**Incidence of Listeria Species in Food and Food Processing Environment: A Review.**

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**Review Article**

**ABSTRACT**

*Listeria* is a ubiquitous organism and can be isolated from a variety of sources such as raw foods, soil, stream water, sewage, plants. It is also been found in uncooked meats, fish, uncooked vegetables, unpasteurized milks, their products, processed foods and food processing environments from different parts of the world. Various methods are used to sanitize the food processing environments and to control the organism from the food and the food processing environments. Proper surveillance, rapid detection of *Listeria* is important to ensure the safety of food products. The article reviews major Listeria incidences in food and food related environments from a global perspective.

**INTRODUCTION**

*Listeria* is a widely distributed bacterium in nature and commonly found in soil, sewage, dust, water and causes listeriosis in humans and animals. Listeriosis is a relatively rare food-borne illness, but can be life threatening with high fatality rates. It is mainly associated with the consumption of processed foods that require no further cooking by the consumer [1, 2, 3], this includes Coleslaw [4], milk [5, 6], cheese [7, 8, 9, 10, 11], butter [12], pate [13,14], ready to eat deli meats [15], raw fruits, vegetables, salads and hot dogs. *L. monocytogenes* is one of the ten phenotypically similar species of *Listeria* (i.e., *Listeria monocytogenes*, *L. innocua*, *L. ivanovii*, *L. welshimeri*, *L. seeligeri*, *L. grayi*, *L. rocourtiae*, *L. martthii*, *L. fleishmanii* & *L. weihenstephanensis*) [16, 17, 18, 19]. Among these ten species of Listeria, two species namely *L. monocytogenes* (pathogenic for human and animals) [20] and *L. ivanovii* (pathogenic for animals) are usually associated with Listeriosis. *L. monocytogenes* is a cause of food-borne disease; it is linked to disproportionately high levels of morbidity and mortality [21, 22]. *L. monocytogenes* is carried within the intestinal tract of seemingly healthy animals [23, 24]. *L. monocytogenes* and other *Listeria* spp. have also been isolated from a variety of raw and processed foods [26]. *Listeria* is considered to be intolerant to the temperatures achieved during food processing, such as cooking and pasteurisation. *L. monocytogenes* in contaminated foods is associated with central nervous system (CNS) diseases, sepsis, endocarditis, focal infections, gastroenteritis and can cause still births and abortions [26]. Non-invasive listeriosis occurs in healthy populations at low infection rates, usually causing only self limiting gastrointestinal diseases [27]. The rate of healthy population infected with listeriosis is low with about 0.7 cases per 100,000 persons. The more severe form of listeriosis is invasive listeriosis with infections commonly occurring in vulnerable individuals like newborns, the elderly, immuno-suppressed patients and pregnant women [28, 29]. Immuno-suppressed patients such as organ transplant recipients, chronic lymphatic leukaemia, AIDS and acute leukaemia are predisposed to higher rates of infection with over 100 cases per 100,000 persons [29]. Food-borne listeriosis outbreaks have been reported since 1975, in industrialized countries in Europe, North America and Oceania with a few or no reports from Africa, Asia and Latin America [30, 31]. Because of the multifaceted properties, *L. monocytogenes* can grow and multiply in various food matrices even under adverse conditions like high pH, low temperature etc.

**Fish and Fish Products**

Lennon et al., in 1984 suggested the involvement of seafood in the transmission of listeriosis, based on epidemiological evidence and proposed that consumption of shellfish and raw fish was responsible for an epidemic...
of prenatal listeriosis in New Zealand in 1980 [32]. The study carried out by Souto et al., in 2007 revealed the incidence of Listeria spp. in the salt-water edible fish and in the environment of fish markets in Thessaloniki, Northern Greece which was less when compared to the level of contamination of the environment of fish markets [33]. It has also been isolated from temperate regions, from fishery products on a regular basis since 1980’s. Embarek reviewed the incidence of Listeria in seafood worldwide and found that the prevalence of L. monocytogenes varied from 4% to 12% in surveys from temperate areas [34]. The presence of L. monocytogenes in salmon from the United States, Chile, Norway and Canada was reported by Farber & Peterkin in the year 1991 [35]. Other studies have found that the prevalence of L. monocytogenes in raw fish is quite low, ranging from 0% to 1% [36, 37] to 10% [38]. Hartemink and Georgesson stated that in Iceland 56% of fresh fish on sale were contaminated with L. monocytogenes and other Listeria spp. [39]. Overall 3% prevalence of L. monocytogenes was observed in European fish [40]. Jørgensen et al., reported the highest prevalence of Listeria monocytogenes in sea food i.e., cold smoked fish and lowest in heat treated and cured sea food [41]. Inoue et al., stated that the ready to eat raw foods and shell fish are relatively high risk foods and 3.3% of Listeria monocytogenes was isolated from retail foods in Japan [42].

There are several reports of incidence of Listeria spp. in fish and fish handling areas as fish and fishery products are acting as a vehicle of transmission of listeriosis [43]. Listeria spp. was isolated from fish and fish handling areas [44] in Mangalore city, India. Similar studies were done by Jayashekaran et al., Vinoth Kumar et al., and found 72.4% of fish and 44.4% of shellfish tested were found to be positive for Listeria species [43, 45]. Karunasagar and Karunasagar reviewed on the incidence of Listeria monocytogenes in tropical fish and fishery products and reported that the incidence in tropical food is very low [46]. Dhanashree et al., reported that the incidence of Listeria species in food samples is 17.5% and highest incidence was in seafood samples of Mangalore city [47]. Moharem et al., reported 37.8% of sea food samples collected from Mysore was positive for Listeria species [48].

In another study, Jallawar et al., isolated Listeria spp. from 200 fresh water fish samples collected from Nagpur, Central India. They could isolate 67% of L. monocytogenes, 21% of L. seeligeri, 8% of L. grayi and 5% L. welshimeri from fresh water fish samples. This showed the predominance of L. monocytogenes in the samples [49]. Das et al., found the prevalence of Listeria spp. in tropical sea food of Kerala, India and found that L. innocua was the most prevalent species of Listeria with an incidence rate of 28.7% and L. monocytogenes with low prevalence i.e., 1.2% of the total samples tested [50]. Swetha et al., observed the higher incidence of Listeria monocytogenes (23.9%) in fish and fish swab samples from Kerala [51].

Ready-To-Eat Foods and Manufacture Environments

Human foodborne infections are defined by high prevalence and low mortality rates. In the case of illness caused by L. monocytogenes the situation is different [52]. It is intended to collect data about the contamination level of certain Ready-To-Eat (RTE) foods in retail outlets in all member states to assess the L. monocytogenes risk [53]. Since the transmission of this infection is primarily by consuming contaminated food (e.g. ready-to-eat food), pre requisite and harmonized programmes are essential to control this microorganism [54] and to calm down the so-called “Listeria hysteria” [55]. As Listeria spp. is highly tolerant to live under extreme pH (some cultures grow at pH 9.6), temperature (<45°C) and salt conditions (20% (w/v) NaCl), they can be found in a variety of environments, foods and clinical samples [35, 29, 56, 57, 58, 59]. Result of the study by Domenech et al., showed survival curves of L. monocytogenes by washing using bleach and washing under tap water. Washing under running tap water reduces the initial load of the organism and dipping lettuces in 1 mL of bleach per litre for 30 min was the most effective treatment [60].

The presence of Listeria spp. in RTE products is particularly troublesome for vulnerable populations. This group which includes pregnant women and their foetus is particularly susceptible to invasive listeriosis, with mortality rates ranging between 20% and 40% [22, 61]. There are evidences that suggest the risk to vulnerable populations may be even higher if virulent strains of L. monocytogenes in RTE foods are encountered [62, 63]. In Canada, eight listeriosis outbreaks have been reported over the period of 24 years and have been linked to a variety of RTE foods [22, 64]. The most notable outbreak was 2008 nationwide outbreak associated with contaminated deli meats that originated from a single food-processing facility (Public Health Agency of Canada 2010), and resulted in 57 invasive listeriosis cases and 23 deaths [21]. The source of contamination was suspected to be a large commercial slicer that harboured L. monocytogenes [21]. Similar scenarios have been reported in other listeriosis outbreaks where Listeria spp. in the processing environment led to contamination of RTE products [65, 66, 67]. It is a known fact that food product contamination is associated with food-processing environments harbouring L. monocytogenes and subsequent post-processing transfer to finished products [68, 69, 66].

Many researchers have focused on the prevalence of Listeria spp. in production environments and contamination patterns in these facilities [70, 71, 72, 73, 74]. Strains of L. monocytogenes capable of persisting in food-processing environments for up to 12 years and intermittently contaminating products have been reported [75, 68, 76, 66]. Establishments of RTE foods have received less attention and consequently fewer data examining prevalence

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Manufactured RTE foods are being consumed in increasing quantities; it has been noted that *L. monocytogenes* has been recognized as an important opportunistic human food-borne pathogen [20]. The unique ability to survive and multiply at refrigeration temperatures may increase the hazard of *Listeria* infection from contaminated foods especially, chilled RTE food products [81]. Freeze injury reversibility of *L. monocytogenes* transforms contaminated frozen foods into a potential source of infection [82]. Many authors have also suggested that some strains which can persist for long periods of time could be specially adapted to colonize the processing plant equipments [83]. Many researchers have also focused on the detection of *L. monocytogenes* in RTE food. The organism has been found in cabbage, celery, carrot, lettuce, cucumber, onion, cabbage, potatoes, tomato and fennel [84]. A study concluded that refrigerated RTE meats with extended shelf life are high risk products for contamination by *L. monocytogenes* [85].

Increased consumption of RTE vegetables was accounted in Brazil, but little is known about the risks associated with the consumption of these products. Although studies have reported that the occurrence of *L. monocytogenes* in RTE vegetables in several parts of the world may be as high as 25%, less studies either in Brazil or abroad have focused on the quantification of this microorganism in these products [86, 87, 88, 89, 90, 91, 92, 93, 94]. *L. monocytogenes* is now a challenge for the safety of RTE foods [95] and that RTE vegetables are consumed without a killing step before consumption, fresh produce plays an important role in listeriosis epidemiology. In the context of risk assessment, the availability of data on the prevalence, counts and growth of food-borne pathogens is of foremost importance for proper building and refining of mathematical models for estimating risks [87].

Prevalence of *Listeria monocytogenes* in RTE foods i.e., smoked fish products, cooked marinated products, meat products and pre-packaged mixed vegetable salads marked in Italy was studied by Meloni et al., and reported that 22% of the samples tested was positive for *Listeria* spp. out of which 37% was *L. monocytogenes*, 29.4% was *L. innocua*, 22.4% was *L. seeligeri*, 5.9% was *L. welshimeri*, 4.8% was *L. ivanovii* and 0.5% was *L. grayi* [96]. In a similar study by Fallah et al., found 33.3% of samples were positive for *Listeria* spp. 34.7% of raw, 33% of Ready to cook and 30.7% Ready to eat products were positive for *Listeria* spp. [97]. The presence of *Listeria* spp. in ready to eat meat and fish products from retail establishments in Metro Vancouver, Canada was studied and 10% of the samples were found positive for *Listeria* spp. *L. welshimeri* was the predominant one followed by *L. innocua* and *L. monocytogenes* [98]. *L. monocytogenes* isolated from retail foods in Florida were characterized by serotyping and concluded that *L. monocytogenes* isolates present in food were diverse with different serogroups [99]. Ready to eat packaged vegetables marketed in Sao Paulo was evaluated by Sant Ana et al., for the presence of *L. monocytogenes* and 3.1% of the samples were found to be positive for it. From the results obtained by the study they concluded that RTE vegetables may act as vehicles of *L. monocytogenes* and the isolates were resistant to disinfectants and were forming strong biofilms in the processing environments [100]. Sant Ana et al., showed that *L. monocytogenes* may proliferate depending on the storage conditions and reach high populations in RTE vegetables [101].

The prevalence of *Listeria* spp. in RTE meats varies from 1.8% to 48.0% [102, 103, 104, 105, 106, 107, 108]. There are several reports of listeriosis-associated with the consumption of RTE meats [35, 109]. Some of these epidemics have resulted in mortalities in consumers particularly among immune-compromised individuals and large-scale recalls of implicated RTE foods [110, 111, 112]. Increased cases of human listeriosis for the period of five years in Sweden paved the way for studying the prevalence of *L. monocytogenes* in three types of RTE foods in retail in Sweden in the year 2010. The results showed 74 positive samples out of 1590 samples tested. The highest prevalence was reported in fish products followed by gravad and cold smoked fish samples [113]. The special risk of listeriosis posed by consumption of RTE meats has led to studies on risk assessments of these products and recommendations by the World Health Organization [114].

**Poultry Products**

Poultry can become contaminated with *Listeria* spp. either environmentally or from healthy carrier birds during production in the farm [115]. In poultry abattoir, processing plant, improper cleaning, disinfecting of environment and equipments, mishandling of the products may lead to *Listeria* contamination of poultry carcasses and the final products [85, 116]. Contamination of RTE poultry products can occur after cooking by cross-contamination environmentally or via workers, surfaces and equipments [117]. Transmission of the resistant strains to human via contaminated food products may have public health consequences [118]. Charpentier and Courvalin
have stated that the antimicrobial resistance of Listeria spp. is due to the acquisition of mobile genetic elements such as self transferable and mobilizable plasmids and conjugative transposons. Many authors have also demonstrated a high prevalence of L. monocytogenes, and other Listeria spp. in meat and poultry product processing environments viz., in chilling and cutting rooms, workers’ hands, conveyor belt rollers, and processing equipments, strongly suggesting that the processing environment represents a significant source of these organisms in finished products. While processed meat and poultry products are cooked to destroy Listeria, these bacteria can recontaminate the product while it is being handled, packaged or distributed. Studies involving knowing transmission routes which depended solely on isolating and counting the organism at different places along the processing line, when the organism was found on any environmental surface, cleaning and sanitizing was then implemented on the contaminated surfaces. But there has been a limitation to find the real source of product contamination. As a consequence, the control and prevention strategies implemented might not correct the contamination source. Thus, recent studies have been greatly facilitated by the use of molecular typing methods with high discriminatory power, including randomly amplified polymorphic DNA (RAPD) profile analysis to trace the route of contamination. Molecular studies on the ecology of Listeria spp. strains present in the food processing environment provide crucial information for the development of better control and prevention strategies for this important food-borne pathogen.

The findings of the study by Yucel et al., in Turkey where L. monocytogenes (6.16%) was isolated in various meat products, support a study by Pesavento et al., conducted in Italy and showed lower percentage of L. monocytogenes (3.15%) from raw meat and retail food samples. A higher incidence of L. monocytogenes was found in retail minced beef (12.2%) in Japan and raw meat and meat products (14.3%) in Switzerland. The differing results of L. monocytogenes prevalence from these studies possibly showed the fact that different food samples and isolation methods were applied. Similarly, studies on foods from local markets around Malaysia (Kuala Lumpur, Melaka, Kuala Terengganu and Selangor) by Hassan et al., and Wong et al., revealed lower percentages of L. monocytogenes. Hassan et al., showed that L. monocytogenes occurred in 35.3% of tested samples constituting frozen beef (75%), local meat (30.4%) and fermented fish (12%). On the other hand, Wong et al., found that 9.30% of vegetarian burger patties sold at various Malaysian markets were contaminated with L. monocytogenes.

Milk, Milk Products and Their Processing Environments

Fleming et al., showed that an outbreak in Massachusetts suggested that Listeria monocytogenes was present in whole and 2% milk which had undergone pasteurization. A second outbreak in Los Angeles was in 1985 which pointed to the possibility of both improper manufacturing practices and post-processing contamination as sources of L. monocytogenes in a Mexican style soft-cheese. Imran et al., tried to analyse the impact of microbial population dynamics on growth of L. monocytogenes in cheese microcosm by using Livarot cheese smear design. A case of Listeriosis in Turkey which was linked to consumption of Turkey frankfurters led to the examination of the production plant. The production plant environment was found to be the source of Listeria. In this case a peeler and the conveyor leading from the peeler were found to be contaminated with L. monocytogenes.

Several outbreaks of listeriosis have also been observed which is caused by the consumption of contaminated cheeses have been reported. The use of raw milk in the manufacture of cheese raises particular safety concerns due to the possible incidence of L. monocytogenes, as well as other pathogenic bacteria. Dairy products have been implicated in several early cases of listeriosis outbreaks. Pasteurized milk, chocolate milk, Mexican-type cheese, the Vacherin Mont d’Or cheese and soft cheeses were involved in listeriosis epidemics. Multinational outbreak from dairy product was reported recently by. Further, Unnerstad et al., showed that certain strains of L. monocytogenes survive within the food processing environment. The ability of L. monocytogenes to form biofilms may contribute to its persistence in food processing plants. The milk processing environment and handling practices may vary among the processing plants. There are chances of increase in cross contamination as 47% of surface of hand of the food handlers and 16% on the processing tables were found to carry L. monocytogenes. As per requirements of the US-FDA, L. monocytogenes should be absent in RTE foods.

Incidence of Listeria spp. in icecreams sold in some retail outlets in Mumbai, India was studied by Rahul Warke et al., and reported that 53.3% and 100% of Listeria spp. incidence in packed and open icecream samples respectively. Very low level of incidence of Listeria monocytogenes was observed among the positive samples. Similar results were reported by. A study carried out by Biswas et al., and concluded that 12% and 9% of branded and non-branded ice creams were contaminated with Listeria spp. Among them 29% and 13% was detected as L. monocytogenes. Percentage of Listeria contamination was higher in branded ice cream samples than in non-branded one. The survey carried out by Kalorey et al., revealed the incidence of Listeria spp. in bovine raw milk from central India. 6.75% samples were positive for Listeria spp., among them 5.1% of L. monocytogenes, 0.1% of L. seeligeri, 0.9% of L. innocua, 0.1% of L. welshimeri were isolated.
A study carried out by Soni et al., [149] in Varanasi, India found that 5.8% of cow milk samples were positive for *L. monocytogenes* and none of the milk products i.e., cheese and ice cream were positive for *L. monocytogenes*. Similar results were reported by Dhanashree et al., [47]. Investigation carried out by Mary et al., (2013) revealed contamination of *L. monocytogenes* in milk samples used in Temples as sacred liquid in Thiruchirapalli, Tamilnadu, India [150]. In 2008 Singh et al., reported that 13 *L. monocytogenes* isolates were isolated from curd and cheese samples [151]. 2.91% *L. monocytogenes*, 2.18% *L. innocua*, 1.45% *L. welshimeri* were isolated from milk samples of cattle of Odisha [152]. *L. monocytogenes* was isolated from Paneer collected from local markets of Haryana and the enrichment procedure was found to be effective for the isolation of *L. monocytogenes* [153]. 60.6% milk and 41.7% of ice cream samples were positive for *L. monocytogenes* sampled from Thiruchirapalli, Tamilnadu, India [154]. 25 *L. monocytogenes* were isolated from 115 milk samples in Meerut; this study gives the preliminary evidence of contamination of milk by *L. monocytogenes* [155].

**Control of Listeria in Food and Food-Related Environments**

Surveillance of *Listeria* in food and food related /food processing environments should be done, as the virulent strains present in the processing environments may act as vehicle for the major outbreaks. According to CDC, approximately 12% of the reported food borne-illnesses in the United States are associated with fresh fruits and produce. CDC has proposed safety measure for preventing food-borne illnesses. Safety measures include promotion of safe handling, cooking and consumption of food. This includes washing raw vegetables and cooking raw food thoroughly [156].

FDA has proposed the food safety requirements for the fresh produce. The food which are eaten raw i.e., fruits and vegetables should be under surveillance during harvesting, packaging and holding. Sanitation of the processing environment, maintenance of sanitation during irrigation and washing of the produce, cleanliness of materials and worker hygiene are the factors which should be monitored by the food processing units [157,158].

Turgis et al., [159] evaluated the combined treatment of trans-cinnamaldehyde and gamma irradiation on *Listeria* in peeled carrots. The combined treatment gave the synergistic action against the organism and increased the radio sensitivity of the organism. Millet et al., has reported that in preparation process of Saint-Nechtaire type cheese, controlling acidification in the early stages of the process helps in controlling the development of *L. monocytogenes* [160]. The efficiency of household decontamination methods for reducing *L. monocytogenes* on salad vegetables i.e., fresh lettuce, cucumber and parsley was studied by Nastou et al., [161]. The results obtained showed that storage of products in lower temperature was effective in reducing the number of *L. monocytogenes*, this shows that storage temperature significantly affects the efficiency of dipping vegetables in water for control of *L. monocytogenes*. Another remarkable result of the study was that acetic acid concentration and the vegetable type are the important factors which affect the efficiency of acetic acid in controlling *L. monocytogenes* on lettuce, cucumber and parsley. 2.0%v/v of acetic acid was found to be the effective minimal concentration for inhibiting *L. monocytogenes* on vegetables. The efficacy of acetic acid for vegetable decontamination is limited and varies with vegetable type.

Many studies have shown that the responses of *L. monocytogenes* to stressors (stressors are chemical or biological agents or environmental condition, external stimulus or an event that causes stress to an organism) in foods and the processing environment are heterogeneous [162, 163, 164]. Sanitization of equipment, disinfection of vegetables and chilling temperature are relevant stressors during minimal processing of RTE vegetables [165]. The responses of *L. monocytogenes* to these conditions may result either in inactivation, survival and persistence in the processing environment or growth in the product during cold storage. The behaviour of *L. monocytogenes* is of great relevance for estimating the risks associated with RTE foods [166, 167, 168].

Stringent cleaning and sanitizing regimes have been implemented in many dairy processing facilities in order to reduce environmental contamination caused by *L. monocytogenes*. Ryser and Marth [169] showed the efficacy of various sanitizing agents by eliminating *Listeria*. It has been investigated by numerous researchers. It is reported that the use of chlorine-based sanitizer at 100 ppm, iodine-based sanitizer at 25-45 ppm, acid anionic-based sanitizer at 200 ppm and quaternary ammonium-type sanitizer at 100-200 ppm were effective in eliminating *L. monocytogenes*.

Geornaras et al., [170] carried out a study evaluating the post-processing application of chemical solutions for their antilisterial effects on smoked sausage formulated with or without potassium lactate and sodium diacetate. Solutions of acetic acid (2.5%), lactic acid (2.5%), potassium benzoate (5%) or nisinapin were studied for the antilisterial activity. Post processing antimicrobial treatments which contained nisinapin showed substantial bactericidal effects on *L. monocytogenes*. Washing with chlorinated water, application of heat was found to be effective measures for the elimination of *L. monocytogenes* in sea food [171]. A study carried out by Wan Norhana et al., [172] showed the effect of heat, chlorine and acids on the survival of *Listeria* on uncooked shrimp carapace and cooked shrimp flesh. The organism was treated with high and low temperatures, 100 ppm of sodium hypochlorite solution, acetic acid, hydrochloric acid and lactic acids with pH 4. Attached and colonized cells showed higher
resistance to all the treatments. Different ClO₂ gas concentrations was used to study the effectiveness of Chlorine dioxide gas (ClO₂) on Listeria on food contact and environmental surfaces by Trinetta et al.,[173] and reported that L. monocytogenes biofilm cells showed more sensitivity compared to planktonic forms. The treatment with 2mg/l for 30 min was found to be the effective concentration. The study concluded that ClO₂ can act as an effective sanitizer in the processing environment.

CONCLUSION

The study of incidence of Listeria spp. in food and their processing units provide information about the contamination status of the fish, chicken, meat, milk and RTE products. The presence of Listeria spp. particularly L. monocytogenes in RTE products and uncooked products could be a potential risk for consumers. Also, the resistance of the Listeria spp. to commonly used antimicrobials is alarming and constitutes a serious hazard for public health[197]. Further studies on the occurrence of Listeria spp. in various food products should be carried out to establish the microbial criteria of foods in many parts of the Country. Strategies to reduce the occurrence of the organism in the food and food processing environment are required to overcome the contamination of the products and to assure the safety of the product.

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