aw] of 0.92, or a pH of #5.0 and an aw of #0.94, or a shelf life of 5 days) [99]. Less than 100 cfu/g throughout the shelflife of the product (5 x 25 g) must be met in ready-to-eat foods and food products that can support *L. monocytogenes* growth while no *L. monocytogenes* must be present in 5 x 25 g samples at the time of leaving the production plant for food that support *L. monocytogenes* growth. Countries such as Germany, the Netherlands, and France have set a tolerance level not above 100 CFU of *L. monocytogenes* per gram of food at the time of consumption while Italy possesses different criteria of total absence of *L. monocytogenes* in 25 g of food [14]. The Food Safety Standard of Ireland prescribed the absence of *L. monocytogenes* in 25 g of RTE food and food products intended for infant consumption and special food for medical purposes in up to 10 collected food samples. Also, the criteria states that *L. monocytogenes* should be absent in 25 g of RTE food after production or the microbial load should not exceed 100 CFU per gram of food throughout its shelf life placed on the market, in up to 5 collected food samples [91].

In 2014, Food Standards Australia-New Zealand prescribed two sets of criteria for *L. monocytogenes* based on the food's ability to support or does not support the growth of bacteria. Such include fewer than 100 CFU of *L. monocytogenes* per gram of food that does not support the growth of *L. monocytogenes* and that *L. monocytogenes* should not be detected in 25 g of food likely to support the growth [100].

In 1987, the USDA initiated testing for *L. monocytogenes* in Ready-To-Eat (RTE) meat and poultry products. The current US policy considers the detectable presence $(≥ 1$ CFU in a 25gram sample) of *L. monocytogenes* in RTE food to be a health hazard [99]. The agency established a "zero tolerance" policy for *L. monocytogenes* in food and the processing environment (no detectable level of viable organisms allowed). However, studies have shown that low numbers of *L. monocytogenes* represent no considerable health risk for most consumers as this bacterium can multiply at refrigeration temperatures, an initially low number of *L. monocytogenes* in food can replicate to levels that could cause an illness even in properly stored food [101].

Risk Reduction in *L. monocytogenes*

L. monocytogenes is prevalent in food processing environments and can survive for extended periods in foods, processing plants, households, or the environment, especially at refrigeration or frozen storage temperatures. Implementing the Hazard Analysis and Critical Control Points (HACCP) is a crucial risk prevention program throughout the food processing environment in high and low-risk environments.

Although *L. monocytogenes* is frequently found in raw foods of both plant and animal origin, it can also be present in cooked foods due to post-processing contamination if handled after cooking. The bacterium is often isolated from cool and wet food processing environments and has been found in foods such as raw and pasteurized fluid milk, cheeses, ice cream, raw vegetables, fermented raw meat and cooked sausages, raw and

cooked poultry, raw meats, and raw and smoked seafood [37]. Even when initially present at low levels in contaminated food, *L. monocytogenes* can grow during refrigerated storage, leading to increased levels in foods that support its growth.

Foodborne pathogens like *Listeria* are primarily associated with fecal contamination from infected animals or humans. To reduce contamination levels, a primary strategy in food processing environments is to decrease the fecal carriage of *Listeria* in livestock. Maintaining hygienic practices, especially during milking and slaughtering, can lower the risk of *Listeria* contamination. Animal manure and contaminated irrigation water introduce a pre-harvest contamination route for field crops such as fruits and vegetables. Despite the risk of contamination in raw foods, many human listeriosis outbreaks result from contaminated processed ready-to-eat foods [102].

Cold stress adaptation and biofilm formation are key attributes of *L. monocytogenes* that facilitate its dissemination in food environments. This attachment to food products leads to significant concern and economic losses due to food spoilage. Biofilms formed by *Listeria* are particularly resistant to detergents, disinfectants, antimicrobial agents, and sanitizers, necessitating greater effort to mitigate risks compared to less resilient microorganisms. The application of antimicrobial agents and ultraviolet radiation has been successfully used to reduce microbial loads in fresh produce [13]. Despite this measure, *L. monocytogenes* may not be inactivated due to its ability to survive in adverse conditions.

Prevention and control of *L. monocytogenes*

Strategies to reduce *L. monocytogenes* contamination in foods and mitigate the risk of listeriosis heavily rely on stringent hygienic and sanitary practices throughout farming, processing, and environmental management. Early detection and timely reporting of a listeriosis outbreak are crucial for halting its spread. Once food becomes contaminated with *Listeria*, refrigerator temperatures may not sufficiently inhibit bacterial survival due to its cold tolerance and biofilm formation. Therefore, implementing the HACCP approach and establishing effective critical control points in food processing plants is essential. Individuals at risk should refrain from consuming raw animal products, raw milk, soft cheeses, and refrigerated ready-to-eat foods that have not been heated to a high temperature.

Total eradication of *L. monocytogenes* in food and food production is unlikely as this pathogen is ubiquitous and found mostly in the animal intestine. Various sources have been identified as sources of contaminating food produce at various stages of the food chain, including contaminated incoming raw materials, inefficient cleaning and poor hygiene, inadequate processing, and factory workers acting as carriers of *L. monocytogenes*. The prevention and control of *L. monocytogenes* contamination in raw and Ready-To-Eat (RTE) foods are paramount in protecting consumers against listeria disease.

Good hygiene practices, such as careful preparation and cooking of food, and interventions like pasteurization,

organic acid washes, and steam vacuuming, along with the use of antimicrobial solutions (e.g., dilute lactic acid and chlorine), can effectively eliminate pathogens from food. Proper sampling of a processing environment should include several areas where contamination is likely, including both food contact and non-food contact surfaces. One of the most common areas susceptible to contamination is floor drains, where *L. monocytogenes* can persist as a harbor site [103]. Continuous monitoring and testing of food processing plants and environments for the presence of *L. monocytogenes* and other foodborne pathogens are effective practices for their control.

A study on the efficacy of X -ray treatment for reducing inoculated *L. monocytogenes* and other pathogens on shredded iceberg lettuce revealed that treatment with X-rays at doses of 1.0 and 2.0 kGy significantly reduced the *L. monocytogenes* population under both conditions [104]. Importantly, this method did not adversely affect the sensory quality, particularly the visual color, of the lettuce leaves during subsequent storage, suggesting X-ray treatment as a viable approach for pathogen reduction in lettuce. Various strategies, including the use of bioprotective meat starter cultures like *L. rhamnosus* E-97800, *L. rhamnosus* LC-705, and *L. plantarum* ALC01 in sausages, have demonstrated antibacterial activity, contributing to reducing *L. monocytogenes* levels in contaminated foods [105]. Combined treatments such as ozone and organic acids have proven efficacy in reducing initial pathogen populations on foods like mushrooms [106] Nitrite has also been identified as a substance that can injure *Listeria*, potentially reducing their numbers [107]. Steam treatment systems and Ultra-High-Pressure Homogenization (UHPH) have rendered *L. monocytogenes* undetectable in grape juice due to their disruptive effect on bacterial cells [108].

Various medicinal plant species have been found to possess compounds with antimicrobial, anti-inflammatory, and antidiarrheal properties, making them potential candidates for listeriosis treatment [109-111]. These extracts often contain bioactive compounds like tannins, flavonoids, alkaloids, and essential oils, which can inhibit the growth of diarrheagenic bacteria, reduce intestinal inflammation, and regulate bowel movements. Clinical studies and trials continue to explore the efficacy and safety of plant extracts for disease treatment, aiming to integrate traditional knowledge with modern medical practices. This approach not only seeks to provide effective treatments but also supports sustainable and accessible healthcare solutions globally. These diverse approaches underscore the importance of multifaceted strategies in ensuring food safety and reducing the risk of *L. monocytogenes* contamination throughout the food chain.

Conclusion

Despite the achievement of developed countries in reporting, curtailing, diagnosing, and monitoring the incidence and outbreak of Listeriosis, many developing countries are yet to have a defined system. The biology, the ubiquitous nature, and the ability of the pathogen to overcome various stresses could make it persist in food and food processing environments for

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a long period, hence the need for good hygiene practices and Good Manufacturing Practices (GMP) from raw material to food consumption. The application of Hazard Analysis Critical Control Points (HACCP) at various stages of food production will reduce the risk of food contamination. One health approach aimed at promoting effective collaboration, coordination, and communication among relevant stakeholders will provide a comprehensive and proactive strategy to tackle zoonotic diseases, mitigate risks, and promote the well-being of both animals and humans while preserving the health of the environment. Proper awareness for the vulnerable group on the risk of eating contaminated food will reduce the disease burden, outbreak, fatality, and morbidity rate. Also, the identification of all the stakeholders involved in the food chain and ensuring proper Good Hygienic Practices (GHP), Good Manufacturing Practices (GMP), and food safety management practices will reduce the disease burden. The manufacturing industries should take proper corrective actions once *Listeria* spp. is found in the environment and monitor the effectiveness of the corrective actions.

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