

Prevalence and Control of *Listeria monocytogenes* in the Food Industry – a Review

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Abstract

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Listeria monocytogenes is a Gram-positive facultative intracellular organism and causative agent of the severe food-borne infection listeriosis. *L. monocytogenes* is more likely to cause death rather than other pathogenic bacteria that cause foodborne illnesses. It is an ubiquitous organism that can be found in food industry equipment and premises. *L. monocytogenes* mainly occurs in the food production chain by cross-contamination, making this pathogen a major threat to the food industry. The pathogen may be found at low or moderate levels in the foodstuffs, but the levels involved in listeriosis outbreaks are relatively very high. The majority of isolates from food products belong to serotype 4b and 1/2a. The control of *L. monocytogenes* can be applied throughout the food chain. Pre- and post-harvest factors such as contact of pigs with pets and pest animals, large group size, hygiene practices, and treatment of manure affected the prevalence of *L. monocytogenes* in the food chain. Good farm-level practices could be utilised to reduce the occurrence of *L. monocytogenes* in the farm environment and possibly further in the food chain. Safety and low level of this pathogen in the food chain can be possible with good agricultural practices, good manufacturing practices, and high-quality raw materials. Therefore, food processing plants must be designed carefully with an emphasis on effective cleaning and disinfecting operations in the production line.

Keywords: Decontamination techniques; foodborne illness; food contamination; foodborne pathogen; outbreaks; risk factors; transmission

From the community's health point of view and in terms of disease burden in various countries *L. monocytogenes* is amongst the supreme bacterial pathogens (TODD 1996; MEAD *et al.* 1999; ADAK *et al.* 2002). Food Standards Agency (FSA) identified that *L. monocytogenes* ranked highest for the cause of deaths in England and Wales and unlike most other common foodborne infections, the mortality rate of listeriosis was found out to be 20–30% (SCHLECH *et al.* 1983; FLEMING *et al.* 1985; TODD 1996; LYYTIKÄINEN *et al.* 2000; ADAK *et al.* 2002, FSA 2015), which highlights and emphasises that its epidemiological scrutiny is a prerequisite.

L. monocytogenes can result in both invasive and non-invasive infections. Being protruding and invasive, it crosses the intestinal mucosal barrier, reaches up to underlying tissues, and consequently within the cytoplasm of the host's cell it infects, multiplies, and spreads in adjacent cells without extracellular space coverage, therefore giving a run around to the immune defences (LUKINMAA 2003; HEIMAN *et al.* 2015). Invasive listeriosis is a rare but severe disease, principally showing an association with a definite risk group of people and the fatality proportion is high, conversely fairly trivial non-invasive infections can also occur in healthy people (CRUM 2002).

Characteristically, about 90% of listeriosis occurs in persons with a predisposing condition or diseased state, such as pregnancy, neo-natality, malignancy, transplantation, alcoholism, immunosuppressive therapy, diabetes, old age, and HIV, thus leading to central nervous system (CNS) infections, premature abortions, septicaemia, neonatal infections, and still births (SCHUCHAT *et al.* 1992; CRUM 2002). Initial clinical symptoms associated with listeriosis are muscle aches with fever and gastrointestinal indications like nausea or diarrhoea while more severe syndromes accompanying the infection are shown in Table 1. In the United States about 1600 people get listeriosis each year.

It is speculated that *L. monocytogenes* is primarily transmitted through the consumption of contaminated foods including processed unpasteurised milk,

Table 1. Syndromes accompanying *L. monocytogenes* severe infection

Diseases	References
Meningitis	PAGLIANO <i>et al.</i> (2015)
Meningoencephalitis	MURAKAMI <i>et al.</i> (2015)
Brain abscess	LIMMAHAKHUN & CHAYAKULKEEREE (2013)
Vertebral osteomyelitis	KHAN <i>et al.</i> (2001)
Epidural abscess	KHAN <i>et al.</i> (2001)
Endocarditis	SUMMA & WALKER (2010)
Hepatitis	WARNER <i>et al.</i> (2012)
Necrotising typhlocolitis	WARNER <i>et al.</i> (2012)
Rhombencephalitis	CARRILLO-ESPER <i>et al.</i> (2013)
Bacteremia	HUANG <i>et al.</i> (2010)
Native or prosthetic valve endocarditis	SUMMA & WALKER (2010)
Arterial infections	CONE <i>et al.</i> (2008)
Spontaneous bacterial peritonitis	TABLANG (2008)
Pneumonia	KOUFAKIS <i>et al.</i> (2015)
Self-limited febrile gastroenteritis	ASAHATA <i>et al.</i> (2015)
Fournier's gangrene	ASAHATA <i>et al.</i> (2015)
Septic/infectious arthritis	DEL POZO <i>et al.</i> (2013)
Endophthalmitis	SHOUGHY & TABBARA (2014)
Conjunctivitis	SHOUGHY & TABBARA (2014)
Chorioretinitis	SHOUGHY & TABBARA (2014)
Keratitis	SHOUGHY & TABBARA (2014)
Sclerokeratitis	SHOUGHY & TABBARA (2014)
Skin infection	LAMBOTTE <i>et al.</i> (2005)
Cholecystitis	BRUMINHENT <i>et al.</i> (2013)

meats, and soft cheeses (ASAHATA *et al.* 2015; KHAN *et al.* 2016a). It is the 3rd leading cause of death from food poisoning. The incidence of listeria outbreaks is highly related to food and its type. In Japan, people consume ready-to-eat (RTE) seafood, including sashimi and sushi. Examination of RTE seafood in Japan revealed that raw minced tuna, which is a common appetiser in Japan, and fish roe products are frequently contaminated with *L. monocytogenes* (5.7–12.1% of the time) (MIYA *et al.* 2010). The United States Food and Drug Administration (USFDA) has established preventive regulatory guidelines for raw seafood, such as RTE foods (processed delicatessen meats, meat spreads, soft cheeses, cooked cold chicken, smoked seafood, and pre-prepared salads) that can support *L. monocytogenes* growth (FDA 2008), and these guidelines have been effective in reducing the incidence of *L. monocytogenes* infection (TAPPERO *et al.* 1995).

Furthermore, not all the strains of *L. monocytogenes* are identical and there exists a prominent variation in adaptation to environments, virulence and resistance to adverse conditions. Vital steps in minimising listeriosis include preventing contamination and controlling the incidence of the pathogen in food chain and foodstuffs. Adopting technologies, agents, and new practices for controlling *L. monocytogenes* are established and their effects on the pathogen and foodstuffs have to be studied carefully. Eradication of the pathogen from the food chain is barely possible, but contamination needs to be decreased and a high level of growth prevented.

Therefore, the purpose of this review article is to highlight the current status of incidence, contamination routes of *L. monocytogenes* in various food products and processing environments, and highlight the future trends.

Human cases of listeriosis. Centers for Disease Control & Prevention (CDC) reported that *L. monocytogenes* is responsible for approximately 1600 confirmed listeriosis cases, resulting in 60 deaths every year in the United States alone (SCALLAN *et al.* 2011). The European Food Safety Authority (EFSA) also reported a total of 1763 human confirmed cases and 191 deaths due to listeriosis. The highest number of deaths was observed in France with 64 cases in 2013 (EFSA 2014). The incidence of *L. monocytogenes* infections is much higher in Western Europe and North America than in Japan. It was estimated that in 2006 the incidence of *L. monocytogenes* infections was 0.65 cases per 1 million inhabitants in

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Japan as compared to the US and Europe with 2.9 and 6.3 cases per 1 million inhabitants, respectively (ASAHATA *et al.* 2015). However, the average annual incidence of human listeriosis in the US was 0.26 cases per 100 000 individuals in 2013 (CRIM *et al.* 2014). About 42% decline was observed by 2012 in the incidence of listeriosis when compared to 1996–1998. However, there was no observed change in the incidence of listeriosis in 2012 compared to 2006–2008 (FRETZ *et al.* 2010).

Foodborne listeriosis occurs rarely but it is usually associated with a high mortality rate. Detailed description of several worldwide listeriosis outbreaks have been reported and serotype 4b is known to be the most common cause of this foodborne disease; however, serotype 1/2a is known to be the cause of sporadic cases (GERNER-SMIDT *et al.* 1995; SWAMINATHAN *et al.* 2001; PAN *et al.* 2009). A total of 2161 human cases of listeriosis were reported in 2014 (EFSA 2014). According to the report, the EU notification rate was 0.52 cases per 0.1 million. There has been a steady and significantly increasing trend in listeriosis in the EU/EEA since 2008. In 2012, there were observed 4 confirmed outbreaks in the US. The largest listeriosis outbreak in the US history occurred in 2011, when 147 illnesses, 33 deaths, and 1 miscarriage occurred among residents of 28 states; the outbreak was linked with the consumption of cantaloupe which was brought from one single farm. Detailed information on *L. monocytogenes* outbreaks from 2010 to the present in the US, Europe, and Australia is illustrated in Table 2.

Transmission/contamination. Historically, *L. monocytogenes* was not strictly recognised till the 1980's as involved in causing foodborne illness. In the 1920's, it was referred to as a human pathogen first associated with infection in farmers and veterinarians who got the disease straight from the farm animals (HELLSTRÖM 2011). *Listeria* infections have been reported in many domestic animals, yet ruminants are most commonly susceptible to animal listeriosis (LOURIA *et al.* 1967). Prolonged excretion of *L. monocytogenes* in milk by not only diseased but also healthy animals was described (WAGNER *et al.* 2000). The foodborne illness is more or less specifically associated with numerous groups of contaminated foods including cheeses, dairy products, beef, and pork (AMAGLIANI *et al.* 2007). Sea food is also considered as a prospective source of the pathogenic bacterium *L. monocytogenes* (CARTER 2005). It has been observed that the application of crude and unprocessed manure onto

pastures has resulted in the contamination of soil and water which consequently transmit the species to fruits and vegetables (MANDAL *et al.* 2011).

There are various sources and routes through which *L. monocytogenes* contamination and transmission may occur. The common routes are given below.

Food and food processing plants. *L. monocytogenes* is a ubiquitous organism that is widely distributed in the environment and can contaminate food through contact with contaminated surfaces. This bacterium has often been found in food processing plants. Thus constituting specific niches from which the pathogen can be spread to food contact surfaces and food products (TOMPKIN 2002). MARTÍN *et al.* (2014) identified a number of factors involved in the adaptation of *L. monocytogenes* in meat processing plants such as strain modification (specific genetic and physiological traits), biofilm formation, and an inefficient cleaning and disinfection procedure. A previous study reported the role of the integrated *comK* prophage in rapid adaptation to specific niches, biofilm formation, and *L. monocytogenes* persistence in food processing plants (VERGHESE *et al.* 2011). However, some authors reported a moderate level of *L. monocytogenes* contamination after cleaning and disinfecting the plants surfaces (CHASSEIGNAUX *et al.* 2001; LOPEZ *et al.* 2008). Studies showed that some strains were able to overcome the different hurdles of meat product processing and were well adapted to the environmental conditions of the plants. The ability to form biofilms could contribute to strain adaptation, persistence, and resistance to sanitisers (MELONI *et al.* 2012; MARTÍN *et al.* 2014). Most likely, biofilm formation occurs in sites within the manufacturing environment that are hard to reach and sanitise and that accumulate food residues and water for long time periods (CHMIELEWSKI & FRANK 2003; VERGHESE *et al.* 2011). Cross contamination may occur when food passes over contaminated surfaces or via exposure to condensate, or aerosols that originate from contaminated surfaces (CHMIELEWSKI & FRANK 2003). The irreversible attachment of *L. monocytogenes* depends upon the strain and contact time between the cell and the substrate in the food processing plants. LUNDÉN *et al.* (2000) reported that the most prevalent serotype of *L. monocytogenes* (serotype 1/2c) found in food processing plants had good adhesion ability and required only a short contact time for attachment. *L. monocytogenes* strains have been isolated from various environmental surfaces such as conveyor floor drains, belts, storage tanks, condensate, and hand

Table 2. *L. monocytogenes* outbreaks since 2010 – present in USA, Europe, and Australia

Country	No of cases	Serotype	Vehicle of infection	Number of deaths	Date	Reference
Austria, Germany, Czech Republic	34	1/2a	quargel cheese	8	2009–2010	FRETZ <i>et al.</i> (2010)
Australia (Victoria, Queensland, New South Wales)	9	NA	rockmelons	2	2010	POPOVIC <i>et al.</i> (2014)
Australia (Victoria)	6	NA	–	4	2010	POPOVIC <i>et al.</i> (2014)
USA (Louisiana)	10	1/2a	hog head cheese	2	January–June 2010	CDC (2015d)
USA (Texas)	10	ND	vegetable, celery poultry, chicken salad	5	January 2010	CDC (2015g)
USA (Oregon)	5	ND	mexican style cheese	0	February 2010	CDC (2015h)
USA (Multistate)	6	NA	mexican style cheese	1	March 2010	CDC (2015a)
Canada (Ontario)	2	NA	meat and sausage, salame, cooked ham, cotto ham	0	March 8–22, 2010	CDC (2015e)
USA (Washington)	2	NA	sushi	0	June 2010	CDC (2015a)
USA (Washington)	4	NA	–	0	December 2010	CDC (2015a)
USA (Multistate)	147	NA	cantaloupe	33	July 2011	CDC (2015b)
USA (New Jersey)	2	NA	mexican style cheese	0	July 2011	CDC (2015a)
	2	NA	ackawi cheese	1	August 2011	CDC (2015a)
	2	NA	–	0	September 2011	CDC (2015a)
USA (Multistate)	15	NA	blue-veined cheese	1	October 2011	CDC (2015a)
USA (Multistate)	23	NA	ricotta salata cheese	5	March 2012	CDC (2015a)
USA (Massachusetts)	3	NA	–	0	April 2012	CDC (2015a)
USA (Massachusetts)	3	NA	–	0	August 2012	CDC (2015a)
USA (New York)	7	NA	–	0	October 2012	CDC (2015a)
Australia (Victoria)	18	NA	soft cheese	2	November 2012	CDC (2015f)
Spain (Gipuzkoa)	27	1/2b, 4b	foie gras	6	January 2013 to February 2014	PÉREZ-TRALLERO <i>et al.</i> (2014)
USA (Multistate)	6	ND	cheese-le frere	1	May 2013	CDC (2015a)
USA (Multistate)	5	ND	–	1	July 2013	CDC (2015a)
USA (Multistate)	8	ND	latin style soft cheese	1	August 2013	CDC (2015a)
USA (Rhode Island)	4	ND	–	2	September 2013	CDC (2015a)
USA (Multistate)	9	ND	mexican style cheese	1	September 2013	CDC (2015a)
USA (Massachusetts)	2	ND	–	0	December 2013	CDC (2015a)
USA (California Maryland)	8	ND	roos foods	1	February 18 to March 4, 2014	CDC (2015c)
USA (Illinois, Michigan)	5	ND	been sprout	2	June–August 2014	CDC (2015c)
USA (Georgia, New York, Tennessee, Texas)	5	ND	hispanic-style soft cheeses	1	June–October 2014	CDC (2015c)
USA (Multistate)	32	ND	caramel apples	0	December 30, 2014	CDC (2015c)
USA (Arizona, Kansas, Oklahoma, Texas)	10	ND	blue bell creameries	3	January 2015	CDC (2015c)

NA – not available; ND – not detectable

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trucks. These are all surfaces on which the biofilm is expected to be formed. Most likely, the growth of *L. monocytogenes* strains in the food processing plant and their biofilm formation increases the general contamination level in the food processing plant. Such contamination is the direct indication of unsatisfactory decontamination procedures which ultimately put exposed food products at risk.

Environmental sources. *L. monocytogenes* is ubiquitous and mostly found in soil, water, and feed as shown in Table 3. However, their populations are low in number. In addition, organic fertilisers of animal origin may be the main route of soil contamination promoting the growth of *L. monocytogenes*. The incidence of *L. monocytogenes* in surface water seems to be linked to direct upstream land use, most specifically, crop land, silage factory, and proximity to dairy farms. Silage is the most common source of *L. monocytogenes* contamination (GISMERVIK *et al.* 2015). Many wild and domestic animals harbour *L. monocytogenes* in their intestines. The farm environment is frequently contaminated with *L. monocytogenes*, and especially ruminant farms may represent an important natural reservoir. Faecal carriage in livestock animals such as goats, cattle, and sheep etc. of *L. monocytogenes* has been widely reported. The prevalence of the pathogen in wild animals ranged from 1% to 60%. However, in Japan, less than 1% of prevalence of *L. monocytogenes* was found in wild animals (LYAUTEY *et al.* 2007a). *L. monocytogenes* has been found in many types of foods, but their numbers are usually low and below the detection limit. The prevalence of *L. monocytogenes* is often high in food products that are minimally processed or the capability of contamination after thermal treatment. As shown in Table 2, the majority of outbreaks are linked to refrigerated and RTE foods that are consumed without proper heating. Generally, *L. monocytogenes* gets more opportunities to reside in foods because of the increased demand and availability of RTE foods and extended shelf life.

Prevention of contamination in food

Pasteurisation/cooking. Pasteurisation as a conventional heating process to eliminate spoilage and pathogenic bacteria from the food products is still a common technique nowadays. Pasteurising temperature ranges from 60°C to 80°C, below the boiling point of water (SILVA & GIBBS 2012). Cooking is

another effective method to control *L. monocytogenes* in foods. However, listericidal effectiveness of the thermal process may not be suitable for all the foods, as these processes bring huge effects on nutritional and sensory characteristics of foods. Many hurdle technologies, including thermal and non-thermal technologies, have been extensively studied to eliminate or reduce the population of *L. monocytogenes* in foods (RAJKOVIC *et al.* 2010). Different technologies and their effect on *L. monocytogenes* in food products are illustrated in Table 4.

Chemical sanitisers. Many chemical sanitisers have been evaluated to control the organism (KHAN & OH 2016; KHAN *et al.* 2016b). Organic acids such as acetic acid, fumaric acid, benzoic acid are food grade substances and have been used as food preservatives in the food industry since its development. European Commission, FAO/WHO, and FDA permitted the use of these organic acids as food preservatives and categorised them as generally recognised as safe (GRAS) (SUREKHA & REDDY 2000; AL-JUHNI & NEWBY 2006; MOLATOVÁ *et al.* 2010; GONZALEZ-FANDOS & HERRERA 2014). All the organic acids showed strong antimicrobial activity against *L. monocytogenes* in laboratory media, in sheep and beef (VERMEULEN *et al.* 2007; MOLATOVÁ *et al.* 2010; TAKALA *et al.* 2011; HE *et al.* 2013; GONZALEZ-FANDOS & HERRERA 2014).

Inorganic acids such as sulphite and nitrite have shown excellent antimicrobial activity against *L. monocytogenes* (BRANDT *et al.* 2011). BRANDT *et al.* (2011) found significant inhibition of *L. monocytogenes* after combined treatment with acidic calcium sulphate and octanoic acid. Moreover, the effect of sodium nitrite with high hydrostatic pressure against *E. coli* BW25113 and *L. monocytogenes* NCTC 11994 showed a synergistic reduction of bacterial count at pH 4.0 (ALBA *et al.* 2013).

Novel decontamination techniques. Food irradiation processing technology, gamma irradiation is highly used as a safe and proven method worldwide for food product preservation. This technology has been evaluated against *L. monocytogenes* in food products to control *L. monocytogenes* (LACROIX & QUATTARA 2000). Irradiation techniques exploit different sources of irradiation production including X-ray machines, gamma rays (γ -rays), and electron accelerators for various food products (SOLANKI *et al.* 2012). Recently, HUQ *et al.* (2015) found a synergetic inhibitory mechanism when γ irradiation in combination with cinnamon essential oil, oregano essential oil, and nisin was applied against *L. monocytogenes* in ready-to-eat ham. The shelf life was extended up

Table 3. Prevalence of *L. monocytogenes* in different areas

Sampling site	Total samples	Positive samples (%)	Prevalence (%)	Reference
Water				
River	36	17	47	FENLON <i>et al.</i> (1996)
	11	3	27.2	MAWAK <i>et al.</i> (2009)
	150	20	7.5	NASSIRABADY <i>et al.</i> (2015)
Stream	15	5	33.3	MAWAK <i>et al.</i> (2009)
Ponds	4	2	50	MAWAK <i>et al.</i> (2009)
Estuary	10	0	0	BERNAGOZZI <i>et al.</i> (1994)
Ground	15	1	5	RENTERGHM <i>et al.</i> (1991)
Surface	314	32	10	LYAUTEY <i>et al.</i> (2007b)
	126	31	24.6	LYAUTEY <i>et al.</i> (2012)
Water (sheep and cattle)	132	1	0.8	ATIL <i>et al.</i> (2011)
Drinking water (troughs, water buckets in barn)	508	100	20	NIGHTINGALE <i>et al.</i> (2004)
Sewage				
Untreated	12	12	100	BERNAGOZZI <i>et al.</i> (1994)
Treated	12	10	83	BERNAGOZZI <i>et al.</i> (1994)
Sewage	136	20	14.7	MACGOWAN <i>et al.</i> (1994)
Soil				
Cultivated	13	1	8	DOWE <i>et al.</i> (1997)
Uncultivated	13	6	31	DOWE <i>et al.</i> (1997)
Garden	136	1	1	MACGOWAN <i>et al.</i> (1994)
Farmyard	36	3	8	GARCIA <i>et al.</i> (1996)
Farm soil	200	10	5	SONI <i>et al.</i> (2014)
Feed				
Feed (sheep and cattle)	132	3	2.3	ATIL <i>et al.</i> (2011)
Environment (sheep and cattle)	132	3	2.3	ATIL <i>et al.</i> (2011)
Pasture grass	68	26	38	HUSU <i>et al.</i> (1990)
Silage	74	11	14.8	OLIVEIRA <i>et al.</i> (2008)
	39	24	62	FENLON <i>et al.</i> (1996)
Feedstuff (silage, haylage, corn)	516	87	17	NIGHTINGALE <i>et al.</i> (2004)
Surfaces				
Open fish market (knives, worker hands, work surfaces)	374	10	2.6	JAMALI <i>et al.</i> (2015)
Environment (wild, farm, vegetation)	107	12	11.2	GELBÍČOVÁ & KARPÍŠKOVÁ (2012)
Defeathering (non-food contact surfaces)	4	1	25	CHIARINI <i>et al.</i> (2009)
Evisceration (food contact surfaces)	95	12	7.9	CHIARINI <i>et al.</i> (2009)
Evisceration (non-food contact surfaces)	11	5	45.4	CHIARINI <i>et al.</i> (2009)
Cutting room (non-food contact surfaces)	33	14	42.2	CHIARINI <i>et al.</i> (2009)
Cutting room (food contact surfaces)	164	35	21.3	CHIARINI <i>et al.</i> (2009)
Freezing room (non-food contact surfaces)	7	4	57.1	CHIARINI <i>et al.</i> (2009)
Sausage processing plant (equipment and environment)	43	9	20.9	VON LAER <i>et al.</i> (2009)
Sausage processing plant (worker hands)	5	1	20	VON LAER <i>et al.</i> (2009)
Sausage processing plant (packing)	5	1	20	VON LAER <i>et al.</i> (2009)
Sausage processing plant (final product)	5	5	100	VON LAER <i>et al.</i> (2009)
Ready-to-eat (RTE) foods				
Foods	396	45	11.4	JAMALI <i>et al.</i> (2013)

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Table 3 to be continued

Sampling site	Total samples	Positive samples (%)	Prevalence (%)	Reference
Foods	109	3	3	WAGNER <i>et al.</i> (2007)
Meals	3830	411	11	NØRRUNG <i>et al.</i> (1999)
Foods	100	4	4	TERZI <i>et al.</i> (2015)
Dairy products				
Milk	317	15	4.7	KARTHIKEYAN <i>et al.</i> (2015)
Raw milk	105	19	18.1	KRAMARENKO <i>et al.</i> (2013)
Raw dairy silo milk	295	58	20	WAAK <i>et al.</i> (2002)
Raw farm tank milk	1459	25	2	MEYER-BROSETA <i>et al.</i> (2003)
Cheese or cheese products	73	14	19	NØRRUNG <i>et al.</i> (1999)
Butter	3229	13	0.4	LEWIS <i>et al.</i> (2006)
RTE milk products	4901	13	0.3	KRAMARENKO <i>et al.</i> (2013)
Meat and meat products				
Meat (raw and processed)	270	65	24.4	AL-NABULSI <i>et al.</i> (2015)
Fresh meat	19	123	15.4	CHEN <i>et al.</i> (2015)
RTE meat products	6746	135	2.0	KRAMARENKO <i>et al.</i> (2013)
Raw meat	343	106	310	NØRRUNG <i>et al.</i> (1999)
Meat pie	6	1	17	AISHA & KAWO (2015)
Raw pork meat	121	41	34	THÉVENOT <i>et al.</i> (2005)
Raw poultry meat	61	38	61	MIETTINEN <i>et al.</i> (2001)
Smoked meat sausage	48	28	58	FELÍCIO <i>et al.</i> (2007)
Pork	76	16	21.1	KRAMARENKO <i>et al.</i> (2013)
Sausages	1392	5	0.4	KRAMARENKO <i>et al.</i> (2013)
Smoked meat sausages	761	1	0.1	KRAMARENKO <i>et al.</i> (2013)
Smoked meat products	1154	25	2.2	KRAMARENKO <i>et al.</i> (2013)
Sea foods				
Fish	12	154	7.8	THÉVENOT <i>et al.</i> (2005)
Frozen raw fish	219	25	11.8	ABDELLRAZEQ <i>et al.</i> (2014)
Aquatic food (fresh fish)	300	23	10.4	MOMTAZ & YADOLLAHI (2013)
Aquatic food (shrimp)	300	1	2.5	MOMTAZ & YADOLLAHI (2013)
Cold-smoked fish products	70	23	32.9	KRAMARENKO <i>et al.</i> (2013)
Cold-treated fish products*	50	6	12	KRAMARENKO <i>et al.</i> (2013)
Salted fish products	391	38	9.7	KRAMARENKO <i>et al.</i> (2013)
Vegetables and fruits				
Vegetables	72	2	2.8	CHEN <i>et al.</i> (2015)
Vegetables	200	20	10	SONI <i>et al.</i> (2014)
RTE salad vegetables	2950	88	3	SAGOO <i>et al.</i> (2003)
Fruit and vegetable based products	717	15	2.1	KRAMARENKO <i>et al.</i> (2013)
Lettuce	6	3	50	AISHA & KAWO (2015)
Balangu	6	2	33	AISHA & KAWO (2015)
Others				
Ice cream (non-branded)	90	11	13	BISWAS & CHANDRA (2011)
Pastry products	663	15	2.3	KRAMARENKO <i>et al.</i> (2013)
Ice cream (branded)	60	17	29	BISWAS & CHANDRA (2011)

*intended to cook after defreezing

to 28 days and the bacterial count was lower than the detection level. However, this treatment is not generally accepted by EU inhabitants.

High pressure processing (HPP) techniques play a vital role in food decontamination. The effectiveness of HPP was reported to be high against Gram-negative bacteria compared to Gram-positive bacteria because of the difference in their membrane composition (SHIGEHISA *et al.* 1991). The effect of HPP on *L. monocytogenes* depends on the pressure applied. When pressures of 300, 500, and 700 MPa were applied against *L. monocytogenes* for up to 9 min, *L. monocytogenes* populations decreased by 1, >3, and >5 logs, respectively. However, pressurisation at 700 MPa showed the fastest inactivation of *L. monocytogenes*, which was reduced from 10^8 to 10^2 CFU/package during the come-up time (LUCORE *et al.* 2000). The mechanism of HPP action is well documented. The effects are very prominent and clear such as cell membrane and cell entity disruption including enzymes and genetic materials (MALONE *et al.* 2002). The demand for safer and RTE foods by consumers, the numbers of HPP techniques have risen exponentially. HPP was commercially used by one industry in 2009, however, by 2009 the numbers increased to 128 (CAMPUS 2010; HEINZ & BUCKOW 2010).

Ozone is considered as a GRAS non-thermal technique highly used in the food industry to destroy bacteria and extend the shelf life of raw food products and minimally processed vegetables and fruits (CONCHA-MEYER *et al.* 2015). Ozone has the advantage of not leaving any residue decomposition into oxygen (WHITE 1992). The efficacy of ozone in combination with heat to control *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 in the apple juice has recently been reported (SUNG *et al.* 2014). The efficacy of aqueous ozone treatment in eliminated *L. monocytogenes* on inoculated lucerne seeds and sprouts has been evaluated. The results showed that ozonated water at the initial ozone concentration of 21.3 ± 0.2 µg/ml for 20 min significantly reduced the population by 1.48 log₁₀ CFU/g. While the treatment of lucerne sprouts for 2 min with water containing 5.0 ± 0.5 , 9.0 ± 0.5 , or 23.2 ± 1.6 µg/ml of ozone resulted in significant reductions of 0.78, 0.81, and 0.91 log₁₀ CFU/g, respectively, compared to populations detected on sprouts treated with water (WADE *et al.* 2003).

Pulsed electric field (PEF) is an alternative non-thermal pasteurisation method used for food product preservation in the food industry for a very long time (YEOM *et al.* 2000). PEF can inactivate the pathogen

using high electric field pulses (>~18 kV/cm) for a short time. The PEF inactivation technique consists of a pulse generator, fluid handling system, treatment chamber, and monitoring systems. The PEF treatment chamber holds two parallel insulated electrodes and food products for the high voltage treatment (MIN *et al.* 2007). SALDAÑA *et al.* (2010) studied the effect of PEF on the inactivation of *L. monocytogenes* STCC 5672 and *S. aureus* STCC 4459 in McIlvaine buffer at different pH. The highest inactivation levels achieved were 3.3 and 6.1 log₁₀ cycles for *L. monocytogenes* and *S. aureus*, respectively, at pH 3.5 after 500 µs of 35 kV/cm (SALDAÑA *et al.* 2010). Similar results were obtained in pasteurised whole, 2%, and skim milk inoculated with *Listeria monocytogenes* Scott A. After treatment with high-voltage PEF, 1–3 log₁₀ cycle reductions of *L. monocytogenes* were observed irrespective of the milk used (REINA *et al.* 1998). In addition, the target organism can be inactivated using PEF facilities for liquid food preservation causing less damage to the sensory and nutritional properties (MATTAR *et al.* 2015).

Ohmic heating is one of the sterilisation techniques practiced in food science since 1980. It is also known as Joule heating, electroheating, and electroconductive heating (GOMATHY *et al.* 2015). Ohmic heating has shown several potential applications in dehydration, blanching, extraction, evaporation, pasteurisation, fermentation, and sterilisation (KNIRSCH *et al.* 2010a, b). LEE *et al.* (2012) studied the effect of ohmic heating on *L. monocytogenes* and other foodborne pathogens in orange and tomato juice. In tomato and orange juices, treatment with 25 and 40 V/cm for 30 and 60 s, respectively, was sufficient to achieve a 5-log reduction in *L. monocytogenes* (LEE *et al.* 2012). However, the inactivation of pathogens depends on applied electric field strength, electrical conductivity, and treatment time. Thus, continuous ohmic heating could effectively control foodborne pathogens in the fruit juice industry over conventional heating methods.

Electrolysed water (EW) as a sanitiser has been studied for the last three decades in the food industry (RAHMAN *et al.* 2010a, 2011). Electrolysis of water produces two different forms of water, i.e. reduced or alkaline water (high pH) and acidic or oxidised water (low pH) (RAHMAN *et al.* 2016). The efficacy of EW alone or in combination with other antimicrobial agents against *L. monocytogenes* and other pathogens has been evaluated (Table 5). MANSUR *et al.* (2015) used fumaric acid (0.5%) and strongly acidic EW against *E. coli* O157:H7,

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Table 4. Effectiveness of various agents/techniques against *L. monocytogenes* in different areas

Agent, technology used	Procedure	Time (min)	Reduction (log CFU)	Temperature (°C)	Suspension, food product	Reference
Ohmic heating	range of 25–40 V/cm	0.5–1.5	> 5/ml	22	orange and tomato juice	LEE <i>et al.</i> (2012)
	range of 10–20 V/cm	1.5–2	> 5/ml	22		SAGONG <i>et al.</i> (2011)
Pulsed electric field	frequency 1700 Hz, pulses 1.5 µs, flow rate 7 ml/s	–	> 4/ml	50	milk	REINA <i>et al.</i> (1998)
	28 kV/cm for 400 mus, pH 3.5	–	6/ml	35	suspension	GÓMEZ <i>et al.</i> (2005)
	20 kV/cm, 10 pulses/min, pulse width 3.25 µs	–	4.5/ml	55		FLEISCHMAN <i>et al.</i> (2004)
	25 kV/cm, pulses width 800 µs, frequency 1 Hz, pH 3.8	–	5.09/ml	35		ÁLVAREZ <i>et al.</i> (2002)
Ozonation	33 mg/min	9	6.3/25 g	NA	chicken (25 g)	MUTHUKUMAR & MUTHUCHAMY (2013)
	0.2 ppm	14	> 5/ml	24	suspension	FISHER <i>et al.</i> (2000)
	5 ppm	5	0.94/g	22	lettuce	YUK <i>et al.</i> (2006)
	0.098 mg/min/ml	5–9	5/ml	22	orange juice	PATIL <i>et al.</i> (2010)
HPP	400	0.01	1.1–3 /ml	25	suspension	DUTILLY (2011)
	345 MPa	10	3.05	25		ALPAS <i>et al.</i> (2000)
	276 MPa	10	8.08	50		ALPAS <i>et al.</i> (2000)
	345 MPa	5	2.64	25		ALPAS <i>et al.</i> (2000)
	600 MPa	2	3.3	20	cooked chicken	PATTERSON <i>et al.</i> (2011)
	400 MPa, pH 4.32	0.4	4/g	18	RTE salad	MARCOS <i>et al.</i> (2008)
HPP + Alginate film	400 MPa, alginate films containing 2000 AU/cm ² of enterocins	10	0.6/g	6	cooked ham	MARCOS <i>et al.</i> (2008)
	500 MPa	1	1.4/g	18	cooked chicken	STRATAKOS <i>et al.</i> (2015)
HPP + <i>Lactobacillus casei</i>	350 MPa, Lb. cell extract 32 CEAU/ml	1–20	> 5/ml	25	suspension	STRATAKOS <i>et al.</i> (2015)
	500 MPa, Lb. cell extract 100 CEAU/g	1	> 5/g	25	meat	STRATAKOS <i>et al.</i> (2015)
HPP + Nisin + <i>tert</i> -butylhydroquinone	600 MPa, TBHQ 300 ppm, nisin 200 IU/ml	5	ND	28	sausage	CHUNG <i>et al.</i> (2005)
	700 MPa	1.8	> 5/g	22	frankfurters	LUCORE <i>et al.</i> (2000)
Bacteriophage P1003	–	–	3.5–5.4/cm ²	22	stainless steel coupon surface	SONI & NANNAPANENI (2010b)
<i>Listeria</i> bacteriophages + bacteriocin coagulin C23	phage FWLLm1 at 5 × 10 ⁶ PFU/ml, FWLLm3 at 5 × 10 ⁵ PFU/ml, coagulin C23 at 584 AU/ml	–	ND	4	milk	RODRÍGUEZ-RUBIO <i>et al.</i> (2015)
Bacteriophage Listex P100	10 ¹¹ PFU/ml	–	8.0/ml	10	melon juice	SONI & NANNAPANENI (2010a)
		–	2.1/ml	10	pear juice	

ND – not detectable; NA – not available; PFU – plaque forming units; AU – arbitrary unit that means the highest dilution showing growth inhibition of the indicator lawn; CEAU/ml – colicin-equivalent activity units/ml

L. monocytogenes, *S. aureus*, and *S. typhimurium*. They found a strong antimicrobial activity against targeted organisms in beef. EW is also effective against biofilm and biofilm-forming pathogens. AREVALOS-SÁNCHEZ

et al. (2013) studied the efficacy of neutralised EW against biofilm forming *L. monocytogenes* EGDe. At a concentration of 70 mg/l of total available chlorine it exhibited the complete inhibition of biofilm after 3 min

Table 5. Applications of electrolysed water (EW) against *L. monocytogenes* in different products

Types of EW	Procedure	Incubation time (min)	Reduction (log CFU)	Temperature (°C)	Suspension/food product	Ref.
Low concentrated	ORP 700, ACC 10, pH 6.8	1.5	6.7/ml	23	suspension	RAHMAN <i>et al.</i> (2012)
Oxidising	ORP 1183, ACC 63, pH 2.4	1	7.4/ml	22		PANGLOLI & HUNG (2013)
Strong acidic	ORP 1150, ACC 20, pH 3.1	2	ND	20		OVISSIPOUR <i>et al.</i> (2015)
Weak acidic	ORP 950, ACC 10, pH 3.5	2	ND	20		OVISSIPOUR <i>et al.</i> (2015)
Strong alkaline	ORP 840, pH 11.1	2	1.9/ml	20		OVISSIPOUR <i>et al.</i> (2015)
Weak alkaline	ORP 715, pH 10.4	2	1.9/ml	20		OVISSIPOUR <i>et al.</i> (2015)
Alkaline	ORP 830–850, pH 11–11.2	5	2.6/g	50	cabbage	RAHMAN <i>et al.</i> (2010b)
Slightly acidic	ORP 898, ACC 5, pH 6.3	3	2.6/g	40	kale	MANSUR & OH (2015)
Low concentrated	ORP 500–520, ACC 5, pH 6.2	3	1.4/g	23	oyster mushroom	DING <i>et al.</i> (2011)
	ORP 700, ACC 100.1, pH 6.8	5	1.7/g	23	pork	RAHMAN <i>et al.</i> (2013)
Strong acidic	ORP 1130, ACC 50.2, pH 2.5	5	1.8/g	23		RAHMAN <i>et al.</i> (2013)
Acidic oxidising	ACC 38, pH 2.3	10	1.3/g	22	beef	AL-HOLY & RASCO (2015)
	ACC 38, pH 2.3	10	1.1/g	22	chicken	AL-HOLY & RASCO (2015)
	ACC 38, pH 2.3	10	1.2/g	22	trout fish	AL-HOLY & RASCO (2015)
Electrolysed	ORP 1080, ACC 50, pH 2.8	5	2.1/g	23	salmon	MCCARTHY & BURKHARDT (2012)
	ORP 1125, ACC 40, pH 2.6	5	1.9/cm ²	21	natural latex gloves	LIU & SU (2006)
	ORP 1125, ACC 40, pH 2.6	5	2.5/cm ²	21	latex (disposable) gloves	LIU & SU (2006)
	ORP 1125, ACC 40, pH 2.6	5	3.8/cm ²	21	nitrile (disposable) gloves	LIU & SU (2006)
	ORP 1211, ACC 50, pH 2.7	5	> 5.4/cm ²	23	stainless steel	PHUVASATE & SU (2010)

ORP – oxidation reduction potential; ACC – available chlorine concentration; ND – not detectable

of treatment. While using a sublethal dose of 40 mg/l of total available chlorine a total reduction of 2 log CFU/cm² of biofilm cells was achieved (AREVALOS-SÁNCHEZ *et al.* 2013).

Other preservatives. Nisin is naturally derived from *Lactococcus lactis* subsp. *lactis*, generally known as bacteriocin. The use of nisin as a preservative in the food industry can be traced back to the 1950s. It is

categorised as GRAS and permitted by the United States Food and Drug Administration in food as a preservative since 1980 (DILLON & BOARD 1994).

DA SILVA MALHEIROS *et al.* (2012) studied the inhibitory effect of nisin and bacteriocin-like substance (BLS) P34. Both substances were encapsulated in partially purified soybean phosphatidylcholine and phosphatidylcholine cholesterol (7:3) liposomes. They

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found remarkable inhibitory effects of partially purified soybean phosphatidylcholine liposome encapsulated with nisin/BLS-P34 in Minas Frescal cheese against *L. monocytogenes* for 10 days of storage. In another study, liposome encapsulated nisin in combination with nisin (1 : 1) exhibited a strong inhibitory effect against *L. monocytogenes* CIP 82110 as compared to free or 100% encapsulated nisin alone (IMRAN *et al.* 2015). Recently, the combined inhibitory effect of nisin and D-limonene nanoemulsion on *L. monocytogenes* was evaluated. The count of *L. monocytogenes* bacteria was reduced by at least 3 log units after 90 min of incubation with the nanoemulsion and it inhibited the organism for four weeks (MATÉ *et al.* 2015). Furthermore, the shelf life of Ricotta-type cheese with the addition of 2.5 mg/l nisin which inhibited the growth of the foodborne pathogen *L. monocytogenes* was longer for more than 2 months compared to the cheese without nisin (1–2 weeks only) (SOBRINO-LÓPEZ & MARTÍN-BELLOSO 2008).

Other lactic acid bacteria have already shown the production of a number of antimicrobial agents, e.g. lactic and acetic acids, hydrogen peroxide, propionic acid, formic acid, diacetyl, phenyllactic acid, 4-hydroxyphenyllactic acid, reuterin, 3-hydroxylated fatty acids (SCHNÜRER & MAGNUSSEN 2005). Phenyllactic acid is highly active against microbes at mg/ml concentrations. In addition, phenyllactic acid in combination with other metabolites produced by lactic acid bacteria certainly showed synergism with the overall antimicrobial effect. The anti-listeria activity of D-3-phenyllactic acid has been evaluated by a number of researchers (DIEULEVEUX & GUÉGUEN 1998; DIEULEVEUX *et al.* 1998; MANU 2012). DIEULEVEUX and GUÉGUEN (1998) evaluated the inhibitory effects of D-3-phenyllactic acid compound isolated from *Geotrichum candidum* against *L. monocytogenes* in UHT whole milk. Results showed that the compound has bacteriostatic effects while reducing the population size by 4.5 log. Reuterin is another antimicrobial agent produced by *Lactobacillus reuteri*. ARQUÉS *et al.* (2008) reported the antimicrobial activity of reuterin against *L. monocytogenes* in cuajada. Results (2 AU/ml) indicated that reuterin reduced the total count of the pathogen by 0.91 log as compared to the control after 3 days of storage. However, no significant difference ($P < 0.05$) was observed between the control and tested sample after 6 days of storage. In another study reuterin caused the complete inhibition of *L. monocytogenes* at a concentration of 8 AU/ml in milk at 37°C after 24 h of incubation (ARQUÉS *et al.* 2004). The inactivation of *L. monocytogenes* by

reuterin can be attributed to the concentration used, because as the concentration increases, the inactivation rate also increases (ARQUÉS *et al.* 2008).

CONCLUSION

The prevalence of *Listeria monocytogenes* in food products and related environments has become a serious concern for the scientific community. The persistence of *L. monocytogenes* strains in food products and facilities owes much to our inability to eliminate them from target sites or to kill them there, and to their own potential to grow in a chilled environment. However, the sources of contamination are different for each food product. For instance, the contamination of pork may originate at farms during its production. Birds may spread *L. monocytogenes* into food processing facilities/premises or directly into foods. Raw materials can be a potent source of *L. monocytogenes* contamination.

To prevent and reduce contamination in the food processing environment and products, it is vital to identify the key sources of contamination and to understand the primary mechanisms. The *L. monocytogenes* reduction at a farm level can be achieved by specific farm management practices and this can contribute to a general decrease of *L. monocytogenes* level in the food chain. Access of wild animals, and especially of birds, to the food processing environment should be prevented. The food processing plants should be subjected to extensive cleansing, disinfecting, and may be disassembled to eliminate any persistent *L. monocytogenes*.

The food products can be subjected to different disinfection techniques. However, some techniques alone are unable to eliminate the pathogen in such cases; the food products can be treated with combined decontamination treatments.

The food processing companies must cautiously consider the industry design, high-quality raw materials, personnel training, good manufacturing, and hygiene practices, and effective cleaning and sanitation to prevent the contamination of the product.

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