

Persistence of *Listeria monocytogenes* in food commodities: foodborne pathogenesis, virulence factors, and implications for public health

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Abstract

Listeria monocytogenes is a ubiquitous pathogen found commonly in vast environment conditions and most commonly found in fresh-cut salads, raw fruits raw vegetables, sausages, cured meat, shellfish, refrigerated ready to eat food, soft cheeses, raw and under pasteurized milk. *L. monocytogenes* is a member of genus *Listeria*, along with five other members, such as *L. innocua*, *L. ivanovii*, *L. seeligeri*, *L. grayi* and *L. murrayi*. However, *L. monocytogenes* is the widely known species found in human cases, although a few cases with *L. ivanovii* have also reported. The infection caused by *L. monocytogenes* is known as listeriosis, and most affected populations are neonates, pregnant women, immune-suppressed and older people. Listeriosis is a severe condition with a higher level of hospitalization (94%) and around 16% fatality. *Listeria* can tolerate wide ranges of temperature, pH values, ions including Chlorides and Nitrites, different Oxygen levels. Therefore, *L. monocytogenes* is widely identified and survived easily on harsh environmental conditions. The ability of biofilm formation makes *Listeria* even harder to eliminate through food processing and associated surfaces. Although outbreaks are not very common, several large outbreaks have been documented in the literature. The extensive incubation period of this pathogen makes it challenging to identify the cause in most of the outbreaks, and most of the *Listeria* associated cases found to be sporadic. *L. monocytogenes* can cause substantial economic and public health impacts due to its effect on humans. Once contaminated, *Listeria* is hard to eliminate due to widespread presence in the environment, intrinsic physiological resistance, ability to adapt to external stresses, and the ability to grow at a wide range of temperatures. The objective of this review was to understand foodborne *Listeria* infections in humans and animals, the occurrence of infections and outbreaks, the growth and survival of *L. monocytogenes* in food-related industries, and risk reduction strategies.

1. Introduction

Listeria monocytogenes is the only known species in the *Listeria* genus that concern for human health (Kathariou, 2002). This organism is a gram-positive,

motile, non-spore forming rod and ubiquitous. It is a facultative anaerobic intracellular foodborne pathogen that can cause severe invasive illness, listeriosis in humans, and other animals, including mammals and

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birds. *Listeria* infection was first identified in 1893 under the microscope as gram-positive rods, from the samples of people who died from the present listeriosis (Gray and Killinger, 1966; Asoss *et al.*, 2014). Though invasive human listeriosis can occur in healthy individuals, many of the reported cases occur in neonatal, pregnant women, elderly, or immune-compromised individuals infected with human immunodeficiency virus (HIV) or undergoing immunosuppressant therapy (Ferreira *et al.*, 2014). Listeriosis is a severe infection that manifests as septicemia, meningitis or other infections related to the central nervous system. In pregnant women, infected infections may lead to spontaneous abortions, stillbirths, or fetal deaths. According to the statistics, *L. monocytogenes* causes approximately 94.0% of hospitalizations, and 15.9% of deaths in laboratory-confirmed listeriosis cases in the United States annually (Scallan *et al.*, 2011). In developed countries worldwide, the incidence of listeriosis is 0.36 to 5 cases annually per million people. However, the number of reported cases can be very low in countries with limited surveillance for this disease (Ferreira *et al.*, 2014). *Listeria monocytogenes* infections have occurred as both sporadic episodes and large outbreaks of human cases in various parts of the world (Jemmi and Stephan, 2006). *L. monocytogenes* is a reasonably resilient organism that can tolerate much higher adverse conditions when compared to general microbial populations. It is capable of slow multiplication at refrigeration temperatures (-0.5°C to 9.3°C) (Gunasena *et al.*, 1995; Ferreira *et al.*, 2014), tolerant to preserving agents such as 10% Sodium Chloride (W/V) (Ferreira *et al.*, 2014), 120 ppm Sodium nitrite (Junttila *et al.*, 1989) and grow and survive outside a host at a wide pH range (5.0 to 9.6) (Ferreira *et al.*, 2014). In nature, the primary habitat of *Listeria* appears to be soil and decaying vegetation (Kathariou, 2002). However, *L. monocytogenes* have been reported in a wide range of environments, including food processing factories, natural, urban, and various farm environments (soil, decaying vegetation, stream water, sewage, and human and animal feces) (Ferreira *et al.*, 2014). The ubiquitous nature of *L. monocytogenes* in food processing, distribution, and storage environments is due to the efficient stress adaptation capacities and biofilm formation (Bonsaglia *et al.*, 2014). Therefore, control of this microbe in food is a significant challenge (Jemmi and Stephan, 2006).

The ability to grow at cold temperatures and pathogenic potential leads to *L. monocytogenes* as a pathogen of particular concern for the safety of fresh, refrigerated, and ready-to-eat (RTE) foods that consumed without reheating, cooking or both (Kathariou, 2002). Though RTE foods are the most common source of *L. monocytogenes*, contaminations from the organism

occur in almost all types of raw food, with vegetables, milk, meat, and seafood most frequently implicated (Wu *et al.*, 2015). Several methods are available to diagnose *L. monocytogenes* in food processing environments, and only zero-tolerance levels of *L. monocytogenes* are accepted in some food safety standards. Therefore risk reduction during food processing is a vital issue that is to be addressed (Norrung, 2000; Chen *et al.*, 2014). This review paper attempts to discuss the *Listeria* infection, foodborne listeriosis cases, contamination and survival of *Listeria* in food, detection methods, impact and practices associated with reducing risk of foodborne listeriosis.

2. Listeriosis

Listeriosis is known as the life-threatening infection caused by *L. monocytogenes*. Listeriosis is an infection that occurs in both humans as well as in animals. It was discovered as a disease-causing bacterium in 1920 and subsequently named *Listeria monocytogenes* (Rocourt and Buchrieser, 2007; Todd and Notermans, 2011). Center for Disease Control and Prevention (CDC) reports from the United States of America to show that the infections caused by *L. monocytogenes* account for the highest rates of hospitalization (91%) among the commonly occurring foodborne pathogens (Mead *et al.*, 1999; Jemmi and Stephan 2006). Listeriosis gained recognition as a major foodborne pathogen as the mortality rates do not diminish over many years, although the occurrence of listeriosis is rather small when compared with salmonellosis and campylobacteriosis (Hussain *et al.*, 2017). However, both sporadic and large outbreaks have been reported by *Listeria* infections (Nakamura *et al.*, 2004).

2.1 Animal Listeriosis

Although many animals can be contaminated and infected with *Listeria*, clinical symptoms can be very rare. The most commonly infected animals with animal listeriosis are known to be ruminants. Meningo-encephalitis, septicemia and uterine infections are the main clinical signs that can be detected in ruminants infected with *Listeria* and in cattle, third-trimester abortions or septicemia in neonates characterize uterine infections (Nightingale *et al.*, 2004). The encephalitic form of animal listeriosis is characterized by neurological signs, including circling, excessive salivation, and unilateral facial paralysis (Donachie and Low, 1997). *L. monocytogenes* also can cause eye infections and keratitis in ruminants; the eye infections are related to the *Listeria* present in feed and silage. In ovine mastitis, *L. monocytogenes* has documented as a responsible pathogen (Schoder *et al.*, 2003). Numerous

reasons are suspected as the causative agent for listeriosis among farm animals. The Primary identified *Listeria* contamination source for animals is grass silage with high pH >5.0 (Jemmi and Stephan, 2006). Though farm animals can directly expose to *L. monocytogenes* in soil and crops while grazing, the number of pathogens acquired through this route is less likely to cause an infection (Nightingale et al., 2004). Feces of the infected ruminants acts as a potential source of the bacterium, which leads to human listeriosis infections by consumption of the food/ raw materials directly contaminated with affected ruminant feces (Pell, 2010). There can be animals which are infected with listeriosis, and may not show any clinical symptoms. However, such animals may still act as carriers for the disease. Fecal contamination of milk and meat by *Listeria* during the milking and slaughtering process of such carrier animals can lead to human listeriosis (Jemmi and Stephan, 2006).

2.2 Human Listeriosis

Human listeriosis occurs by the consumption of the food commodities contaminated with and is known as a possible severe life-threatening condition (Pini and Gilbert, 1988; Gillespie et al., 2010). Not all the population is generally affected by listeriosis. A specific group of people who are lacking general immunity is susceptible to be infected with human listeriosis (Lomonaco et al., 2009).

Infection caused by *Listeria* can cause invasive and non-invasive listeriosis. Invasive type of listeriosis generally affects the sterile parts of the body, including liver, spleen, cerebral spinal fluids, and blood (Hussain et al., 2017). Septicemia, meningitis, and meningoencephalitis are the commonly occurring symptoms of listeriosis in human cases. Abortions or stillbirths are caused by *Listeria monocytogenes* in pregnant mothers by establishing natural localized immunosuppression at the maternal-fetal interface (Jemmi and Stephan, 2006). Although the vast majority of the listeriosis cases affect the immuno-suppressed individuals, healthy individuals can also be affected when large numbers of *Listeria* organisms are involved. It is known as the non-invasive type of listeriosis, also known as febrile gastroenteritis or non-invasive gastroenteritis, and it has often been linked to outbreaks (Hussain et al., 2017). This infection, however, can be occurred as a milder form of infection compared to status occurs with immune-suppressants with the onset of fever, muscle aches, headache, and diarrhea occurring 9–48 h after exposure (Frye et al., 2002). This type of disease caused by *L. monocytogenes* has not been as well studied as the invasive type of disease, but strains

causing febrile gastroenteritis have also caused listeriosis (Todd and Notermans, 2011).

Listeriosis is not a typical foodborne outbreak when compared to other foodborne infections. The incubation period of *L. monocytogenes* to cause listeriosis usually takes a more extended period when compared to other food poisoning pathogens: varies from 11 days and up to 70 days, which has a median of 31 days (Schuchat et al., 1991). Therefore, sometimes the source of infection may difficult to trace back (Johnsen et al., 2010). However, listeriosis is considered as a disease that shows a higher hospitalization rate compared to similar foodborne pathogens. The average death rate in individuals infected by *Listeria* is around 20-30% despite the use of adequate antimicrobial treatments (Swaminathan and Gerner-Smidt, 2007). Most outbreaks have occurred in Europe, the U.S., Canada, and to a lesser extent, Australia and New Zealand (Todd and Notermans, 2011).

3. Foodborne listeriosis

3.1 Sources of *Listeria monocytogenes* in the environment

The most common cause of human listeriosis is the consumption of RTE foods contaminated by the pathogen (Garner and Kathariou, 2016). Most of the listeriosis cases are found to be sporadic (Ferreira et al., 2014). The primary source of *Listeria* contamination has been the food processing environment. Widely distributed *Listeria* found in the environment may enter the food processing facility through various routes, which include contaminated equipment, food products, and person to person infections (Gandhi and Chikindas, 2007). In a food processing environment, pathogens like *L. monocytogenes* can colonize on equipment surfaces and in crevices. Once inoculated and colonized, they can remain in the surfaces and food processing environment without destroying, sometimes for years (Kathariou, 2002). This persistent colonization of the microorganisms, which is hard to eliminate, is known as biofilm formation (Kumar and Anand, 1998).

3.2 Fresh produce—associated listeriosis outbreaks and level of fresh produce contamination

First reported *Listeria* outbreak from fresh produce was the transmission of the pathogen via contaminated coleslaw in 1981, in Maritime Provinces, Canada (Schlech et al., 1983; Donachie and Low 1997). Both pre-harvest and post-harvest contamination of fresh produce with the pathogenic *L. monocytogenes* can affect human health. There are numerous direct and indirect causes of contamination, including animals or insects, soil, water, dirty equipment and human handling (Hussain et al.,

2017). *L. monocytogenes* is responsible for approximately 1,600 cases of foodborne listeriosis resulting in an estimated 1,500 hospitalizations and 260 deaths in the USA., each year (Scallan *et al.*, 2011). The occurrence of listeriosis is around 5 cases per million per year in France, Germany, and Switzerland (Asoss *et al.*, 2014).

Most of the sporadic cases and outbreaks of listeriosis are reported due to the consumption of contaminated fresh produce and RTE. Most of the RTE foods are animal originated. Most commonly documented food with *Listeria* include hot dogs, deli meats (Gombas *et al.*, 2003), other processed specialty meat products such as ham (Shen *et al.*, 2006), seafood (Rahimi *et al.*, 2012), and dairy products (Chen *et al.*, 2009), especially soft cheeses (Massa *et al.*, 1990), butter and raw milk (Moura *et al.*, 1993), vegetables (Mena *et al.*, 2004), especially cabbage (Garner and Kathariou, 2016), salads (Garner and Kathariou, 2016), fruits and fruit juices (Kuan *et al.*, 2017).

3.3 Outbreaks of foodborne listeriosis

A disease outbreak is known as the occurrence of a higher number of disease cases than what is usually expected in a defined community, geographical area, or season. An outbreak may be occurred in a restricted geographical area or can be occurred over several countries, which may last from a few days up to years in some instances. *Listeria* outbreaks occur when a high number of people in the population in a particular area are affected by listeriosis within a short period. Most of the outbreaks that happen by the ingestion of contaminated food commodities usually happen with a batch of contaminated food or fresh produce by the same origin. Outbreaks generally occur with the infection of a potentially virulent type of a microorganism, as they seek medical care than a less-virulent type of pathogen. Less virulent food infections usually go underdiagnosed, as they do not seek for medical assistance.

Several *Listeria* outbreaks have documented in the literature, although a vast majority of the listeriosis cases are reported as sporadic cases rather than an outbreak (Kathariou, 2002). A massive outbreak, the Maritime Provinces of Canada, provided the first evidence for transmission of listeriosis by foodborne organisms (Schlech *et al.*, 1983).

The pattern of listeriosis outbreaks in the last 40 years shows an increasing trend. Only five large listeriosis outbreaks were reported between 1980-1990, but the number has increased to 13 cases from 1990 to 2000. Outbreaks between 2000- 2010 are 10 in number, and there are nine outbreaks reported from 2010 to up to

date around the world. Most of the reported outbreaks were from Europe, while the USA and Canada showed reported a slightly low number of cases than Europe (Table 1).

The majority of the reported *Listeria* outbreaks were associated with dairy products, both pasteurized and non-pasteurized milk, cheeses, and butter made of such milk (Autio *et al.*, 1999; McLauchlin *et al.*, 2004; MacDonald *et al.*, 2005; Swaminathan and Gerner-Smidt, 2007). Fresh produce such as fresh vegetables, fruits, sprout, and salads also showed as emerging food sources as listeriosis outbreaks around the world (Schlech *et al.*, 1983; Aureli *et al.*, 2000; Garner and Kathariou, 2016; Hussain *et al.*, 2017). RTE meat, processed meat products, and smoked meat associated outbreaks were also common around the world (Sim *et al.*, 2002; McLauchlin *et al.*, 2004; Swaminathan and Gerner-Smidt, 2007; Thomas *et al.*, 2020).

The largest reported listeriosis outbreak of 1599 cases was reported in Italy in 1997 after the consumption of sweet corn salad, and no deaths were reported (Aureli *et al.*, 2000). A large number of deaths associated with listeriosis occurred in an outbreak in the USA in 1985, where 63% of the associated neonates were died (Schuchat *et al.*, 1991). This outbreak was associated with soft cheese that was made of unpasteurized milk (Schuchat *et al.*, 1991). The largest outbreak reported in recent history was from South Africa between 2017-2018, after the consumption of polony- a processed meat sausage, reporting 937 cases of listeriosis. Fairly a large number of deaths (27%) were reported during this outbreak (Thomas *et al.*, 2020).

4. Growth, survival, prevalence and stress resistance

The growth of bacteria in food commodities is usually controlled by the interaction of several environmental and nutritional parameters, including temperature, pH, oxygen content, sodium chloride concentration, sodium nitrite concentration (Buchanan *et al.*, 1989).

4.1 Growth, prevalence, and survival of *Listeria monocytogenes*

L. monocytogenes is a pathogen that can tolerate many adverse environmental conditions such as low and high temperatures, adverse pH values, salt and nitrite concentrations, and lower atmospheric Oxygen (Kathariou, 2002; Jemmi and Stephan, 2006; Gandhi and Chikindas, 2007; Ferreira *et al.*, 2014; Tasara and Stephan, 2016). *L. monocytogenes* can show a slow growth even in refrigerated temperatures, and up to much higher temperatures than the ambient temperature,

Table 1. Large outbreaks of *Listeria* infections in the world

Year	Area/Country	Associated Food	Group Associated and Number	Death Rate	Reference
1979	Boston, USA	Raw vegetables	20 cases	3 (15%)	Ho <i>et al.</i> (1986)
1981	Maritime Province, Canada	Coleslaw	41 cases, 7 adults and 34 infants	27% in infants, (5 spontaneous abortions, 4 still births)	Schlech <i>et al.</i> (1983)
1983	Massachusetts, UK	Pasteurized whole or 2% fat milk	49 cases, 42 Non pregnant and, 7 pregnant women	(14 out of 49), 29% perinatal cases.	Mclauchlin <i>et al.</i> (2004)
1985	Los Angeles, California, USA	Mexican-style soft cheese made of unpasteurized milk	142 cases, 93 pregnant women and 49 non pregnant women	63% for early neonatal, and 37% for non-neonatal infections	Schuchat <i>et al.</i> (1991)
1983 – 1987	Switzerland	Soft cheese	122 cases, 65 pregnant women and 57 non pregnant women	34 deaths (28%)	Mclauchlin <i>et al.</i> (2004)
1987 – 1989	UK	Pate	355 cases, 185 pregnant women and	94 (27%)	Mclauchlin <i>et al.</i> (2004)
1993	France	Pork tongue in aspic	279 cases	Not reported	Mclauchlin <i>et al.</i> (2004)
1993	France	Pork rillettes	38 cases, 31 pregnant women and 7	10 (26%)	Goulet <i>et al.</i> (1998)
1994	USA	Commercially pasteurized chocolate milk	45 cases, 1 pregnant woman and 44 non pregnant women	0 (0%)	Mclauchlin <i>et al.</i> (2004)
1994	Georgia	Prawns	10 Individuals including 2 pregnant mothers	0 (0%)	Riedo <i>et al.</i> (1994)
1994 -	Sweden	Cold-smoked and	9 cases	2 (22.22%)	Ericsson <i>et al.</i>
1995	France	Soft cheese	20 cases, 11 pregnant women and 9 non pregnant women	4 (20%)	Mclauchlin <i>et al.</i> (2004)
1997	Italy	Sweet corn salad	1599, non-pregnant women	0 (0%)	Aureli <i>et al.</i> (2000)
1998 - 1999	USA	Hot dogs and delicatessen meats	50 cases	>8	Mclauchlin <i>et al.</i> (2004)
1998 - 1999	Finland	Butter	25 cases, all non-pregnant women	6 (24%)	Lyytikäinen <i>et al.</i> (2000)
1999 – 2000	France	Pork rillettes	10 cases, 3 pregnant women and 7 non pregnant women	2 (20%)	Mclauchlin <i>et al.</i> (2004)
1999	Finland	Smoked Fish	5 individuals, including 1	0 (0%)	Miettinen <i>et al.</i>
2000	USA	Turkey meat	29 cases, 8 pregnant women and 21 non pregnant women	7 (24%)	Mclauchlin <i>et al.</i> (2004)
1999 – 2000	France	Pork tongue in jelly	32 cases, 9 pregnant women and 23 non pregnant women	10 (31.25%)	Mclauchlin <i>et al.</i> (2004)
2000	New Zealand	Corned beef ham	21 Individuals	Unknown	Sim <i>et al.</i> (2002)
2000	USA	Fresh Mexican style cheese	13 Individuals, 11 pregnant women	0 deaths, 5 stillbirths, 3 premature deliveries, 3 infected newborns	Macdonald <i>et al.</i> (2005)
2000 – 2001	USA	Mexican style soft cheese made of unpasteurized milk	12 cases; 10 pregnant women and 2 non pregnant women	5 (42%)	Mclauchlin <i>et al.</i> (2004)
2001	Sweden	Fresh cheese prepared from cow and goat milk	48 Individuals; 3 pregnant woman, 1 elderly	0 (0%)	Carrique-Mas (2003)
2002	USA	Delicatessen turkey ready -to-eat meats	54 cases including 12 perinatal cases	8 (14.81%)	Swaminathan and Gerner-smidt (2007)
2002	Quebec, Canada	Cheese made from raw milk	17 cases, 3 perinatal cases	0 (0%)	Swaminathan and Gerner-smidt (2007)
2003	Texas, USA	Mexican-style cheese	12 cases	Unknown	Swaminathan and Gerner-smidt (2007)

Table 1 (Cont.). Large outbreaks of *Listeria* infections in the world

Year	Area/Country	Associated Food	Group Associated and Number	Death Rate	Reference
2007	Norway	Camembert cheese made from pasteurized milk	17 individuals; 15 patients under risk age	3 (18%)	Odd <i>et al.</i> (2010)
2008-2009	Multistate	Sprouts (Multiple types)	20 cases; 4 perinatal cases	0 (0%)	Garner and Kathariou (2016)
2010	Texas, USA	Diced celery	10 cases	5 (50%)	Garner and Kathariou (2016)
2011	Multistate	Whole cantaloupe	147 cases including 7 Perinatal cases	33 (22.45%), 1 miscarriage	Garner and Kathariou (2016)
2011	USA	Romaine lettuce	84 cases	15 (17.85%)	Hussain <i>et al.</i> (2017)
2014	Multistate	Stone fruit (Nectarines, peaches)	2 cases	1 (50%)	Garner and Kathariou (2016)
2014	Multistate	Mung bean sprouts	5 cases, all hospitalized	2 (40%)	Garner and Kathariou (2016)
2014	Multistate	Commercially produced, prepackaged caramel apples	35 cases, 34 were hospitalized, 11 perinatal cases were reported	5 (13.51%)	Garner and Kathariou (2016)
2013 - 2105	Denmark	Smoked fish	20 cases	7 individuals (35%) and a 38-week fetus died	Gillesberg Lassen <i>et al.</i> (2016)
2015	USA	Ice cream	5 cases	N/A	Chen <i>et al.</i> (2017)
2016	Ohio, USA	Packaged salads	19 cases	1 (5%)	Hussain <i>et al.</i> (2017)
June 2017 - April 2018	South Africa	Polony (a type of processed meat)	937 cases; 465 were pregnant women, 97 HIV patients	193 (27%)	Thomas <i>et al.</i> (2020)

growth of *L. monocytogenes* occurs in the temperatures between 3°C and 45°C, but the optimum temperature range is known as 30-37°C (Donachie and Low, 1997). *L. monocytogenes* can grow among a wide range of pH values (4.3-9.4) (Zhu *et al.*, 2017). Although the most favorable *Listeria* growth occurs on pH values of 5.0 and above, there are documented cases of *Listeria* survival in silage, on pH 4.0 or even lower. Therefore, although generalized pH range for *Listeria* growth is established, the food cannot be guaranteed as *Listeria* free in pH value less than 5.0 (Junttila *et al.*, 1989).

When considering the salt concentration in the survival environment, it has become a pathogen that withstands higher concentrations, such as 10% (W/V) Sodium Chloride levels (Ferreira *et al.*, 2014). According to the above fact, most food commodities are usually having a 3.0-3.5% level of Sodium Chloride, especially meat products and sausages as a preserving agent, cannot be indicated as they are eliminated from the presence. During the meat preserving process, the most commonly added curing agent is sodium nitrite (NaNO₂). Bacteriostatic activity of sodium nitrite is highly dependent on the initial pH of the medium. Sodium nitrite has a lower potential activity when initial pH levels are 6.0 or above and more effective against when

the incubation temperature is lowered to 5°C on *L. monocytogenes*. The elevated sodium chloride level would reduce the bacteriostatic effect of sodium nitrite when *L. monocytogenes* are cultured aerobically (Buchanan *et al.*, 1989).

L. monocytogenes is a ubiquitous and facultative anaerobic bacterium, which survives in both aerobic and microaerophilic environments successfully. The pathogen can survive modified atmospheric packages with 10% carbon dioxide (CO₂), and 100% nitrogen (N₂). Bacterium shows increased growth in atmospheric packages with 97% N₂ and 3% oxygen (O₂) (Francis and Beirne, 1998). It requires 20% CO₂ modified atmospheric packages to reduce the growth of *L. monocytogenes* by 0.5-1 log unit between 0-5 days, with an extended log phase, although the final population is not affected (Bennik *et al.*, 1996). Therefore, more than 20% of CO₂ level is required to inhibit the growth of *L. monocytogenes* and, modified atmospheric packages with low oxygen levels are not sufficient as it can enhance the growth of the bacterium. The food industry currently uses Modified Atmosphere Packaging (MAP) and vacuum packaging to inhibit spoilage and pathogenic organisms. Combinations of oxygen (O₂), carbon dioxide (CO₂), and nitrogen (N₂) are used in

MAP In most packages, the bacteriostatic effect is obtained by a combination of decreased O₂ and increased CO₂ concentrations (Jydegaard-Axelsen *et al.*, 2004). However, the facultative nature of *L. monocytogenes* enables it to overcome these low oxygen barriers. The ability of *L. monocytogenes* to survive the various external environments, including low O₂ conditions before infection as well as in the various microenvironments of the gastrointestinal tract, is essential for this pathogen to cause disease (Lungu *et al.*, 2009).

4.2 Stress resistance of *Listeria monocytogenes* by biofilm formation

One reason for *L. monocytogenes* to attach to and survive on various working contact surfaces may be its ability to form biofilms (Jemmi and Stephan, 2006). Microbial populations present as planktonic cells or as communities in biofilms, which they usually attached to a surface and enclosed in a polysaccharide matrix. These biofilms have decreased growth rates and show variations in the transcribed genes (Donlan *et al.*, 2002). A higher rate of gene transfer by conjugation (Hausner and Wuertz, 1999) and high exo-polysaccharide production (Sutherland, 2001), showed enhanced resistance to sanitizers, disinfectants and antimicrobial agents (Robbins *et al.*, 2005). The formation of biofilms is not limited to one surface. Due to the ubiquitous nature, *Listeria* has the ability to form biofilms on a wide variety of surfaces, including medical devices, water system piping, industrial equipment, as well as in food processing facilities (Donlan *et al.*, 2002). Often biofilms can be present on food handling surfaces, food storage areas such as conveyor belts, and stainless steel equipment (Kumar and Anand, 1998; Bonsaglia *et al.*, 2014). The primary concern of the biofilm formation is the smooth transfer of microorganisms from the biofilms to the food commodities in such processing environments and surfaces. Most frequent contaminations are observed in surfaces associated with meat and dairy processing plants (Carpentier and Cerf, 1993; Midelet and Carpentier, 2002). It shows that the formed biofilms are more resistant to disinfectants and sanitizing agents, which makes it more difficult to remove them from the associated surfaces (Spoering and Lewis, 2001). *Listeria* is capable of existing in monoculture biofilms (a biofilm with a single microorganism, such as *Listeria*) or be a part of mixed culture biofilms with bacteria such as *Flavobacterium* (Gandhi and Chikindas, 2007). Mixed culture biofilms usually have a significantly higher number of *L. monocytogenes* cells attached to stainless steel surfaces, compared to the single culture biofilm. Further, *L. monocytogenes* cells from mixed culture biofilm could

survive for a comparatively more extended period than monoculture biofilms (Bremer *et al.*, 2002).

5. Detection of *Listeria*

Several methods are available to detect *L. monocytogenes* in food commodities, animal feed, or in clinical samples. In food, the classic detection method for *L. monocytogenes* is generally performed in a two-step cultural enrichment process, which is designed to detect the single target cell in a particular sample (Dwivedi and Jaykus, 2011). Microbial methods can be classified as qualitative and quantitative methods. Qualitative methods detect the presence of a bacterium as present or absent, where the quantitative tests enumerate the number of pathogens present directly or indirectly.

Food processing companies usually depend on quality control tests, which deliver the result of contamination within a shorter period. Thus it helps to dispatch each batch of prepared products after the completion of tests. More rapid tests (<48 hrs) include immunological and nucleic acid-based techniques that have developed to detect the presence of the pathogen in rapid detection periods (Jemmi and Stephan, 2006).

Several rapid diagnostic methods are available to detect *L. monocytogenes* and other *Listeria* spp. in food processing environments. Rapid detection methods such as immunoassays, biosensors, and molecular biological methods can be used as alternatives for conventional culture methods in food processing laboratories.

5.1 Classical detection methods

Classical detection methods include the enrichment of the sample in order to apply a plating technique to culture them. Classical detection methods usually involve the detection of the presence of a bacterium (Jasson *et al.*, 2010).

Culture-based methods are usually involved in two steps, where pre-enrichment is done in a general medium to increase the number of viable microorganisms in the sample. Pre-enrichment step dilutes the inhibiting compounds present, provides nourishment for the bacteria to grow, and provides re-hydration to the sample (Dwivedi and Jaykus, 2011). Another enrichment is done in a selective medium that contains different salts and antibiotics to suppress the growth of undesirable microorganisms and favor the growth of the target bacterium and to result in a million times multiplication of the target (Jasson *et al.*, 2010). Presumptive colonies can be cultured on a selective agar medium to observe the colonies of the target, which morphologically confirms the presence or absence of the target organism.

If no colonies were grown, the test results as a negative result, or if any colonies were present, they could be confirmed with an appropriate morphological, biochemical, physical, or serological testing (Dwivedi and Jaykus, 2011). An average one week is required for the identification of a suspected *Listeria* colony to be cultured and confirmed through culture methods (Jemmi and Stephan, 2006). Chromogenic media development or usage of fluorogenic substrates are an advantage for the identification of microorganisms as it enhances the accuracy by inhibiting the unwanted micro-flora growth and enabling the favored microflora to appear in colored colonies with pre-determined color guidelines (Gasnov et al., 2005). Chromogenic media avoids the requirement of subculturing steps or biochemical tests to confirm an unknown microbial culture (Mandal et al., 2011).

5.2 Rapid alternative detection methods

Rapid or alternative detective methods are the combinations of several methods, such as culture methods, immunoassays, and nucleic acid-based methods (Välilmaa et al., 2015). Rapid tests are usually faster than the convenient detection methods (<48 hrs), and the detection levels can be differed according to the method that is using.

5.2.1 Immunoassays

Immunoassays are based on the ability of specific binding between an antibody and an antigen. An antigen can detect the epitopes present in the antibody and binds to them. There are two types of antibodies in immunoassays, namely monoclonal antibodies and polyclonal antibodies. A monoclonal antibody can detect an epitope, where a polyclonal antibody can detect several antigens. The detection level of an immunoassay is around 10^3 - 10^7 (Välilmaa et al., 2015).

Several immunological methods are used for the detection of *L. monocytogenes* in diagnostic, enzyme-linked immunosorbent assay (ELISA), enzyme-linked fluorescence assay (ELFA), immunomagnetic separation (IMS), and lateral flow immunoassays (Välilmaa et al., 2015). Enzyme-linked immunosorbent assay (ELISA) is the most widely used immunoassay method due to its simplicity of application and the rapidness of generating results. ELISA is a specific, accurate, and low-cost, qualitative screening test, and it uses the immunochromatography technique. Immunoassays are famous as the testing can be carried out directly without an enrichment media or laborious sample preparation. Many of the immunoassays are available as commercial kits, and regulatory authorities approve them (Gasnov et al., 2005). It can be a monoclonal or polyclonal antibody-based according to the target pathogen. ELISA test to

detect *L. monocytogenes* is standardized and validated in ISO 16140:2003. Positive and negative results for the samples were detected by the optical densities of the control and the target pathogen with a microplate reader (Portanti et al., 2011). Relative accuracy, sensitivity, relative detection levels, and specificity are needed to be evaluated with the type of antibody that is using. ELISA technique to detect *L. monocytogenes* shows cross-reactions with *L. innocua* and *L. Ivanovii*, while no other cross-reactions are not identified in common foodborne pathogens such as *Bacillus* spp., *Enterobacter*, *Escherichia coli* and *Klebsiella* (Portanti et al., 2011). The relative detection level of ELISA is 5-10 CFU/mL (Portanti et al., 2011; Välilmaa et al., 2015).

Enzyme-Linked fluorescence assay (ELFA) was developed to enhance the sensitivity of ELISA by adding fluorescent labels to antibodies (Zunabovic et al., 2011). According to the literature, time-resolved fluorescent immunoassay correlates with the commercial ELISA kit, although it shows results within less time frame and shows more sensitivity detection levels; 20 CFU/mL (Jaakohuhta et al., 2007). Various other fluorescent immunoassays have been developed in order to increase the sensitivity of ELISA (Välilmaa et al., 2015).

Immunomagnetic separation (IMS) is used in many detection methods to capture the target pathogen. In IMS, paramagnetic beads are coated with antibodies to capture target pathogens from the heterogeneous sample matrixes (Välilmaa et al., 2015). Skjerve et al. (1990) discusses the approach of using IMS to detect *L. monocytogenes* in food matrixes with adequate sensitivity and specificity, to use IMS successfully in the food industry, where the detection levels of less than 100 CFU/ mL can be detected and <200 CFU/mL in pure culture spiked samples. A recent study has been conducted in order to increase the specificity and sensitivity with the rapid detection of IMS by coupling with Real-Time PCR (Yang et al., 2007).

Lateral Flow Immunoassay is an immunochromatography method that detects the presence of the target pathogen visually based on the pathogen's ability to bind to a specific antibody. In this technique, the target pathogen migrates from the sample to the antibody, then pathogen is immobilized on a membrane surface and can be detected as a band on the membrane surface (Välilmaa et al., 2015). A study conducted by Koets et al. (2009) discusses the usage of lateral flow immunoassay as a simultaneous detection for *L. monocytogenes* and *Listeria* spp. in food. Another study conducted by (Cho and Irudayaraj, 2013) discusses the lateral flow chromatographic enzyme immunoassay coupled with a magnetic concentration step that detects

100 CFU/mL in milk spiked with *L. monocytogenes* within 2 hrs. A lateral flow assay is identified as specific as a PCR assay, although the sensitivity is lower with >300 samples (Välilä et al., 2015).

5.2.2 Biosensors

Biosensors are defined as the indicators of biological compounds that used to detect a target pathogen. They convert a biological response into an electric signal. It consists of a bioreceptor (microorganism, tissue, cell, enzyme, nucleic acid biomimetic, or antibody) and a transducer. Transduction may be optical, electrochemical, thermometric, piezoelectric, magnetic microchemical, or combinations of one or more of the above. Bioreceptor receives the target analyte, and corresponding biological responses are converted to electric signals by a transducer. An amplifier enhances the primary input signal and delivers a detectable output signal as a waveform signal (Velusamy et al., 2009). Application of biosensors to food testing has several advantages; many of the systems are portable, which enables them in the field application or on the spot analysis. They also provide rapid test results that are capable of testing multiple samples simultaneously (Velusamy et al., 2009).

Applications of biosensors to detect *L. monocytogenes* has been studied in Ohk et al. (2010) could detect 10^3 CFU/mL in *L. monocytogenes* pure cultures spiked into ready to eat meat products such as sliced beef, chicken and turkey after 18 hours enrichment. However, although the detection levels are higher than culture-based methods, the sensitivity of the biosensors are not as high as in amplification methods (Välilä et al., 2015).

5.2.3 Bacteriophage-based detection methods

Bacteriophage-based detection methods have many advantages such as; high sensitivity, specificity and ease of detector amplification. Further, bacteriophage-based methods can distinguish between live and dead target cells. Bacteriophage based detection kits designed to detect *Listeria* spp. and *L. monocytogenes* are commercially validated and are available to be used in the food industry. The use of bacteriophage-based methods to detect *L. monocytogenes* has been studied by Kretzer and colleagues in 2007 with the application of paramagnetic beads coated with phage-based proteins and culturing. 100 CFU/g was detected from lettuce, cheese salmon, meat, and milk after 6 hours of pre-enrichment. The sensitivity of bacteriophage detection methods is better than the level of sensitivity of culture-based methods (Gasnov et al., 2005; Kretzer et al., 2007; Välilä et al., 2015).

5.2.4 Amplification methods

Standard amplification methods used in literature are; conventional Polymerase Chain Reaction (PCR), multiplex PCR, Real-Time PCR (RT-PCR), and quantitative PCR (qPCR) for the pathogen detection in food manufacturing environments (Välilä et al., 2015).

Conventional PCR is a commonly identified diagnostic tool for the detection of *Listeria* spp. and *L. monocytogenes* in the laboratory. It is an in-vitro method that can increase the number of target DNA sequences in a sample. They can be performed with suitably designed primers. Amplified results indicated by a conventional PCR are analyzed through electrophoresis or fluorescence. The usual drawbacks on conventional PCR are; high cost, the need for expert knowledge, influences to the results by the inhibitory compounds in food or certain microbiological media, and conventional PCR is usually qualitative (Zunabovic et al., 2011; Law et al., 2015; Välilä et al., 2015). Furthermore, PCR does not distinguish between live and dead cells in a sample; thus, false-positive results can be obtained (Mandal et al., 2011). Molecular detection methods are often preferred due to its specificity, sensitivity, and since there are no prior enrichment processes, the reaction can be completed within nearly 4 hrs (Velusamy et al., 2009).

Conventional PCR detects one species at a time. A modification to conventional PCR is multiplex PCR, where it enables the detection of multiple species at the same time in a single run or can detect different target locations in the same species. There are developed and validated multiplex PCR programs available to detect several *Listeria* spp. in the same sample. A study conducted by Rattanachaikunsopon and Phumkhachorn (2012) has designed a reverse transcription-based multiplex PCR to detect *L. monocytogenes*, *L. innocua*, *L. grayi*, and *Listeria* spp. The detection level for the above study was 50 CFU/mL in pure cultures. Many other different studies have been carried out in literature targeting different target genes to detect *Listeria* spp. and *L. monocytogenes* in the food industry (Rattanachaikunsopon and Phumkhachorn, 2012b).

Real-time PCR (RT PCR) is a modification of conventional PCR, where RT PCR can quantify the amplified products using fluorescence or in real-time. RT PCR is usually faster, and it is widely used to detect food pathogens (Välilä et al., 2015). The sensitivity of RT PCR in *L. monocytogenes* spiked samples (milk, juice, meat) showed a detection level of 100 CFU/mL with a 93.3% correction rate (Jin et al., 2012).

5.2.5 Comparison of several diagnostic tests used for *Listeria monocytogenes* detection

Based on the principles behind each diagnostic technique to detect *L. monocytogenes*, advantages, and limitations of each method (conventional culture method, immunoassays, or molecular-based methods) are indicated in Table 2.

6. Impact of *Listeria monocytogenes*

Listeriosis is considered as a significant public concern as it can cause a substantial impact through associated food commodities. Therefore, *Listeria* infection is considered as an infection with a large scale hospitalization and a fatality rate (Hedberg, 2011).

Furthermore, *L. monocytogenes* contamination can affect the food industry as it can reject or detain the food products making the higher economic cost for the inspection or re-inspection. Any product detected to be contaminated with *Listeria* is unsuitable for human consumption and needs to be discarded. Therefore, the manufacturers' encounter an economic loss as the manufacturing costs and the invested capital cannot be converted to profits (Jemmi and Stephan, 2006).

If any food commodity is detected with possible *Listeria* contamination, the whole batch can be rejected immediately or may be subjected to another confirmation test series, which makes them delaying the dispatch. In addition to the direct costs of rejection and detention, inevitable delays due to food safety measures are taken to ensure the elimination of *L. monocytogenes* can delay the distribution and transportation can cause expiry of

shelf-life, and the costs of holding products may also be applied (Jemmi and Stephan, 2006).

7. Risk reduction strategies

In order to reduce the level of risks, various risk management strategies are conducted in food processing environments at different levels. Foodborne pathogens like *Listeria* are mainly associated with fecal contamination by infected animals or humans. In order to reduce the level of contamination, one primary strategy taken in the food processing environments is to reduce the fecal carriage of *Listeria* by the livestock strongly correlated with milk and carcass contamination (Ferreira et al., 2014). Maintaining hygienic practices, especially during milking and slaughtering, can reduce the risk of contamination by *Listeria*. Implementation of the hazard analysis and critical control points (HACCP) is a vital risk prevention programme implemented throughout the food processing environment, especially in high-risk groups (Jemmi and Stephan, 2006).

The application of antimicrobial agents and Ultra-Violet radiation has successfully been used to reduce the microbial load in fresh produce (Zhu et al., 2017). Although the reduction of the microbial load is possible, *L. monocytogenes* might not be inactivated entirely due to its ability to survive in adverse conditions (Hussain et al., 2017).

Understanding of the cellular physiology of *Listeria* is vital to develop more efficient and effective control

Table 2. Comparison between different diagnostic methods used to detect *L. monocytogenes*

Diagnostic Method	Advantages	Drawbacks	References
Conventional methods (Culture based or chromatographic)	<ul style="list-style-type: none"> • Cost effective • Requires less expertise knowledge • Sensitive • Reliable • Not inhibited by compounds present in food 	<ul style="list-style-type: none"> • Time consuming • Labor intensive • Less specific 	Jasson et al. (2010), Dwivedi and Jaykus (2011) and Välimaa et al. (2015)
Immunological methods	<ul style="list-style-type: none"> • Fast • Reproducible • Can be automated • Less sensitive to food interferences 	<ul style="list-style-type: none"> • Antigen-antibody binding is not always strong • Less sensitive than nucleic acid-based methods 	Jasson et al. (2010)
Nucleic acid-based methods	<ul style="list-style-type: none"> • More specific • High sensitivity • Stable • Rapid • Reproducible • Can be automated • Multi parameters can be tested at once 	<ul style="list-style-type: none"> • Involves high cost • Can be sensitive to interfering agents present in food 	Jasson et al. (2010) and Välimaa et al. (2015)

measures for *L. monocytogenes* contamination in food processing environments. Cold stress adaptation and biofilm formation are the fundamental attributes of *L. monocytogenes* that is essential for its dissemination in food environments. The attachment of the bacteria to the food product or the product contact surfaces leads to serious hygienic problems and economic losses as it tends to spoil food (Donlan and Costerton, 2002; Gandhi and Chikindas, 2007). Especially the biofilms formed by *Listeria* are more resistant to detergents, disinfectants, antimicrobial agents, and sanitizing agents, more effort needed to be taken place to eliminate the level of risks than less hardy microorganisms. Fresh produce comes into contact with many different working surfaces and different temperatures during processing or transport, and according to a study by Bonsaglia *et al.* (2014), these two factors influence the *L. monocytogenes* biofilm formation. They compared *L. monocytogenes* biofilms growing on three kinds of touched surfaces, polystyrene, glass, and stainless steel, at three different temperatures (4°C, 20°C, and 35°C). The results showed that *L. monocytogenes* attaches more easily to hydrophilic surfaces (glass and stainless steel) than to hydrophobic surfaces (polystyrene). Higher temperatures and longer incubation times decreased the extent of adherence to surfaces, but the results were not significant. In many foods processing plants involve the usage of glass or stainless-steel surfaces, which are more or less hydrophilic, need more effort to reduce the risks of contamination (Bonsaglia *et al.*, 2014)

8. Conclusion

L. monocytogenes is a virulent foodborne pathogen that is responsible for many outbreaks and sporadic cases of listeriosis worldwide. Several adaptations strategies, including surviving in harsh environmental conditions and biofilm formation, are found in *L. monocytogenes* to thrive in various adverse conditions. Therefore, risk management methods need to be applied to prevent the contamination of *L. monocytogenes* and ensure food safety. The main priority is the early detection of *Listeria* contamination in food to prevent foodborne outbreaks and comply with regulatory requirements. Since there is a 'zero-tolerance policy' for the presence of *Listeria* in certain foods for human consumption in many countries (FDA, 2003; Välimaa *et al.*, 2015), it is crucial to prevent possible listeriosis outbreaks, associated hospitalizations, and deaths. Therefore, research into developing rapid, sensitive, specific, and cost-effective methods are essential to ensure food safety.

Further, the development of cost-effective minimal processing and chemical-free technologies can effectively eliminate pathogens and simultaneously

allow a shelf-stable and fresher product to be produced.

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