Chapter 6

Contamination, Prevention and Control of *Listeria monocytogenes* in Food Processing and Food Service Environments

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Additional information is available at the end of the chapter

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Abstract

This chapter reviews issues related to the occurrence and growth of *Listeria monocytogenes* in food processing and food service environments. *L. monocytogenes* is a food-borne pathogen with the capacity to contaminate raw or minimally processed foods such as chilled ready-to-eat (RTE) foods. The consumption of food contaminated with *L. monocytogenes* can result in a disease known as listeriosis among vulnerable groups of people such as pregnant women and fetuses, newborns, adults between the ages of 65 and 75, and people with weakened immune systems. *L. monocytogenes* is ubiquitous and has been isolated from soil, vegetation, sewage, water, animal feed, fresh and frozen meat including poultry, slaughterhouse wastes and the feces of healthy animals and humans. The bacterium is both acid tolerant and salt tolerant. It is able to grow at refrigerator temperature, and is therefore often associated with the consumption of raw or minimally processed and often chilled RTE foods. *L. monocytogenes* is able to form biofilms on food processing and preparation surfaces, which protects it from antimicrobial action. Continuous education of vulnerable groups regarding food safety will increase their awareness of the importance of practicing safer food handling practices such as hand washing and safe storage of RTE foods as a means to prevent listeriosis.

Keywords: *Listeria monocytogenes*, ready to eat food, listeriosis, food safety

1. Introduction

Listeria monocytogenes is a bacterium which is ubiquitous in nature, and occurs frequently in food processing and handling environments [1]. The consumption of food contaminated with

L. monocytogenes can result in a disease known as listeriosis, to which pregnant women and their newborns, adults aged 65 or older, and people with weakened immune systems are particularly vulnerable [2]. In healthy adults, listeriosis is most likely to manifest as mild gastroenteritis. However, in some instances it can result in more severe symptoms, which can lead to life-threatening illnesses such as endocarditis, encephalitis or meningitis, and severe sepsis [3].

Inadequate food hygiene practices during food preparation are primarily responsible for the propagation of the bacterium and contamination of ready-to-eat (RTE) foods (**Table 1**) during processing, distribution and handling [4]. Small to medium-sized enterprises (SMEs) are more likely to experience *L. monocytogenes* outbreaks than renowned large-scale food processing enterprises owing to differences in the implementation of food safety measures [5].

RTE foods, which are often stored at low temperatures, are the type most susceptible to contamination with *L. monocytogenes* since the bacterium is psychrotrophic and possesses the ability to survive and grow in the presence of many food preservation systems, such as low pH and high salt concentrations [6]. The contamination of minimally processed fruit and vegetable products with *L. monocytogenes* is often a concern, considering that these foods, which are attractive to consumers, are often not subjected to lethal treatments during processing to inactivate potential pathogens [7]. Moreover, the manner in which RTE vegetables are sliced can affect the survival of *Listeria* and the effectiveness of decontamination procedures in the finished products. Hand tearing or manual slicing with a razor blade reduced the survival and growth of *E. coli* and *L. innocua*, probably because of less damage to the vegetable tissues and minimal leakage of nutrients from damaged plant tissues [8].

Year	Foodstuff implicated	Country of outbreak
2017	Creamy, soft, raw-milk cheeses [2]	USA
2016	Frozen vegetables [62]	USA
2016	Raw milk chocolate milk products [63]	USA
2016	Packaged salad [64]	USA
2015	Soft cheese [65]	USA
2015	Ice cream [66]	
2014	Commercially produced, pre-packaged caramel apples [67]	USA
2014	Mung bean sprouts [68]	USA
2014	Soft cheese [69]	USA
2014	Cheese products [70]	USA
2017–2017	Various food products [71]	South Africa
2017	Not determined [72]	Australia
2014	Various food products [73]	28 EU/EEA countries

CDC: Centre for Disease Control, **NICD**: National Institute for Communicable Diseases, **ECDC**: European Centre for Disease Prevention and Control, **USA**: United States of America, **EU**: European Union, **EEA**: European Economic Area.

Table 1. Some records of global ***Listeria*** outbreaks between 2014 and 2017.

L. monocytogenes has the ability to attach itself to food preparation contact surfaces and grow to form protective biofilms, which generally protect the bacterial cells from antimicrobial action during cleaning and sterilisation processes [9]. However, low concentrations (<10 μg/mL) of paenibacterin have been found to suppress the growth of *L. monocytogenes* within the biofilm matrix as well as to down-regulate the genes involved in biofilm formation [10]. Considering that *L. monocytogenes* is a food-borne pathogen of public interest [11], the objective of this paper is to review issues related to the occurrence and growth of *L. monocytogenes* in food processing and food service environments.

2. Health and economic impacts of listeriosis

Globally, billions of people are at risk every year and thousands die as a result of consuming unsafe food [12]. In the United States of America (USA), listeriosis has been identified as the third leading cause of death from food-borne illness, after non-typhoidal *Salmonella* and *Toxoplasma gondii*, despite its rarity [13]. In Africa, food-borne illness continues to be a major health threat, especially for vulnerable groups such as infants, pregnant women and their newborns as well as immune-compromised individuals such as elderly people and those with HIV/AIDS [14].

In humans, invasive listeriosis is characterized by septicemia, meningitis, and abortion in pregnant women [15]. Listeriosis in pregnant women can result in premature labor, stillbirth, abortion, and neonatal infection, with high neonatal mortality [16]. It should be noted that *L. monocytogenes* infection in healthy individuals does not necessarily result in invasive disease. The incubation period of listeria-related gastroenteritis can range from 1 to 24 days, but the average incubation period has been found to be less than 24 hours. After the incubation period, prominent symptoms will include fever, then diarrhea, arthralgia, myalgia, and headache. Other common symptoms are nausea, vomiting, abdominal pain and watery diarrhea. In healthy individuals, the illness tends to last between 1 and 3 days, with a very low rate of hospitalization [17].

Listeriosis may have an economic impact in the form of costs incurred by the government in funding health institutions to deal with the problem [18]. Other costs can take the form of legal costs emanating from lawsuits imposed on food production companies arising from illness and death due listeriosis [19].

3. Ecology and growth conditions of *Listeria monocytogenes* in the food chain

L. monocytogenes are ubiquitous bacteria that can be found in different environments such as soil and water, and especially in food-manufacturing environments [20]. Many *Listeria* species have been isolated from soil, vegetation, sewage, water, animal feed, fresh and frozen meat including poultry, slaughterhouse wastes and the feces of healthy animals, including humans [21]. Animals have been found to be carriers of *L. monocytogenes*, hence the contamination of foods of animal origin, such as meats and dairy products [22].

L. monocytogenes can survive a low pH of 5.5 through a phenomenon known as the acid tolerance response (ATR), which causes cells to be more resistant in adverse acidic conditions [23]. The bacterium, which is notable for its persistence in food-manufacturing environments, is relatively salt-tolerant and is able to grow at refrigerator temperature, and is therefore often associated with the consumption of raw or minimally processed and often chilled RTE foods (e.g., soft and semi-soft cheese and smoked fish products), which are consumed without further processing [24, 25].

4. The occurrence of *L. monocytogenes* in the food processing environment

L. monocytogenes is able to attach to food processing surfaces and multiply to form biofilms in inaccessible locations in processing facilities [9]. Biofilms protect the bacterium against antimicrobial action, enabling it to colonize food processing equipment, conveyor belts, pipes, floors and drainage systems and to persist for months or even years, cross-contaminating different surfaces in food processing plants [26]. The formation of biofilms on various food contact surfaces by *L. monocytogenes* makes it extremely difficult to control this pathogen effectively, especially in processing plants where inadequate cleaning has been carried out [27].

5. The occurrence of *L. monocytogenes* in RTE foods and food contact surfaces in food service facilities

RTE foods have gained considerable popularity in many developing and developed countries because of their perceived better flavor, affordability and accessibility [28]. However, numerous *L. monocytogenes* outbreaks have been associated with RTE foods [29]. The prevalence of *L. monocytogenes* in RTE food is a major concern relating to food safety because RTE foods are consumed without further processing (cooking) or washing at home (**Table 2**). It is for this reason that stringent microbiological guidelines need to be formulated and followed to ensure that processors produce RTE food that is safe [30]. Implicated RTE foods include RTE deli meats, raw milk and other raw milk dairy products (soft cheese) (**Table 1**). Between 1999 and 2011, 73% of all food-borne outbreaks of listeriosis that occurred in the United Kingdom (UK) were attributed to the consumption of sandwiches [31].

The presence of *L. monocytogenes* in RTE food is attributed to contamination during production, distribution or storage [32]. *L. monocytogenes* contamination in various food factory environments has been reported at nearly all stages of processing ([5] and Rodrigues et al. [33]). When compared with other food-borne pathogens such as *Staphylococcus aureus*, *E. coli* 015:H7, and *Salmonella* and *Shigella* species, *L. monocytogenes* has been found to be most prevalent on food contact surfaces in food service establishments [34]. Owing to its ability to grow in contaminated food during storage at refrigeration temperature, *L. monocytogenes* has

Food groups	Susceptible food products
Meat	Processed meat products such as ground beef, sausages, deli ham, beef hot dogs and meat-related sandwich products (e.g., pork, beef)
Poultry	Processed chicken such as deli chicken, deli turkey, eggs, and related sandwich products
Fish	Cooked shrimps, sushi, smoked salmon, seafoods, and related sandwich and salad dishes
Dairy	Cheese, yogurt
Fruit and vegetables	Cabbage, lettuce, cucumber, frozen green beans, peanut butter, vegetable salads, raw sprouts, cantaloupe melon and related salad dishes
Cereal and baked products	Pasta, cakes, pies, sausage rolls

Table 2. Listeria in food: foods that are susceptible to contamination by ***Listeria monocytogenes*** [57, 74].

been found in raw and processed RTE foods that required low temperature storage [35]. The high volume of food products such as meat, vegetables, dairy products and fruits that pass through the cold chain in food service establishments could contribute to the high incidence of *L. monocytogenes* in RTE food and on food contact surfaces [34, 36].

Inadequate cleaning procedures and hygiene practices can promote the formation of biofilms on food contact surfaces in food service establishments, thereby increasing the chances of *L. monocytogenes* cross-contamination within food service facilities [37]. Because biofilms are able to resist most sanitisers and disinfectants used, cross-contamination by *L. monocytogenes* poses a serious food safety risk in food service establishments, including domestic kitchens [38]. The ease with which *L. monocytogenes* is able to adhere to food contact surfaces and form biofilms increases the likelihood of its persisting on food contact surfaces, and hence cross-contaminating the final food products [39, 40]. The presence of food debris on food contact surfaces encourages the formation of *L. monocytogenes* biofilms [41].

6. The occurrence of *L. monocytogenes* on food contact surfaces in domestic kitchens

Inadequate hygiene practices in domestic kitchens may contribute to the persistence of food-borne pathogens, thereby compromising the safety of foods produced there [42]. Home kitchens have been found to be a significant location where food-borne illnesses are acquired. A survey conducted in the domestic kitchens of consumers aged 60 and above in the UK indicated that a large number of foods in home refrigerators were beyond the use-by date and up to 66% of opened RTE foods had been stored beyond the recommended 2 days after opening [43]. A study of the occurrence of *Listeria* spp. on food contact surfaces in domestic kitchens in the Netherlands found high levels of *L. monocytogenes* on dish-cloths and in bathrooms, but low levels on kitchen sinks, washing-up brushes and refrigerators [44]. Many researchers have found high levels of *L. monocytogenes* on refrigerator surfaces in domestic kitchens [45].

7. Legislation relating to the occurrence of *Listeria monocytogenes* in foods

Most food legislation stipulates the microbial criteria for food-borne bacteria such as *L. monocytogenes* or their toxins and metabolites in specific foods. These criteria often prescribe the acceptable levels of these bacteria or their toxins in food products available on the market [46]. Most foods that support the growth of *L. monocytogenes* should be the focus of risk management efforts. Countries such as Germany, the Netherlands and France have set a tolerance level of 100 colony forming units (cfu) of *L. monocytogenes* per gram of food at the time of consumption while others, such as the USA and Italy, require a total absence of *L. monocytogenes* in 25 g of food [47]. The new criteria for *L. monocytogenes* in RTE food gazetted by Food Standards Australia-New Zealand on 31 July 2014 prescribe two sets of criteria for *L. monocytogenes* for application based on whether the growth of the bacterium does or does not occur inherently in a particular RTE food. These criteria include fewer than 100 cfu of *L. monocytogenes* per gram of food in which the growth of *L. monocytogenes* is not likely to occur, and that *L. monocytogenes* should not be detected in 25 g of food in which the growth of *L. monocytogenes* is likely to occur [48].

The Food Safety Standard of Ireland has prescribed the following in relation to *L. monocytogenes*: *L. monocytogenes* should be absent in 25 g of RTE food destined for infant consumption or for serving as a special food for medical purposes in up to 10 collected food samples. Similarly, in the case of RTE foods that are able to support the growth of *L. monocytogenes*: *L. monocytogenes* should be absent in 25 g of RTE food following production or should not exceed 100 cfu per gram of food placed on the market during its shelf life, in up to 5 collected food samples. Lastly, in the case of RTE foods that are not able to support the growth of *L. monocytogenes*: *L. monocytogenes* should not exceed 100 cfu per gram of food placed on the market during its shelf life, in up to 5 collected food samples [49].

8. Methods commonly used for the identification of *L. monocytogenes* in foods

8.1. Culture methods

L. monocytogenes can be isolated from contaminated samples by subjecting them to pre-enrichment. This entails mixing samples with enrichment media such as *Listeria* Enrichment Broth (Sigma), after which the enrichment samples can be cultured on *L. monocytogenes*-specific agar plates such as Listeria Mono Differential Agar (Sigma). Isolation can be performed using various other media and procedures [50]. Thereafter, pure cultures of *L. monocytogenes* to be used for downstream identification and characterization analysis can be prepared by isolating individual colonies from agar plates [51]. The culture-based methods are often used in combination with immunoassay- or molecular PCR-based methods for accurate detection of *L. monocytogenes* in food samples [52].

8.2. Immunoassay

During immunoassay, monoclonal antibodies specific to *L. monocytogenes* can be incorporated into various techniques for identification. Immunoassay tests usually have high specificity

and are fast and easy to use, but do not permit identification to species level. Another disadvantage of this method is that the presence of a low number of listeria cells in a sample can give rise to a false positive [53]. Various variants of immunoassays are available, including sandwich-type enzyme-linked immunosorbent assay (S-ELISA) [54], nanoparticle immunoassay [55], and enzyme-linked fluorescent assay (ELFA) [56].

8.3. PCR-related methods

PCR-based techniques involve the amplification of a specific gene segment of *L. monocytogenes* such as HlyA-, Iap-, PrfA and SsrA using specific primers followed by monitoring of the amplified segment using agarose gel electrophoresis or other detection techniques such as SYBR Green [57]. Similarly, the 16S rRNA genes of *L. monocytogenes* can be amplified, sequenced, and searched against existing databases for identification [52]. The disadvantage of PCR-based techniques is related to the costs associated with the purchase of the instrument and reagent, as well as the expertise required to conduct the experiments [58].

9. Prevention and control of *Listeria monocytogenes* in food systems

The prevention and control of *L. monocytogenes* in RTE foods is paramount in protecting consumers against listeriosis. In a document entitled "Guidelines on the application of general principles of food hygiene to the control of *L. monocytogenes* in foods" the World Health Organization has provided guidelines that can be followed to minimize the likelihood of the occurrence of *L. monocytogenes* in RTE foods. According to the [59], food safety measures need to be carried out at different levels of a food production environment, and must include:

1. Establishing the design and adequacy of a production facility: proper location and layout, and adequate equipment and facilities such as water supply, drainage, toilets, temperature control, storage, and hand washing basins.
2. Control of food safety hazards and implementation of hygiene practices throughout the food production line. Accredited HACCP implementation programme.
3. Establishment of adequate sanitary conditions and maintenance of the production facilities; effective cleaning programmes; pest control and proper waste management; and effective monitoring of cleaning programmes.
4. Ensuring adequate implementation of personal hygiene, health status, personal cleanliness and personal behavior of staff.
5. Ensuring adequate and properly functioning transport facilities; these should be well maintained and fit for purpose.
6. Continuous training of staff working in the food production environment, including refresher training.

While the food industry is taking numerous measures to protect foods from *Listeria*, consumers of RTE food, especially those belonging to the vulnerable groups, must take suitable

Vulnerable consumer group	Reason for vulnerability	Recommended preventive food hygiene measures
Pregnant women	Weak immune system due to hormonal changes	• Wash and dry your hands before and after touching and preparing ready-to-eat food. • Refrigerator food contact surfaces should be clean and sanitized regularly, and operate below 5°C. • Kitchen utensils such as knives, cutting boards and graters must be washed before and after being used in preparing ready-to-eat foods. • Minimally processed fruits and vegetables must be washed thoroughly in flowing water prior to consumption. • Store raw meat separately from and below cooked and ready-to-eat food in the refrigerator. • Protein-rich foods containing meat, fish, chicken, egg, sprouts and dairy foods that have cooled to room temperature must be discarded. • Protein-rich foods must be kept either hot (60°C or hotter) or cold (5°C or colder).
Unborn fetuses and newborn babies	Undeveloped immune system	
People over the age of 65	Weak immune system due to ageing	
People with diseases such as cancer, leukemia, AIDS, diabetes, or liver or kidney disease	Weak immune system due to disease	
People on drugs that can suppress the immune system such prednisone or cortisone	Suppressed immune system due to drugs	
People undergoing organ transplant	Suppressed immune system due to drug administration	

Table 3. Listeria in food: Advice to people vulnerable to listeriosis [75].

precautions during the handling of food in their households to prevent the growth and contamination of food by *L. monocytogenes* (**Table 3**).

10. Consumer awareness of listeriosis

Continuous provision of food safety education to consumers through various channels such as social media increases consumer awareness of the need for safer food handling practices such as hand washing and safe storage of RTE food [60]. The food standard agency of the UK has identified and targeted consumers who are at risk of contracting listeriosis. Vulnerable people, many of whom live and obtain their food independently include those with various forms of cancer, diabetes, alcoholism and diseases of the kidneys, liver, cardiovascular system (e.g., heart disease), digestive system (e.g., Crohn's disease) and musculoskeletal/connective tissue system (e.g., lupus) [61]. Even though most consumers of food sold by street vendors may not have confidence in the safety of RTE foods sold on the street, this often does not affect their preference for such foods because of their affordability, availability and convenience [28].

11. Conclusions

RTE foods have gained considerable popularity in many developing and developed countries because of their perceived better flavor, affordability and convenience. Consumers will continue to consume RTE foods despite their association with *L. monocytogenes* outbreaks. While

most food processing industries are taking measures to protect foods from listeria, consumers of RTE food, especially those belonging to vulnerable groups, must take suitable precautions during the handling of food to prevent the growth of *L. monocytogenes* and contamination of food by this organism. Continuous identification of those groups of consumers vulnerable to listeriosis and food safety education directed at them specifically will increase their awareness of the need for safer food handling practices such as hand washing and safe storage of RTE food in an effort to prevent listeriosis.

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Conflict of interest

I declare that I have no conflict of interest regarding the publication of this research chapter.

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