

Chapter 2

What Is the Problem with *L. monocytogenes*?

2.1 Public Health Concern

L. monocytogenes is the causative agent of listeriosis, a food-borne disease of particular concern for risk groups including pregnant women, the young, the elderly and the immunocompromised, for all of whom it can be life-threatening.

2.1.1 Disease Characteristics

Healthy adults are generally unaffected by *L. monocytogenes*. However, in the susceptible populations (elderly, pregnant women and their unborn children, infants, and the immunocompromised) listeriosis is a serious disease that can occur in different forms: neuromeningeal (meningitis, encephalitis), maternal-neonatal (intra-uterine infection, spontaneous abortion) and febrile gastroenteritis, and in serious cases it can lead to brain infection, sepsis and even death. Fatality rates of 20–30 % are common among hospitalized patients (Goulet et al. 2012). The infective dose is unknown and is likely to vary, depending on the state of health of the individual affected.

Despite the extensive research on *L. monocytogenes*, the host factors that determine susceptibility to disease are poorly understood, as studies on oral transmission in a small animal model have not been standardised, making comparison of results difficult (D’Orazio 2014). There have been studies on the intravenous and intra peritoneal routes of infection (Kernbauer et al. 2013), however, oral infection is more realistic as it better represents the mode of ingestion of *L. monocytogenes* as a foodborne pathogen. Mice are an ideal animal model for studies with *L. monocytogenes* as it is possible to mimic all phases of disease in large-scale experiments. However, the interaction between the protein internalin A and E-cadherin (necessary

for epithelial invasion) is impaired due to sequence incompatibilities between the mouse E-cadherin and the internalin A of listeria. A transgenic mouse (expressing a ‘humanised’ E-cadherin) (Disson et al. 2008) or a ‘murinised’ strain of *L. monocytogenes* (expressing a modified internalin A) (Bergmann et al. 2013) have been used to overcome this limitation. These studies have shown the importance of internalin A (and other proteins) in pathogenicity and suggest that a different molecular mechanism is required to cross the blood–brain barrier than that required to cross the intestinal epithelium.

2.1.2 Disease Outbreaks Associated with *L. monocytogenes*

It is estimated that 99 % of listeriosis cases are caused by contaminated food (Mead et al. 1999). The vast majority of cases of listeriosis are sporadic cases, and in these cases determination of the source of infection is generally not possible. Table 2.1 shows foodborne disease outbreaks associated with *L. monocytogenes* in the last number of years. Foodborne outbreaks of *L. monocytogenes* have been associated with many different food product categories, including dairy products, seafood, vegetables and various meat products. High risk food products are generally ready-to-eat (RTE) foods that do not require further cooking before consumption.

In the European Union, according to the latest EU summary report on zoonoses, zoonotic agents and food-borne outbreaks (EFSA 2014), 1,642 confirmed human cases of listeriosis were reported in 2012, representing a 10.5 % increase compared with 2011. The EU notification rate was 0.41 cases per 100,000 population, with the highest member state specific notification rates observed in Finland, Spain and Denmark. On average, 91.6 % of the cases were hospitalised. This is the highest proportion of hospitalised cases of all zoonoses under EU surveillance. A total of 198 deaths due to listeriosis were reported by 18 member states in 2012, which was the highest number of fatal cases reported since 2006.

2.1.3 Outbreak Investigation

Outbreak investigation can help to identify the source of on-going outbreaks and prevent additional cases. Even when an outbreak is over, a thorough epidemiological and environmental investigation can often increase knowledge on listeriosis and prevent future outbreaks (Reingold 1998). The main steps for the investigation of an outbreak are the following: preliminary assessment to confirm the existence of an outbreak, case definition, case confirmation, analytical studies to establish the background rate of disease and find cases of infection, generation of an hypothesis, verification of the hypothesis, environmental investigation, adoption of control measures, and communication to the public (Reingold 1998).

Table 2.1 Major outbreaks of foodborne listeriosis

Year	Place	No. of cases (deaths)	Food type	Serovar	References
<i>United States of America</i>					
1979	Maryland	20 (3)	Raw vegetables or cheese	4b	Ho et al. (1986)
1983	Maryland	49 (14)	Past. milk	4b	Fleming et al. (1985)
1985	California	142 (48)	Mexican-type cheese	4b	Linman et al. (1988)
1986–1987	Pennsylvania	36 (44 %)	Ice cream, brie, salami	4b, 1/2b, 1/2a	Schwartz et al. (1989)
1986–1987	California	2	Eggs	4b	Schwartz et al. (1989)
1987	California	11	Butter		FDA (2003)
1989	US	9 (1)	Shrimp	4b	Riedo et al. (1994)
1994	US	45	Chocolate milk	1/2b	Dalton et al. (1997)
1998–1999	US	101 (21)	Deli meats	4b	CDC (1999)
1999	US—three states	11 (2)	Pate	1/2a	Carter (2000)
2000	N. Carolina	12 (5)	Queso Fresco	4b	CDC (2001) and MacDonald et al. (2005)
2000	US—ten states	30 (7)	Deli turkey meat		Hurd et al. (2000)
2001	US	16	Turkey meat	1/2a	Frye et al. (2002)
2002	US—eight states	54 (8)	Turkey meat		Gottlieb et al. (2006)
2003	Texas	13 (2)	Mexican type fresh cheese		Swaminathan and Gerner-Smidt (2007)
2003–2007	Texas+seven states	74 (10)	Queso Fresco		Smith (2008)
2006	Oregon	3 (1)	Past. cheese		CDC (2006)
2007	US	5 (3)	Past. milk		Cumming et al. (2008)
2008	Multi-state	8	Mexican		Jackson et al. (2011)

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Table 2.1 (continued)

Year	Place	No. of cases (deaths)	Food type	Serovar	References
2009	Washington	2	Cheese		Anonymous (2009b)
2010	Texas	10 (5)	Celery		Anonymous (2010)
2011–2012	US	146 (31)	Cantaloupe	Multiple strains of 1/2a and 1/2b	CDC (2011)
2012	14 states	20 (4)	Ricotta salata cheese		CDC (2012)
2013	5 states	6 (1)	Farmstead cheeses		CDC (2013)
2014	California and Maryland	8 (1)	Dairy products		CDC (2014b)
2014	US	2	Ice cream		
2014 to January 2015 (outbreak not over)	11 states (to date)	32 (6) (to date)	Caramel apple		CDC (2014a)
Not specified	Texas	7 (3)	Frozen vegetables	4b	Simpson (1996)
Not specified	24 States	108 (14)	Frankfurters	4b	Mead et al. (2006)
<i>Europe</i>					
1981	England	11 (5)	Dairy products	1/2a	FDA (2003)
1983–1987	Switzerland	122 (33)	Soft cheese	4b	Bula et al. (1995)
1986	Austria	28 (5)	Unpasteurised milk		Allerberger and Guggenbichler (1989)
1987–1989	UK, Ireland	355 (94)	Pate	4b	McLauchlin et al. (1991)
1989–1990	Denmark	26 (6)	Blue-mold cheese/hard cheese	4b	Jensen et al. (1994)
1992	France	279 (85)	Jellied port tongue	4b	Jacquet et al. (1995)
1993	France	39 (12)	Pork rillettes	4b	Goulet et al. (1998)
1993	Scandinavia	1	Goat's milk cheese		Eilertz et al. (1993)
1993	Italy	23	Rice salad	1/2b	Salamina et al. (1996)
1994–1995	Sweden	9 (2)	Smoked trout	4b	Ericsson et al. (1997)

1995	France	37 (11)	Soft cheese	4b	Goulet et al. (1995)
1997	France	14	Soft cheese	4b	Jacquet et al. (1998)
1997	Italy	1566	Corn and tuna salad	4b	Aureli et al. (2000)
1999	Finland	5	Smoked trout	1/2a	Miettinen et al. (1999)
1998–1999	Finland	25 (6)	Butter	3a	Lyytikäinen et al. (2000)
1999	England	2	Cheese	4b	Craig (2007)
1999–2000	France	10 (3)	Pork rillettes	4b	de Valk et al. (2001) and Swaminathan et al. (2007)
1999–2000	France	26 (7)	Pigs tongue	4b	Dorozynski (2000)
2001	Sweden	120	Soft cheese	1/2a	Carrique-Mas et al. (2003)
2001	Belgium	2	Frozen ice cream		Yde and Genicot (2004)
2003	UK	5	Sandwiches	1/2	Dawson et al. (2006)
2005	Switzerland	10 (3)	Soft cheese	1/2a	Bille et al. (2006)
2006	Czech Republic	75	Cheese	1/2a	Vit et al. (2007)
2006/2007	Germany	189	Acid curd		Koch et al. (2010)
2008	Austria	16	Jellied pork	4b	Pichler et al. (2009)
2009/2010	Austria/Germany Czech Republic	34 (8)	Quargel	1/2a (2 strains)	Fretz et al. (2010)
2012	Spain	2	Fresh cheese	1/2a	de Castro et al. (2012)
2014	Denmark	>38 (15)	Spiced lamb roll, pork, bacon, sausages, liver pâté and other meat products		Food Safety News (2014)
<i>Canada</i>					
1981	Canada	41 (17)	Coleslaw mix	4b	Schlech et al. (1983)
1996	Canada	2	Crabmeat	1/2a	Farber et al. (2000)
2001	Manitoba	7	Cream		Pagotto et al. (2006)
2002	British Columbia	47	Cheese	4b	Pagotto et al. (2006)

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Table 2.1 (continued)

Year	Place	No. of cases (deaths)	Food type	Serovar	References
2002	Quebec	17	Soft and semi-hard cheese	4b	Gaulin et al. (2003)
2002	British Columbia	86	Past. cheese	4b	Pagotto et al. (2006)
2008	Quebec	38 (2)	Cheese	1/2a	Gilmour et al. (2010)
2008	Canada	57 (22)	Maple Leaf RTE deli meat	1/2a	PHAC (2008)
<i>Australia/New Zealand</i>					
1978–1979	Australia	12	Vegetables		Niels le Souef and Walters (1981)
1980	New Zealand	22 (6)	Raw vegetables	1/2a	Lennon et al. (1984)
1990	Australia	11 (6)	Pate	1/2a	Watson CaO (1990)
1991	Tasmania	4	Smoked mussels	1/2a	Mitchell (1991)
1992	New Zealand	4 (2)	Smoked mussels	1/2a	Brett et al. (1998)
1996	South Australia	5 (1)	Diced chicken		Hall et al. (1996)
1998–1999	Australia	9 (6)	Fruit salad		Jelfs et al. (2000)
2000	New Zealand	31	RTE corned beef	1/2	Sim et al. (2002)
2009	Multi-state	40	Chicken meat		Anonymous (2009b)
2008–2009	Australia	29	Chicken wrap		Anonymous (2008)
<i>Japan</i>					
2001	Japan	38	Washed-type	1/2b	Makino et al. (2005)
<i>Chile</i>					
2008		119 (5)	Brie	ND	Anonymous (2009a)

Typing methods for discriminating different bacterial isolates are essential epidemiological tools in outbreak investigation. Pulsed-field gel electrophoresis (PFGE) has been considered as the “gold standard” among molecular typing methods for *L. monocytogenes*. However, there are other traditional (serotyping, phage-typing) or molecular-based (amplified fragment length polymorphism, variable-number tandem repeat typing, single locus and multilocus sequence typing, comparative genomic hybridisation, among others) methods that can be used to examine the relatedness of isolates (Sabat et al. 2013). More recently, next-generation sequencing (NGS) of the genomes is becoming a highly powerful tool for outbreak investigation and surveillance schemes in routine clinical practice (see Sect. 4.2.8) (Rychli et al. 2014; Holch et al. 2013), due to the continuous decline in the costs of NGS. NGS costs in US dollars (USD) can be as little as USDs 100 per bacterial genome, including sample preparation, library quality control and sequencing (Koser et al. 2012). NGS allows the development of a genome-wide gene-by-gene analysis tool, through extended multilocus sequence typing (eMLST) or through a “pan-genome approach”. Instead of the traditional MLST based on seven genes, the eMLST method would be based on the whole core genome including all genes present in all isolates of a species. On the other hand, with the “pan-genome approach”, the relatedness of isolates would be measured by the presence or absence of genes across all genomes within a species (Sabat et al. 2013).

In 2013 the Centre for Disease Control and Prevention in America (CDC) and the Food and Drug Administration (FDA), through the Genome TRAKR Network, and in parallel with their on-going surveillance, launched a pilot study on Whole Genome Sequencing (WGS) as a ‘proof-of-concept’ project on tracking *L. monocytogenes* isolates during disease outbreaks. To date (January 2015), over 2,000 *L. monocytogenes* strains have been fully sequenced (<http://www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgramWGS/ucm403550.htm>), with the numbers increasing monthly. This will provide a large database of well characterized environmental (food, water, processing facility, clinical etc.) isolates that will facilitate a better understanding of *L. monocytogenes* and its switch from saprophyte to virulent pathogen (Toledo-Arana et al. 2009).

2.2 Occurrence of *L. monocytogenes*

2.2.1 Occurrence in Ready-to-Eat Food Processing Facilities

Knowledge on the occurrence (or absence) of *L. monocytogenes* in a food processing environment is valuable information for a food business operator (FBO) as they can target measures for control of the contamination and reduce the risk of cross-contamination of food, thus reducing the risk to public health.

L. monocytogenes is widely distributed in the environment and has been isolated from a variety of sources, including soil, vegetation, silage, faecal material, sewage and water. It is frequently present in raw foods of both plant and animal origin, and

it can be found in cooked foods due to post-processing contamination. Thus, it has been isolated from foods such as raw and unpasteurized milk, cheese, ice cream, raw vegetables, fermented meats and cooked sausages, raw and cooked poultry, raw meats, and raw and smoked seafood. In addition, its ubiquitous presence also leads to the potential for contamination of the food processing environment, where occurrence and persistence of *L. monocytogenes* is frequent (Fox et al. 2011a; Nakari et al. 2014; Vongkamjan et al. 2013).

A number of surveys of *L. monocytogenes* in foods (especially RTE foods) and environments within food processing plants have been performed in recent years, revealing its presence at variable frequencies ranging from 0 % to around a 20 % (Table 2.2). For instance, in the particular case of Ireland, the occurrence and persistence of *L. monocytogenes* in foods and food processing environments of 48 food businesses by regular sampling and characterization of isolates by serotyping and Pulsed Field Gel Electrophoresis (PFGE) has recently been monitored (Leong et al. 2014). In that study, 2,006 samples (1,574 environments and 432 foods) were analyzed for the presence of *L. monocytogenes* between March 2013 and March 2014 and a prevalence of 4.6 % was observed, with slightly higher incidences in food samples (5.3 %) than in environmental samples (4.4 %). Positive food samples included cheese, smoked salmon, apple juice, mushrooms, milk, sausages, pudding, gammon, stuffing and chicken meat. The highest *L. monocytogenes* prevalence was observed in that survey for the vegetable sector (9.4 %), followed by the meat (4.2 %), the dairy (3.9 %) and the seafood (1.6 %) sectors. Interestingly, 30 of the 48 food business taking part in the survey showed at least one positive sample for *L. monocytogenes* over the course of the study, and this shows the widespread occurrence of *L. monocytogenes* in foods and food industry-related environments.

2.2.2 Occurrence at Retail Level

Contamination of RTE foods by *L. monocytogenes* can occur at various stages of the processing and distribution chain, including at retail level, although studies of occurrence at retail level do not necessarily imply that contamination occurred at retail. There is a gap in knowledge on a distinction between contamination at retail and contamination at processing. Cross-contamination with *L. monocytogenes* at retail has been identified as the main source of *L. monocytogenes* in RTE deli products (Sauders et al. 2009; Tompkin 2002; Vorst et al. 2006). Data from some surveys have indicated that RTE deli products handled at retail have a significantly higher *L. monocytogenes* prevalence than products pre-packed by the manufacturer and not handled at retail (Gombas et al. 2003). For instance, Gombas et al. (2003) analysed 31,705 samples from retail markets in the USA and found an overall *L. monocytogenes* prevalence of 1.82 %, with the prevalence ranging from 0.17 to 4.7 % among the product categories tested. Interestingly, these authors observed significantly

Table 2.2 Occurrence of *L. monocytogenes* in different recent studies

Country	Foodstuffs/samples	Procedure	Prevalence	Prevalence by food category and other observations	References
Spain	RTE fruits, whole fresh vegetables, sprouts and RTE salads (300 samples)	EN ISO 11290-2	0.7 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 3.4 % (lettuce); 0.8 % (mixed salads); not determined in the rest of products	Abadias et al. (2008)
Spain	Fruits and raw and RTE vegetables (445 samples)	EN ISO 11290-1 and RT-PCR	2.7 % (<i>Listeria</i> spp.); 0.9 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 4.8 % (edible leaves); 4.8 % (mixed salads); not determined in fresh fruits, roots and sprouts	Badosa et al. (2008)
Spain	RTE fish products, RTE meat products, RTE dairy products and RTE dishes and desserts (1,226 samples)	Enrichment in Fraser broth; plating onto ALOA and Oxford agar	3.5 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 20 % (frozen Atlantic bonito small pies), 7.9 % (smoked salmon), 11.1 % (pork luncheon meat), 6.2 % (frozen chicken croquettes), 16.9 % (cured dried sausage), 12.5 % (cooked ham), 20 % (cooked turkey breast), 1.3 % (fresh salty cheese), 15.1 % (frozen cannelloni); not determined in other RTE products	Cabedo et al. (2008)
Korea	Kimbab (popular RTE food in Korea) (30 samples)	Multiplex-PCR	6.7 % (<i>L. monocytogenes</i>)		Cho et al. (2008)
South Africa	RTE filled baguettes and RTE salads (209 samples)	Two steps enrichment in half-Fraser and Fraser broth. Plating on RAPID TM L. Mono TM Agar	4 % (<i>L. monocytogenes</i> —RTE foods)	<i>L. monocytogenes</i> : 6 % (filled baguettes); 3 % (assorted salads)	Christison et al. (2008)

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Table 2.2 (continued)

Country	Foodstuffs/samples	Procedure	Prevalence	Prevalence by food category and other observations	References
Brazil	Gravlax salmon (refrigerated RTE fish product) and different points of the processing line of a single processing factory	Two steps enrichment (<i>Listeria</i> enrichment broth and modified Fraser broth). Plating onto Palcam agar	56 % (<i>Listeria</i> spp.); 38 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 41 % (salmon), 32 % (food contact surfaces), 43 % (non-food contact surfaces) and 34 % (food handlers' samples)	Cruz et al. (2008)
India	Raw/fresh seafoods from fish markets (115 samples)	USDA and FDA methods	24.3 % (<i>Listeria</i> spp.); 8.7 % (<i>L. monocytogenes</i>)		Parihar et al. (2008)
Jordan	RTE foods (dairy products, vegetables, traditional dishes and miscellaneous samples) (360 samples)	EN/ISO 11290-1 and EN/ISO 11290-2	16.9 % (<i>Listeria</i> spp.); 5.3 % (<i>L. monocytogenes</i>)	15.8 % of positive samples showed <i>L. monocytogenes</i> counts >100 CFU/g	Awaisheh (2009)
Latvia	RTE vacuum-packaged meat products (211 samples)	EN/ISO 11290-1 and EN/ISO 11290-2	18 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 42 % (cold-smoked, sliced, vacuum-packed beef and pork products); 0.8 % (cooked, sliced, vacuum-packaged meat products) 16 % of positive samples showed <i>L. monocytogenes</i> counts >100 CFU/g	Bertzins et al. (2009)
Chile	Frozen salads (intended for cooking), cooked or raw RTE vegetable salads freshly prepared in supermarkets, and industrial minimally processed raw RTE salads with a 10-day shelf life (717 samples)	FDA Bacteriological Analytical Manual	15.3 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 25.4 % (frozen vegetable salads), 10.2 % (freshly prepared, cooked or raw RTE vegetable salads), 0 % (raw minimally processed salads industrially prepared)	Cordano and Jacquet (2009)

Greece	Milk products, RTE salads, raw meat and raw meat products, and fish purchased in ten open-air market places (210 samples)	EN ISO 11290-1	14.3% (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 8 % (milk products); 27.5 % (raw meat); 18 % (meat products); 30 % (fish); 0 % (salads)	Filiouis et al. (2009)
Ireland	Environmental samples in farm milking facilities (298 samples)	FDA method	19 % (<i>L. monocytogenes</i>)		Fox et al. (2009)
Spain	Deli meat products, smoked fish and pâté (783 samples)	EN/ISO 11290-1 and EN/ISO 11290-2	12.7 % (<i>Listeria</i> spp.); 6.2 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 10.8 % (vacuum-packed smoked salmon); 25 % (vacuum-packed smoked trout); 2.7 % (vacuum-packed deli meat products); 8.5 % (opened deli meat products); 0.8 % (vacuum-packed pâté) Some brands of smoked fish with 60 % of positive lots	Garrido et al. (2009)
United Kingdom	RTE foods (sliced meats, hard cheese, sandwiches, butter, spreadable cheese, confectionery products containing cream, and probiotic drinks) (6,984 samples)	Health Protection Agency standard microbiological methods	4.9 % (<i>Listeria</i> spp.); 2.3 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 7.0 % (sandwiches); 4.2 % (sliced meats) <i>L. monocytogenes</i> at >100 CFU/g: 0.4 % (sandwiches), 1.0 % (sliced meats)	Little et al. (2009)
Italy	Smoked fish products, marinated products, meat products, pre-packaged mixed vegetable salads (200 samples)	EN/ISO 11290-1 and EN/ISO 11290-2	22 % (<i>Listeria</i> spp.); 9.5 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 12 % (smoked fish); 4.76 % (cooked marinated products); 20 % (meat products), 2 % (vegetable salads)	Meloni et al. (2009)

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Table 2.2 (continued)

Country	Foodstuffs/samples	Procedure	Prevalence	Prevalence by food category and other observations	References
Ethiopia	RTE foods (pasteurized milk, cheese, ice cream, and cakes) and raw meat products (minced beef, pork, and chicken carcasses) from supermarkets and pastry shops (711 samples)	EN/ISO 11290-1	26.6 % (<i>Listeria</i> spp.); 4.8 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 11.7 % (ice cream); 6.5 % (cakes); 3.9 % (soft cheese); 3.7–5.1 % (meat products)	Mengesha et al. (2009)
Botswana	Food samples obtained randomly from selected supermarkets and street vendors (1,324 samples)	Enrichment and plating onto Modified <i>Listeria</i> Selective Agar	4.3 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 2.75 % (cheese); 1.08 % (raw milk); 0 % (biltong); 10.11 % (frozen cabbage); 7.41 % (coleslaw salads)	Morobe et al. (2009)
Germany	Vacuum packed meat products (50 samples)	Real-time PCR, immunoassay and culturing	64 % (<i>L. monocytogenes</i> —rPCR); 4 % (<i>L. monocytogenes</i> —immunoassay and culturing)	<i>L. monocytogenes</i> at >100 CFU/g: 12 % (vacuum-packed RTE products)	Netschajew et al. (2009)
Ireland	Cheese (351 samples)	FDA method	6 % (<i>L. monocytogenes</i>)		O'Brien et al. (2009)
Belgium	Mayonnaise-based deli-salads, cooked meat products, smoked fish (1,974 samples)	AFNOR validated VIDAS LMO method and EN/ISO 11290-2	6.3 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 6.7 % (mayonnaise-based deli-salads); 1.1 % (cooked meat products); 27.8 % (smoked fish) Positive samples with <i>L. monocytogenes</i> at >100 CFU/g: 16 % (smoked fish)	Uyttendaele et al. (2009)

United Arab Emirates	RTE salads	EN/ISO 11290-1	0 % (<i>L. monocytogenes</i>)		Almualia et al. (2010)
Brazil	Minimally processed leafy vegetables from retail market (162 samples)	Immunooassay <i>Listeria</i> Rapid Test	3.7 % (<i>Listeria</i> spp.); 1.2 % (<i>L. monocytogenes</i>)		Aparecida de Oliveira et al. (2010)
Jordan	RTE meat products (beef and poultry) (240 samples)	EN/ISO 11290-1 and EN/ISO 11290-2	17.1 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 19.2 % (beef meat products); 15 % (poultry products)	Awaishah (2010)
Finland	RTE cold-smoked pork products and plant environment of a single processing plant (183 samples)	EN/ISO 11290-1	21 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 18 % (raw pork); 26.8 % (environmental samples); 0 % (RTE cold-smoked pork after dry salting); 35 % (RTE cold-smoked pork after dry salting and brining injections)	Bertzins et al. (2010)
Switzerland	Ingredients, final product from a single sandwich-producing plant (2,245 samples)	EN/ISO 11290-1	7.4 % (<i>L. monocytogenes</i> —ingredients and sandwiches)	3.5 % (<i>L. monocytogenes</i> —environmental swabs)	Blatter et al. (2010)
Italy	RTE salads from retail stores (1,158 samples)	Immuno-enzymatic method mini-VIDAS SLM	0 % (<i>L. monocytogenes</i>)		Caponigro et al. (2010)
Italy	Traditional Chinese food from restaurants and take-away establishments (118 samples)	EN/ISO 11290-1	0 % (<i>L. monocytogenes</i>)		Catellani et al. (2010)
Korea	RTE foods (ready-to-cook foods and samples of fresh-cut produce purchased from hyper chain stores) (145 samples)	Enrichment in <i>Listeria</i> enrichment broth. Plating onto <i>Listeria</i> selective agar (Oxoid)	0.7 % (<i>L. monocytogenes</i>)		Chung et al. (2010)

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Table 2.2 (continued)

Country	Foodstuffs/samples	Procedure	Prevalence	Prevalence by food category and other observations	References
Italy	Whole vegetables and RTE salads (964 samples)	EN/ISO 11290-1 and PCR Bax System	0.3 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 0.4 % (whole vegetables); 0.3 % (RTE salads)	De Giusti et al. (2010)
Australia	RTE peanuts, cashews, almonds, brazil nuts, hazelnuts, and mixed packs (564 samples)		0.4 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 4.7 % (mixed packs); not determined in the rest of products	Eglezos (2010)
United Kingdom	Specialty meats (continental sausages, cured/fermented, dried meats) sampled from markets and specialty food shops (2,359 samples)	HPA Standard Microbiological Methods	2.3 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> at >100 CFU/g: 0.3 %	Gormley et al. (2010)
Turkey	Cheese samples (white cheese, processed cheese, dil cheese and kasar cheese) purchased from supermarkets (280 samples)		2.5 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 4.8 % (white cheese), 1.4 % (processed cheese), 1.7 % (kasar cheese), 0 % (dil cheese)	Kahraman et al. (2010)
Japan	Asazuke (Japanese light pickles) from local supermarkets (108 samples)	EN/ISO 11290-1	11.11 % (<i>L. monocytogenes</i>)		Maklon et al. (2010)

United Kingdom	RTE foods (5,840 samples)	Enrichment in Fraser broth; plating onto <i>Listeria</i> selective agar (Oxford formulation)	4.45 % (<i>Listeria</i> spp.); 2.55 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 4.08 % (crustaceans); 6.74 % (smoked fish); 2.20 % (cooked meat); 0.24 % (paté); 3.71 % (pasta- and rice-based salads); 1.90 % (fermented meats); 5.23 % (sandwiches); 2.00 % (sushi); 0.90 % (green salad); not determined in other food products <i>L. monocytogenes</i> at >100 CFU/g; 0.05 %	Meldrum et al. (2010)
Japan	Seafood products and RTE foods from grocery stores and delicatessens (701 samples)	Two-step enrichment procedure; plating onto Palcam agar. Mini-VIDAS LMO	5.4 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 12.1 % (minced tuna); 2.6 % (tuna blocks); 5.7 % (salmon roe); 9.1 % (cod roe); 3 % (smoked salmon); not determined in other products	Miya et al. (2010)
Spain	Food and environmental samples from a single Iberian pork-processing plant (2,127 samples)		24% (<i>L. monocytogenes</i>)		Ortiz et al. (2010)
Spain	Cooked meat RTE products (Cooked ham and chopped pork) (68 samples)	EN/ISO 11290-1 and EN/ISO 11290-2	14.7 % (<i>Listeria</i> spp.); 7.35 % (<i>L. monocytogenes</i>)		Perez-Rodriguez et al. (2010)
Italy	Raw meat and retail RTE products (1,268 samples)	EN/ISO 11290-1	11.7 % (<i>Listeria</i> spp.); 3.2 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 5.7 % (raw meat); 0.8 % (fresh soft cheese); 2.2 % (ham); 1.7 % (sandwiches); not determined in other products	Pesavento et al. (2010)

(continued)

Table 2.2 (continued)

Country	Foodstuffs/samples	Procedure	Prevalence	Prevalence by food category and other observations	References
Thailand	Meat and meat products, dairy and dairy products, fresh vegetables, fresh seafood, and RTE food samples from super markets (380 samples)	EN/ISO 11290-1	16.8 % (<i>Listeria</i> spp.); 4.7 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 15.6 % (meat and meat products); 0 % (dairy and dairy products); 3.3 % (fresh vegetables); 0 % (fresh seafood); 1.1 % (RTE foods)	Stonsaovapak and Boonyaratankomkit (2010)
Israel	Salads/dips, dairy, fish, poultry and meat products (10,413 samples)	USDA Laboratory Guidebook	12.1 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 26.9 % (poultry), 14.8 % (fish), 9.2 % (salads/dips), 12 % (meat), 2.1 % (dairy products)	Vasilev et al. (2010)
New Zealand	Mussel processing plants (raw material, environment, food contact surfaces, and final product) (40,565 samples)	FDA Bacteriology Analytical Manual	1.1 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 1 % (environmental samples); 4.7 % (raw material samples); 1.3 % (final product samples); 3 % (waste water)	Cruz and Fletcher (2011)
Spain	RTE salads, vegetables, turkey or chicken meat, fish, desserts, sweets and organic by-products (99 samples)	Enrichment in Fraser broth followed by VIDAS, conventional PCR and qPCR analysis	18.1 % (<i>L. monocytogenes</i> —VIDAS); up to 22.2 % (<i>L. monocytogenes</i> —PCR); up to 19.1 % (<i>L. monocytogenes</i> —qPCR)		Elizaquível et al. (2011)

Egypt	Street-vended RTE foods, including sandwiches and dishes of traditional food (576 samples)	Enrichment in <i>Listeria</i> Selective Broth; plating onto Oxford agar	24 % (<i>Listeria</i> spp.); 14 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 16 % (meat products); 9 % (poultry products); 8 % (seafood products); 14 % (dairy products); 24 % (plant products)	El-Shenawy et al. (2011)
Ireland	Raw milk, cheese, processing environmental and external environmental samples from 16 farmhouse cheese manufacturing facilities (1,591 samples)	EN/ISO 11290-1	15.7 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 6.3 % (milk); 13.1 % (processing environment samples); 12.3 % (external environment samples)	Fox et al. (2011a)
USA	Food and environmental samples from 120 retail deli establishments (1,981 samples)	Enrichment in Fraser broth; detection using the VIDAS <i>L. monocytogenes</i> (LMO) II assay; plating onto modified Oxford media	11.4 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 1.5 % (food products); 13.2 % (environmental samples); 4.8 % (food contact surfaces); 17 % (non-food contact surfaces)	Hoelzer et al. (2011)
Serbia	Fresh fish (cooled), frozen food (fish and sea products), canned fish products; smoked fish, salted fish, thermally treated fish and fish products, semi-canned fish and canned fish (470 samples)	EN/ISO 11290-1	12.34 % (<i>Listeria</i> spp.); 1.92 % (<i>L. monocytogenes</i>)		Kuzmanovic et al. (2011)

(continued)

Table 2.2 (continued)

Country	Foodstuffs/samples	Procedure	Prevalence	Prevalence by food category and other observations	References
Brazil	RTE sliced foods (cooked ham and salami) (130 samples)	EN/ISO 11290-1 and EN/ISO 11290-2	13.1 % (<i>Listeria</i> spp.); 3.5 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 6.2 % (salami); 0.8 % (cooked ham)	Martins and Leal Germano (2011)
Brazil	Luncheon meat (industrially vacuum-packaged and sliced and re-packaged at retail stores) (300 samples)	Pre-enrichment in UVM Listeria Enrichment broth and Fraser broth; plating onto tryptose agar with nalidixic acid and Palcam agar	5.3 % (<i>Listeria</i> spp.); 2 % (<i>L. monocytogenes</i>)		Mottin et al. (2011)
Italy	RTE salads (48 samples)	USFDA Bacteriological Analytical Manual and/or ISO protocol 11290-1	31.2 % (<i>Listeria</i> spp.); 6 % (<i>L. monocytogenes</i>)		Nabi et al. (2011)
Jordan	Traditional foods and dairy products (120 samples)	–		Four <i>Listeria</i> species were detected in all tested samples except for Motabbal Al-bathinjan	Omar et al. (2011)
Jordan	Raw chicken and RTE chicken products (chicken-shawirma, chicken-burger, chicken-sausage and mortadella) (280 samples)	EN/ISO 11290-1	50 % (<i>Listeria</i> spp.); 18.2 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 9.4 % (fresh broiler chicken); 13.3 % (chicken-shawirma); 76.7 % (chicken burgers); 30 % (chicken sausages); 0 % (mortadella)	Osaili et al. (2011)
Trinidad	RTE meat products of two popular local brands and food samples and surfaces from one manufacturing plant (480 samples)		18.3 % (<i>Listeria</i> spp.); 8.1 % (<i>L. monocytogenes</i>)		Syne et al. (2011)

USA	Environmental samples from small or very small RTE meat processing plants (688 samples)	USDA-FSIS procedure	9.5 % (<i>Listeria</i> spp.); 6.1 % (<i>L. monocytogenes</i>)	Williams et al. (2011)
Switzerland	RTE lettuce, fresh-cut fruit, and sprouts (233 samples)	EN/ISO 11290-1	2.1 % (<i>L. monocytogenes</i>)	Althaus et al. (2012)
Algeria	Dairy products, unpacked sliced meat products, cooked meat dishes, salads, mayonnaise from school and company cafeterias, restaurants, manufacturers and retail trade (227 samples)	AFNOR V08-055	9.3 % (<i>Listeria</i> spp.); 2.6 % (<i>L. monocytogenes</i>)	Bouayad and Hamdi (2012)
Iran	Raw, ready-to-cook and RTE poultry products (402 samples)	Selective enrichment and isolation protocol recommended by USDA	33.3 % (<i>Listeria</i> spp.); 12.9 % (<i>L. monocytogenes</i>)	Fallah et al. (2012)
Italy	RTE seafood products (38 samples)	EN/ISO 11290-2	23.68 % (<i>L. monocytogenes</i>)	Gambarin et al. (2012)

(continued)

Table 2.2 (continued)

Country	Foodstuffs/samples	Procedure	Prevalence	Prevalence by food category and other observations	References
Canada	Deli meat (beer sausage, bologna, salami, cheese loaf, chicken and turkey breast, cooked ham, corned beef, meat macaroni loaf, mortadella, variety pack sausages, and different types of pepperoni) and fish (flavoured, candied and/or smoked fish jerky, nuggets, as well as lox, sockeye sticks, smoked steelhead trout, and tuna) (80 samples)	Health Canada's MFLP-74 enumeration and MFHPB-30 two-step enrichment methods	10 % (<i>Listeria</i> spp.); 2.5 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 5 % (fish products); 0 % (deli meat products)	Kovacevic et al. (2012a)
Canada	RTE food samples (dairy, fish, and meat) and swabs from food processing environments (508 samples)	Health Canada's MFLP-74 enumeration and MFHPB-30 two-step enrichment methods	11.8 % (<i>Listeria</i> spp.); 6.7 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 7.8 % (environmental swabs); 5.6 % (food samples); 19.7 % (fish products); 0 % (meat and dairy products)	Kovacevic et al. (2012b)
Sweden	Soft and semi-soft cheeses (mould- and smear-ripened); heat-treated meat products; smoked and gravad fish (1,590 samples)	EN/ISO 11290-1 and EN/ISO 11290-2	4.7 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 0.4 % (cheese); 1.2 % (meat products); 12 % (fish products); 14 % (gravad and cold-smoked fish); 2 % (hot-smoked fish) <i>L. monocytogenes</i> at >100 CFU/g: 0.2 % (cheese); 0.5 % (fish)	Lambertz et al. (2012)

Brazil	Minimally processed vegetables (172 samples)	Enrichment in Listeria Enrichment Broth; plating onto Oxford and Lithium chloride-phenylethanol moxalactam media Alternative methods: Vidas, Vip, Reveal	1.2 % (<i>L. monocytogenes</i>)		Maistro et al. (2012)
Malaysia	Raw and RTE foods (sausages, burgers, canned fish, minced, fish, chicken and meat) from local wet markets, mini markets and supermarkets (140 samples)	Enrichment in Listeria enrichment broth. Plating onto PALCAM selective agar	8.57 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 13.3 % (sausages); 33.3 % (burgers); 25 % (minced meat); 0 % (chicken, beef, fish, and canned fish)	Marian et al. (2012)
United Kingdom	Food samples, including cooked meat, cakes, RTE fruit and vegetables, and sandwiches (16,881 samples)	Enrichment in buffered peptone water. Plating onto agar Listeria (Ottaviani and Agosti) (ALOA)	No prevalence data presented	<i>L. monocytogenes</i> at >100 CFU/g: 0.04 %	Meldrum et al. (2012)
Germany	RTE poultry products (emulsion type sausages, turkey breast and raw RTE spreadable sausages of turkey meat and onion) (300 samples)	EN/ISO 11290-2	6 % (<i>Listeria</i> spp.); 3 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> at >100 CFU/g: 1 %	Meyer et al. (2012)

(continued)

Table 2.2 (continued)

Country	Foodstuffs/samples	Procedure	Prevalence	Prevalence by food category and other observations	References
Spain	Fresh, frozen and fresh-cut vegetables packaged under modified atmosphere (191 samples)	EN/ISO 11290-1 and EN/ISO 11290-2; multiplex-PCR and DVC-FISH	4.19 % (<i>L. monocytogenes</i> —by culture); 10.47 % (<i>L. monocytogenes</i> —by multiplex-PCR); 32.98 % (<i>L. monocytogenes</i> —by DVC-FISH)		Moreno et al. (2012)
Japan	Imported cheese and non-cooked meat products (150 samples)	Bacteriological Analytical Manual	4.1 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 7.8 % (non-cooked meat products); 0 % (cheese)	Okada et al. (2012)
Iran	Seafood (fresh and frozen fish and shrimp) (264 samples)	USDA protocol	7.6 % (<i>Listeria</i> spp.); 1.9 % (<i>L. monocytogenes</i>)		Rahimi et al. (2012)
Brazil	Packaged RTE vegetables (512 samples)	EN/ISO 11290-1	3.1 % (<i>L. monocytogenes</i>)		Sant'Ana et al. (2012)
Turkey	Cig kofte without meat (a novel bulgur ball product) (70 samples)	Primary enrichment in Half-Fraser broth followed by immunomagnetic separation (IMS). Plating on PALCAM and Oxford agars. Confirmation by PCR	17.1 % (<i>L. monocytogenes</i>)		Taban (2012)
Iran	Raw/fresh, frozen, and RTE seafood products (245 samples)	Multiplex-PCR		<i>L. monocytogenes</i> : 1.4 % (raw/fresh fish and shrimp); 0 % (RTE seafood products)	Zarei et al. (2012)

European Union (except Portugal) and Norway	Packaged hot or cold smoked or gravad fish, packaged heat-treated meat products and soft or semi-soft cheeses (13,088 samples)	EN/ISO 11290-1 and EN/ISO 11290-2	5.5 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 10.3 % (hot or cold smoked or gravad fish); 2.07 % (packaged heat-treated meat products); 0.47 % (soft and semi-soft cheese) <i>L. monocytogenes</i> at >100 cfu/g: 1.7 % (fish), 0.43 % (meat) and 0.06 % (cheese)	EFSA (2013)
Turkey	RTE and traditional food products (239 samples)	PCR	2.09 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 2.58 % (salads); 6.66 % (abagannus samples); 0 % (broad bean paste, hummus, parsley salads, traditional salted yoghurt, thyme salads, and walnuts with red pepper)	Elmali et al. (2013)
Iran	Popular seafood products and their market and processing environments (1,525 samples)	Most probable number (MPN) technique	14.5 % (<i>L. monocytogenes</i> —raw and RTE foods); 17 % (<i>L. monocytogenes</i> —processing environments)	<i>L. monocytogenes</i> : 11.9 % (whole raw fish); 4 % (whole raw shrimp); 29.3 % (fish fillets); 21.7 % (shrimp flesh); 9.75 % (RTE fish products); 5.55 % (RTE shrimp products)	Fallah et al. (2013)
Spain	Refrigerated ready-to-eat seafood products at retail (young eels, crabstick and smoked salmon) (250 samples)	EN/ISO 11290-1/A1 and EN/ISO 11290-2/A	2.4 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 4.8 % (smoked salmon); 0 % (young eels and crabstick)	González et al. (2013)

(continued)

Table 2.2 (continued)

Country	Foodstuffs/samples	Procedure	Prevalence	Prevalence by food category and other observations	References
Malaysia	RTE food samples purchased from hypermarkets and street-side hawkers stalls (396 samples)	EN/ISO 11290-1. Different selective agar were used, CHROMagar-Listeria, Listeria selective agar and PALCAM agar	17.9 % (<i>Listeria</i> spp.); 11.4 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 14.7 % (salads and vegetables), 13.2 % (chicken and chicken products), 10 % (beverages), 9.5 % (eggs and egg products), 6.7 % (beef and beef products), 6.7 % (lunch boxes) and 6.7 % (seafood and seafood products)	Jamali et al. (2013)
Greece	RTE foods (sandwiches, oven baked bakery products, desserts oven baked, desserts with dairy cream) and ready-to-bake frozen pastries from university canteens (479 samples)	Enrichment in <i>Listeria</i> enrichment broth. Plating on Palcam agar. Confirmation by API <i>Listeria</i> strip	6.8 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 7.7 % (sandwiches), 0 % (desserts oven baked), 20 % (desserts with dairy cream), 3.8 % (oven baked pastries), 8.7 % (frozen pastries)	Kotzekidou (2013a)
Greece	Raw food ingredients, RTE products and ready-to-bake frozen pastries (356 samples)	Enrichment in <i>Listeria</i> enrichment broth. Plating on Palcam agar Real-time PCR	10.4 % (<i>L. monocytogenes</i> —by culture); 11.0 % (<i>L. monocytogenes</i> —by RT-PCR)	<i>L. monocytogenes</i> : 19.4 % (raw meat and meat products); 14.9 % (frozen pastries); 17.7 % (desserts); 7.3 % (sandwiches); 0 % (fish, eggs, vegetables and dairy products)	Kotzekidou (2013b)
Croatia	RTE minimally processed and refrigerated vegetables (100 samples)		20 % (<i>Listeria</i> spp.) and 1 % (<i>L. monocytogenes</i>)		Kovacevic et al. (2013)

Estonia	Raw and RTE meat, milk, fish, pastry, crop, culinary, fruit and vegetable foods (21,574 samples)	EN/ISO 11290-1 and EN/ISO 11290-2	2.6 % (<i>L. monocytogenes</i>)	<i>L. monocytogenes</i> : 2 % (RTE meat); 0.3 % (RTE dairy); 5.4 % (fish); 2.2 % (smoked meat); 3.6 % (mixed meat products) <i>L. monocytogenes</i> at >100 CFU/g: 2.9 % (fish products)	Kramarenko et al. (2013)
Taiwan and Philippines	Chicken- and pork-based street-food samples (119 samples)	Enrichment in Fraser broth; plating onto CHROMagar	0 % (<i>L. monocytogenes</i>)		Manguiat and Fang (2013)
Spain	Plant-based foods (cereals, legumes, fruits and vegetables) (781 samples)	EN/ISO 11290-1	0 % (<i>L. monocytogenes</i>)		Sospedra et al. (2013)
USA	Environmental samples from a mushroom production facility (184 samples)	Enrichment in University of Vermont broth; plating onto Oxford agar	15.9 % (<i>Listeria</i> spp.); 1.6 % (<i>L. monocytogenes</i>)		Viswanath et al. (2013)

($p < 0.001$) higher prevalence for in-store packaged samples than for manufacturer-packaged samples of luncheon meats, deli salads, and seafood salads. Occurrence data from other *L. monocytogenes* surveys performed at retail level in the last five years are also included in Table 2.2.

It is important to note that recently conducted risk assessments for *L. monocytogenes* in deli meats indicated that the majority of listeriosis cases and deaths associated with deli meats are probably due to contamination of products at retail (Endrikat et al. 2010; Pradhan et al. 2010). Endrikat et al. (2010) estimated that 83 % of human listeriosis cases and deaths attributable to deli meats are due to retail-sliced products, and Pradhan et al. (2010) performed a risk assessment using product-specific growth kinetic parameters that indicated that 63–84 % of human listeriosis deaths linked to deli ham and turkey can be attributed to contamination at retail. Occurrence and cross-contamination at retail level do not attract much research, but are obviously an important source of listeriosis.

2.2.3 Identifying Routes of Contamination

Tracing the source of *L. monocytogenes* is critical in the control of the organism in a localised environment, although the ubiquitous nature of the organism makes it difficult to positively identify the source of contamination. However, it has been proven that recontamination during processing is a major source of *L. monocytogenes* contamination in food (Chen et al. 2010; Lomonaco et al. 2009; Vitas and Garcia-Jalon 2004). Sub-typing of isolates, using methods such as PFGE, allows analysis of the molecular diversity of *L. monocytogenes* strains present (Fox et al. 2011b). Strains recurring in the environment over time (persistent strains) can be identified (Stessl et al. 2014). Persistent strains in the environment represent an increased risk of contamination of food products. Control of these persistent strains, in particular, is an important part of a food processing facility food safety programme. After characterising the molecular diversity of isolates in the environment in question, putative routes of transmission and/or sources of entry into the environment can be identified. Muhterem-Uyar et al. (2015) identified three potential contamination scenarios that can increase the risk of food contamination, hot-spot contamination, widespread contamination and sporadic contamination (Muhterem-Uyar et al. 2015). Visualisation of the contamination on a facility map (Fig. 2.1) can help identify the putative contamination routes. Thus, control strategies can be adjusted/targeted to remove the source of contamination and interrupt the route of transfer to the food. Analysis of such results can not only identify persistent strains, but can also identify an area which may be colonised by a particular strain, leading to possible recontamination events. It can also be used to prevent the spread of strains throughout the facility.

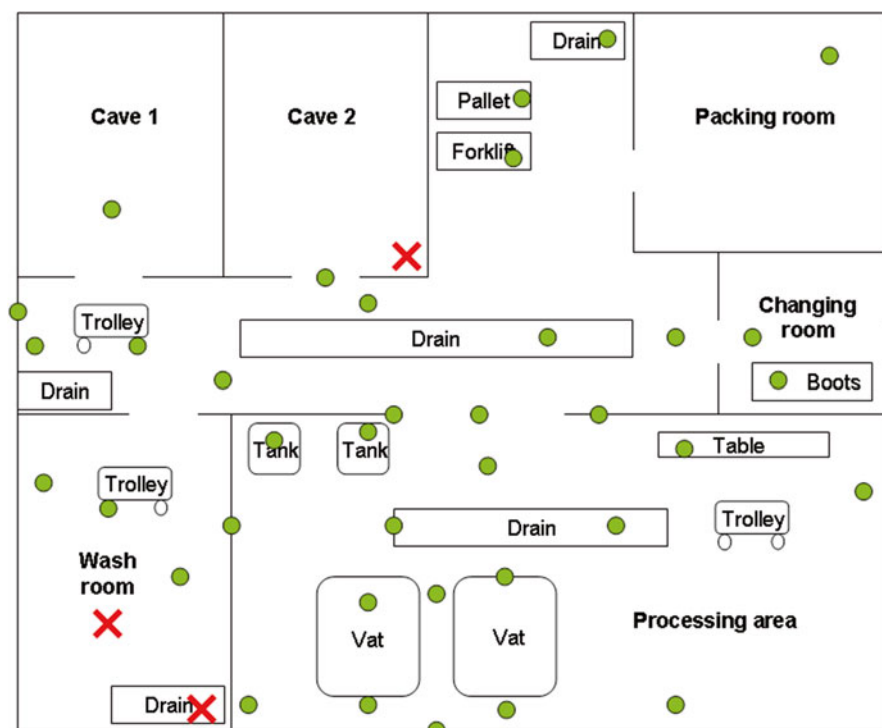


Fig. 2.1 A factory map (rough drawing; not to scale) showing potential sampling points. (a) green circle indicates negative sampling sites and, (b) red 'x' indicates positive sampling sites

2.2.4 Economic Burden: Litigation, Sampling Costs, Etc.

Economic losses linked to *L. monocytogenes* include the costs linked to illness outbreaks and industry costs of regular monitoring programmes, and possible recalls of contaminated food—including the reputational damage incurred.

Various efforts have been made to estimate the cost of cases of listeriosis in the US (Mead et al. 1999; Scallan et al. 2011), and foodborne gastrointestinal disease in general (Flint et al. 2005; Scharff 2010). These estimates cannot be compared as different methodologies were used in each case. However, they all take hospitalisation, loss of income, etc. into account.

In the USA in particular, litigation in the case of a foodborne illness or death is becoming the norm. There are specialised law firms and lawyers promoting such litigation (<http://www.pritzkerlaw.com/listeria/alfalfa-sprouts-listeria-lawyer.html>). Such claims are made against the growers, processors, distributors, restaurants or

other eating establishments that may be implicated in the case. Compensation can include claims for the following:

- Medical expenses
- Pain and suffering (includes physical pain, suffering, emotional distress and disability)
- Loss of income
- Loss of potential earnings
- Punitive damages upon clear and convincing evidence that the acts of the defendant show deliberate disregard for the rights or safety of others
- Other damages

In the case of death, the following expenses can be included in the litigation:

- Funeral expenses
- Medical expenses
- Potential loss of earnings
- Loss of advice, comfort, assistance, protection, counsel and society
- Punitive damages upon clear and convincing evidence that the acts of the defendant show deliberate disregard for the rights or safety of others

In Europe, there is an onus on industry to protect the consumer from *L. monocytogenes* (European Regulation No 2073/2005 (EC 2005)). This responsibility for consumer protection entails considerable costs on industry—(necessary) implementation of hygiene controls, testing (processing environment and product) and possible recalls. In addition to the financial costs, there are also the costs of reputational damage that can in some cases lead to closure.

Hygiene controls are a necessary part of any food business. They help to maintain product quality, extend product shelf-life, and protect against foodborne pathogens in general, including *L. monocytogenes*. In addition, in all regulatory systems, end product testing is a requirement in food processing. Such testing is a considerable cost on the manufacturer and the number of samples required for testing varies with the regulatory regime. In addition to end-product testing, there is also a requirement for process control testing and processing environment testing. Such testing is not specific for *L. monocytogenes*.

L. monocytogenes contamination of a product can be very low and not evenly distributed on the food. As some foods can support growth of *L. monocytogenes*, and this microorganism can grow at refrigeration temperatures, it is possible that contaminated food can be released onto the market. If such contamination is detected after product release, then a product recall, either voluntary or compulsory, may be instigated. Such recalls impose considerable costs on the manufacturer, increasing with the scale and extent of the recall. Table 2.3 shows a number of product recalls that have been instigated recently.

Table 2.3 Some food recalls associated with *L. monocytogenes*

Year	Country	Number of recalls	Associated products
1991–2008	Canada	6	Frankfurters, pork, salami and others
1998–2008	United States of America	216	Frankfurters, sandwiches, ham, chicken, cheese, hot dogs, beef jerky and others
2009	United States of America	7	Meat, RTE meal
2010	Australia/New Zealand	31	Meat, cheese
2010	Canada	12	Meat, fish, eggs, cheese
2008	England	6	Meat, cheese
September 2010 to December 2011	Ireland	6	Meat, fish, cheese
2014	United States of America	3	Dairy products
2014	United States of America	1	Soy products

2.2.5 Regulations with Respect to *L. monocytogenes*

In Europe, Regulation (EC) No 2073/2005 (EC, 2005) lays down the microbiological criteria for certain microorganisms in foods and the implementing rules to be complied with by FBOs when implementing general and specific hygiene measures. In relation to *L. monocytogenes*, this regulation covers primarily RTE food products, and requires the following: (1) in RTE products intended for infants and for special medical purposes, *L. monocytogenes* must not be present in 10×25 g; (2) in RTE products other than those for infants and special medical purposes different microbiological criteria apply depending on the ability of the food product to support growth of *L. monocytogenes*. Thus, for RTE foods unable to support the growth of *L. monocytogenes*, the levels should be <100 cfu/g throughout the shelf-life of the product (n=5; c=0). On the other hand, in RTE foods that are able to support the growth of the bacterium, *L. monocytogenes* must not be present in 5×25 g samples at the time of leaving the production plant; however, if the producer can demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout its shelf-life, the level should be <100 cfu/g throughout the shelf life of the product (n=5, c=0). In addition, this regulation establishes that the safety of the food is the responsibility of the FBO who can conduct studies to evaluate the growth of *L. monocytogenes* that may be present in the product during the shelf-life under reasonably foreseeable storage conditions of distribution, storage and use in order to investigate compliance with the criteria throughout the shelf-life of the product.

In Canada (http://www.hc-sc.gc.ca/fn-an/legislation/pol/policy_listeria_monocytogenes_2011-eng.php) and Australia/New-Zealand (<http://www.foodstandards.gov.au/code/microbiolimits/Pages/Criteria-for-Listeria-monocytogenes-in-ready-to-eat-foods.aspx>), the regulations are in line with European regulations, allowing a differentiation between foods that can and cannot support growth. However, in the USA there is ‘zero tolerance’ of *L. monocytogenes* (absence in 5×25 g of food is required at all times, and in the processing environment), where any occurrence in is considered an offence (<http://www.fsis.usda.gov/wps/portal/fsis/topics/regulatory-compliance/listeria>).

2.2.6 *L. monocytogenes* Numbers in Food

If a food processing facility is contaminated with *L. monocytogenes*, there is an increased risk of cross-contamination to the food being processed. As the European regulations allow 100 cfu/g of food under certain circumstances, the ability of the food to support the growth of the organism becomes important. This is not as relevant in jurisdictions where there is a ‘zero tolerance’ of *L. monocytogenes*.

The ability of *L. monocytogenes* to grow in food products may be estimated based on specifications of the physico-chemical characteristics of the product, consultation of the available scientific literature, or predictive mathematical modelling. There are many tools that support predictive modelling of *L. monocytogenes* in food. These include, for example, general pathogen models such as Combase (www.combase.eu) and Pathogen Modelling Programme (PMP; <http://pmp.errc.ars.usda.gov/PMPOnline.aspx>), and more specific *L. monocytogenes* models such as those at <http://safesmokedfish.food.gov.uk/>, or <http://fssp.food.dtu.dk/>. Such predictive models are useful, but for many reasons, including the possibility of overestimation/underestimation of growth in food products, in most cases growth assessment will involve laboratory-based studies, so-called challenge tests. A challenge test can be defined as a laboratory-based study that measures the growth of *L. monocytogenes* in artificially contaminated food stored under foreseeable abuse conditions of transportation, storage at retail and at consumer level. From a public health perspective, overestimation of growth is a ‘fail-safe’ scenario, although such overestimation can be inaccurate from a food producer’s perspective. For example, in 40 % of cases Combase predicted growth in cheese when no growth was seen in growth experiments (Schvartzman et al. 2011). It was further shown that the growth characteristics of *L. monocytogenes* were different in liquid and solid matrices (Schvartzman et al. 2010).

Determining the ability of foods to support the growth of *L. monocytogenes* is not simple, since many RTE foods are traditionally produced in local regions using variable formulations which may have an impact on the growth of *L. monocytogenes*.

The Food Standards Agency of New Zealand has recently published guidelines for undertaking challenge studies (FSANZ 2014), although this document is not specifically related to *L. monocytogenes*. On the other hand, Canada also has guidelines which specifically relate to *L. monocytogenes* (Health-Canada 2012). In Europe, in order to facilitate the task of performing challenge studies, the European Union Community Reference Laboratory for *L. monocytogenes* (EURL *Lm*) prepared a Technical Guidance document in 2008 in collaboration with seven laboratories, including six National Reference Laboratories (NRL) for *L. monocytogenes* (EC 2008). This guidance document was aimed at describing the microbiological procedures for determining growth of *L. monocytogenes* using challenge tests in the frame of the application of Regulation (EC) No. 2073/2005. The content of this Technical Guidance document has been reviewed by Beaufort (2011). However, feedback from food processors and independent laboratories indicated a need for the revision of the guidance document and to develop a more user-friendly set of guidelines to facilitate such analyses. In September 2012, the revision of the “EURL *Lm* Technical Guidance Document for conducting shelf-life studies on *Listeria monocytogenes* in ready-to-eat foods” commenced. The EURL *Lm* established a working group of representatives from 10 NRLs, 1 associate NRL and 1 laboratory on behalf of a NRL, and the updated version of the Technical Guidance document has recently been published (EC 2014).

Table 2.4 summarizes the main factors that must be considered when performing a laboratory challenge test to assess growth potential by following the updated version of the EURL *Lm* Technical Guidance document for conducting shelf-life studies on *L. monocytogenes* in RTE foods. An indication of the growth potential is obtained from the difference between the $\log_{10}$ cfu/g at the end of the shelf-life and the $\log_{10}$ cfu/g at the beginning of the test. When this difference is greater than 0.5 $\log_{10}$ cfu/g the food is classified into RTE foods able to support the growth of *L. monocytogenes*. Alternatively, when the difference is less than 0.5 $\log_{10}$ cfu/g, the food is classified into RTE foods unable to support the growth of *L. monocytogenes*. A challenge study using this protocol has recently been undertaken demonstrating that mushrooms (*Agaricus bisporus*) do not support the growth of *L. monocytogenes* (Leong et al. 2015), while smoked salmon does (Fig. 2.2).

In cases where growth potential is demonstrated, the growth rate is then important, in order to determine if numbers will exceed the limit of 100 cfu/g during shelf-life. In these circumstances the initial numbers and the growth rate are both important. The EURL *Lm* Technical Guidance document includes a section on undertaking challenge trials to determine growth rate. The major differences between the challenge trials to determine growth potential and the challenge trials to determine growth rate are that each strain must be tested individually in the growth rate experiments and also sampling must be undertaken at regular intervals and food storage must be carried out at a uniform temperature.

Table 2.4 The main factors to be considered when designing a laboratory challenge test to assess the growth potential of *Listeria monocytogenes* on a food matrix

	2014 European Guidance Document
Number of batches	– If growth probability is low or inter-batch variability of pH and water activity is negligible: 1 batch – If growth probability or inter-batch variability are high: at least three batches
Choice of strains	A mixture of at least two strains. One of them must be a strain with known growth characteristics (EURL <i>Lm</i> strain collection available to this aim). The other strain/s can be freely chosen from food, environment, outbreak, or collection
Inoculum preparation	First subculture in a non-selective medium at an optimal temperature (e.g. 30 or 37 °C) Second subculture at a temperature close to the actual storage temperature of the product
Food inoculation	Inoculum volume must not exceed 1 % of the mass (or volume) of the test unit The contamination level must be targeted at around 100 cfu/g Several methods of inoculation can be considered depending on the product tested. The inoculation procedure should mimic natural contamination
Storage conditions	– When FBO has its own data on the first two stages of the cold chain (from manufacturing to retail, and storage at retail) or there exists national information available, the use of this information is preferred to select the storage temperature to be used – If no data are available: 8 °C (1/3 of shelf life), 12 °C (1/3 of shelf life), and 12 °C (1/3 of shelf life)
Analysis of inoculated test units	– Enumeration of <i>L. monocytogenes</i>: at least at the beginning of the challenge test and at the end of the shelf life of the product (three test units at each time) by following the standard method EN ISO 11290-2 – Associated microflora: at the start and end of the challenge test following relevant standard methodology – Physico-chemical characteristics of the food (at least pH and water activity): at least at the beginning and end of the challenge test
Analysis of non inoculated test units	Analysis of 1 test unit following relevant standard methodology for: – Detection of <i>L. monocytogenes</i> (EN ISO 11290-1) – Associated microflora – Physico-chemical characteristics of the food (at least pH and water activity) – Gas concentration
Calculation of standard deviation of cell numbers (log ₁₀ cfu/g) at Day 0	The standard deviation of the results obtained for enumeration of <i>L. monocytogenes</i> on the inoculated batches at Day 0 should not exceed 0.5 log ₁₀ cfu/g. If this value is exceeded the challenge study is unacceptable
Calculation of the growth potential	The growth potential (log ₁₀ cfu/g) is the difference in the median of the results at the end of the challenge test and the median of the results at the beginning of the challenge test

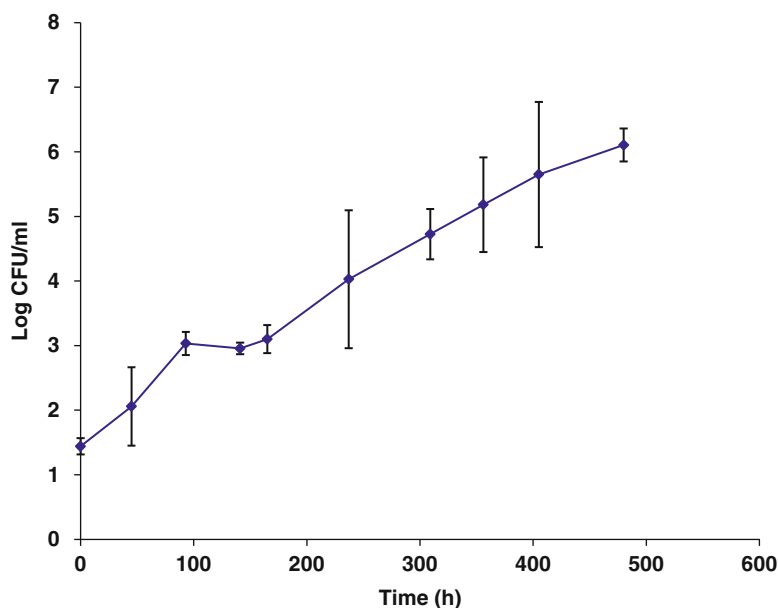


Fig. 2.2 Growth of *L. monocytogenes* on the surface of smoked salmon artificially inoculated with a cocktail of three strains and incubated at 8°C for 7 days and 12°C for 14 days

2.2.7 Addressing Occurrence and Regulatory Issues in Industry

In addition to the costs in human terms, *L. monocytogenes*-related economic losses now run into the billions per year worldwide, highlighted by the recent high profile epidemic related to some foodborne outbreaks. For some food businesses, an outbreak of listeriosis associated with their products would be extremely detrimental. Sporadic and persistent contamination of RTE food processing facilities with *L. monocytogenes* creates a problem for RTE food industries. *L. monocytogenes* is a ubiquitous bacterium that can be widely distributed in food processing environments. Because it can resist various stresses additional measures to the normal HACCP, GMP practices may be required to manage and control it. The challenge for the food industry is to develop, implement, and maintain programmes for monitoring and control of *L. monocytogenes*.

2.3 Organism Characteristics

2.3.1 Persistence in the Food Processing Environment

The persistence of *L. monocytogenes* in the food-processing environment is well-documented but poorly understood (Carpentier and Cerf 2011; Lomonaco et al. 2009; Leong et al. 2014). This is partly due to the loosely defined term “persistence”.

Persistence of bacterial strains in a food processing facility refers to repeated isolation of the same strain (characterised by PFGE) for months or even years at the same sites (Unnerstad et al. 1996). Non-persistent strains, which are isolated infrequently in a sampling programme, could be identified more frequently, if a more extensive sampling programme was undertaken. Therefore, these strains are referred to as 'presumed' non-persistent strains. Persistence can cause repeated food contamination, and an increasing risk of food safety violation, thus impacting on public health (Pricope et al. 2013). Strains of *L. monocytogenes* that have been repeatedly isolated from the same environment over a long period of time are therefore thought of as being persistent. However, although it is probable that these strains are surviving and persisting in the food-processing environment, it is also possible that constant contamination from outside sources, for example, from raw materials, may act as a continuous source of particular *L. monocytogenes* strains (Carpentier and Cerf 2011). Persistence of *L. monocytogenes* isolates has been shown at food processing facilities, often for many years (Tompkin 2002). In addition to strains persisting at larger scale cheese production facilities (Lomonaco et al. 2009), persistence has also been documented at smaller artisan facilities (Fox et al. 2011a), in the salmon industry (Fonnesbech Vogel et al. 2001; Wulff et al. 2006), in meat processing plants (Giovannacci et al. 1999; Nesbakken et al. 1996) and in poultry production plants (Lawrence and Gilmour 1995; Ojeniyi et al. 2000).

The persistence of *L. monocytogenes* in conditions which would be inhospitable to most bacteria may be due to several factors including: (1) the existence of harbourage sites that are difficult to clean and disinfect properly, (2) or alternatively the ability of particular strains to grow at a wide range of temperatures, especially refrigeration temperatures, resist acid stress, desiccation, or disinfectants, or to form biofilms on industrial environments (Galvão et al. 2012; Gandhi and Chikindas 2007; Schmid et al. 2009; Takahashi et al. 2011). This ability to grow or survive where other bacteria cannot allows *L. monocytogenes* to thrive with little competition from other bacteria.

Harbourage sites are probably a very important factor in the persistence of *L. monocytogenes*. When used correctly, cleaning and sanitising procedures should be adequate to remove *L. monocytogenes* from the environment (Cruz and Fletcher 2011). However, a harbourage site could be an area where cleaning procedures do not reach properly, so *L. monocytogenes* is not properly removed. When used correctly and at the correct concentration, *L. monocytogenes* does not seem to have increased resistance to disinfectants when compared to other bacteria (Lourenço et al. 2009). However, a harbourage site may be an area where the product reaches but it cannot be properly dried so a sub lethal amount of the product remains in the site. This may allow *L. monocytogenes* strains sufficient time to develop a resistance to the product so that a community of *L. monocytogenes* which is resistant to the cleaning product develops. This strain could then be spread out from the harbourage site to contaminate other areas of the facility (Carpentier and Cerf 2011).

A major step to discourage bacterial growth in food processing is exposure to refrigeration temperatures. Although the majority of foodborne pathogens cannot grow at these temperatures, *L. monocytogenes* can. Therefore, refrigeration

temperatures may essentially select for *L. monocytogenes* growth. Cold shock proteins have been shown to be essential for *L. monocytogenes* ability to survive at low temperature as well as its ability to survive osmotic stress (Schmid et al. 2009). An alternative sigma factor, sigma factor B (σ^B), encoded by *sigB*, plays a vital role in *L. monocytogenes* response to stress (see Sect. 2.3.2). Indeed, the *sigB* gene has been shown to be vital in the survival of *L. monocytogenes* to prolonged cold storage (Moorhead and Dykes 2004).

Tolerance to disinfectants has been studied among groups of persistent and presumed non-persistent *L. monocytogenes* strains but the results from different studies are contradictory. Some studies have demonstrated that persistent strains showed higher resistance than presumed non-persistent strains (Aase et al. 2000; Fox et al. 2011b; Lunden et al. 2003). Other studies have shown that there is little difference between persistent and presumed non-persistent strains with respect to disinfectant tolerance (Holah et al. 2002; Kastbjerg and Gram 2009).

A five-gene stress survival islet (SSI-1) has been identified in certain *L. monocytogenes* strains and has been seen to contribute to growth in suboptimal conditions. Ryan et al. (2010) created a deletion mutant which lacked SSI-1 and it was seen to have reduced growth capabilities in low pH and high salt conditions. Expression of the islet gene was seen to be regulated by SigB, the global stress regulator which has previously been seen to be an important virulence factor in gastrointestinal invasion in listeriosis (Zhang et al. 2011).

The SSI-1 was seen to be present in approximately 50 % of strains tested and is present in the commonly used laboratory strain *L. monocytogenes* EGD-e (Ryan et al. 2010). SSI-1 has been frequently identified in 1/2c, 3b and 3c strains tested but is not generally found in serogroup 4 strains (Hein et al. 2011). From the reduced growth capabilities of the deletion mutant in suboptimal conditions, SSI-1 appears to contribute to stress survival in suboptimal conditions such as those encountered in certain foods or even in the gastrointestinal tract.

Biofilm formation is an important factor in the survival of *L. monocytogenes* strains in the environment (Harvey et al. 2007). Strong adherence to surfaces, and especially biofilm formation may contribute to the ability of *L. monocytogenes* to survive cleaning procedures. Bacteria in a biofilm display altered behaviour in comparison to the behaviour of planktonic cells. This can include increased adherence, increased resistance to stresses and increased tolerance to disinfectants (Bremer et al. 2006). Bacteria in a biofilm may display altered gene expression, cell morphology, growth rate and can produce extracellular polysaccharide (EPS) which has a protective effect and has been seen to be important in biofilm formation (Chae et al. 2006). The biofilm structure itself helps to protect *L. monocytogenes* from both physical and chemical stresses (Cruz and Fletcher 2011). Although the adhesion ability of *L. monocytogenes* is affected by conditions of low temperature, varying pH and low nutrient availability commonly found in a food processing facility (Galvão et al. 2012), biofilms have been routinely identified in multiple food processing facilities worldwide (Cruz and Fletcher 2011; Latorre et al. 2010). The wear of equipment over time may facilitate the formation of biofilms as the bacteria can attach to scratches or imperfections which develop in the equipment (Latorre et al. 2010).

Although disinfectants and sanitizers may be effective against planktonic cells, their effect on biofilms can be variable (Bremer et al. 2006). Norwood and Gilmour (1999) found statistically greater mean adherence ability among persistent strains compared to presumed non-persistent strains. However, the results were not entirely consistent as some individual non-persistent strains showed high adherence. Using a microtitre plate assay method, Djordjevic et al. (2002) did not find higher adherence among persistent strains. In a study by Lunden et al. (2000), it was shown that persistent strains showed enhanced attachment over short periods of time, although some presumed non-persistent strains matched, or in some cases surpassed, the levels of attachment of persistent strains after 72 h. A recent study found better adherence of persistent strains than sporadic strains from the dairy environment (Latorre et al. 2011). Higher biofilm formation among persistent compared to presumed non-persistent strains from bulk milk samples was also described by Borucki et al. (2003). Latorre et al. (2010) conducted a study monitoring the epidemiology of *L. monocytogenes* strains on a dairy farm, in which they postulated that biofilm formation was responsible for repeated contamination events during the study period. The work, including typing of *L. monocytogenes* strains isolated from bulk milk and milking equipment, and examination of biofilms on the milking equipment, supported the view that the ability of *L. monocytogenes* to form biofilms is important in persistence of strains.

In addition, it has been shown that strongly adherent *L. monocytogenes* strains have an increased invasive ability in both cell cultures (Kushwaha and Muriana 2010b) and in vivo in mouse assays (Kushwaha and Muriana 2010a). Therefore, the *L. monocytogenes* strains in a biofilm may have increased virulence compared to planktonic *L. monocytogenes* cells. This further increases the need to eliminate persistent *L. monocytogenes* biofilms from the food processing environment.

2.3.2 Stress Response and Sigma B

L. monocytogenes has the ability to survive and even grow under stress conditions e.g. at refrigeration conditions, or in the host. Survival and adaptation in the host has recently been reviewed by Gahan and Hill (2014).

Sigma factors contribute to stress survival in bacteria. A sigma factor (σ) is a specialised protein subunit that is required for initiation of RNA synthesis. Along with the RNA polymerase, it binds to a specific promoter sequence and in that way determines which genes are transcribed. Different bacteria have a different number of sigma factors, but all cells have primary sigma factors which direct transcription of essential genes, and alternative sigma factors, the activity of which depends on the environmental conditions in which the cells exist. The larger the number of sigma factors a cell has, the greater the ability it has to adapt to stressful environmental conditions. Some of the common sigma factors include σ^{70} , σ^{38} , σ^{28} and σ^{32} . σ^{32} (RpoH) for example (the heat shock sigma factor), is turned on when the bacteria are exposed to heat. Due to the higher expression, the factor will bind with a high

probability and in doing so other heat shock proteins are expressed. This enables the cell to survive higher temperatures. Some of the enzymes that are expressed on activation of σ^{32} include chaperones, proteases and DNA-repair enzymes. The system is quite complex as there are anti-sigma factor proteins and anti-anti-sigma factor proteins.

In *L. monocytogenes*, *sigB* encodes σ^B which contributes to stress survival of *L. monocytogenes* under acid and osmotic stress and also has a role in stationary phase stress response (O'Byrne and Karatzas 2008). It also directly upregulates virulence genes, and is responsible for regulation of >100 genes (Mujahid et al. 2013). Gene deletion (see Chap. 3) has been used to study the function of σ^B in regulating stress and virulence genes (Wiedmann et al. 1998). For a review of alternative sigma factors and their role in virulence, see Kazmierczak et al. (2005).

2.3.3 Virulence and Virulence Factors

In order to cause an infection, *L. monocytogenes* has many obstacles to overcome. It must first resist the passage throughout the intestinal tract, recognize and target human cells, adhere to and enter into them, delay phagosome maturation, escape into the cytoplasm, control the production of different factors such as toxins, and identify pathways to infect other cells (Camejo et al. 2011). The expression of several virulence factors makes all this possible. Having developed a large arsenal of virulence determinants, *L. monocytogenes* is capable of infecting a large variety of cells, tissues and organs.

A major group of virulence factors are internalins. Internalins are a family of proteins characterised, in part, by the presence of one or more leucine rich repeats (LRR) at their amino (N)-terminal ends. Genome sequencing has revealed 24 different types of internalins in *L. monocytogenes* (Pizarro-Cerdá et al. 2004). Internalin A (InlA) is the best understood member of the internalin family and is vital to bacterial invasion of non-professional phagocytic cells such as epithelial cells in the intestine (Fig. 2.3). InlA binds to E-Cadherin of the host cells; this facilitates bacterial adhesion and invasion (Chen et al. 2011). The LLR region of InlA binds to the cytoplasmic region of E-Cadherin and *L. monocytogenes* uses the ability of E-Cadherin to form junctions and facilitate crossing the epithelial cell barrier (Schubert et al. 2002). Variations in the genes encoding InlA have been shown to occur in natural populations of *L. monocytogenes*. These mutations result in excretion of a truncated InlA which has limited or no activity (Chen et al. 2011; Nightingale et al. 2005). Several premature stop codons (PMSC) that result in truncated InlA have been reported, and further PMSCs continue to be described. The truncated InlA is interpreted as virulence reduction for these strains. However, some strains with truncated InlA have been shown to cause outbreaks, so a truncated InlA is not definitively indicative of an avirulent strain. In the future, it may be possible to ascertain the risk posed by strains with truncated InlA, but currently they are considered virulent by regulatory authorities. Internalin B (InlB) has been shown to

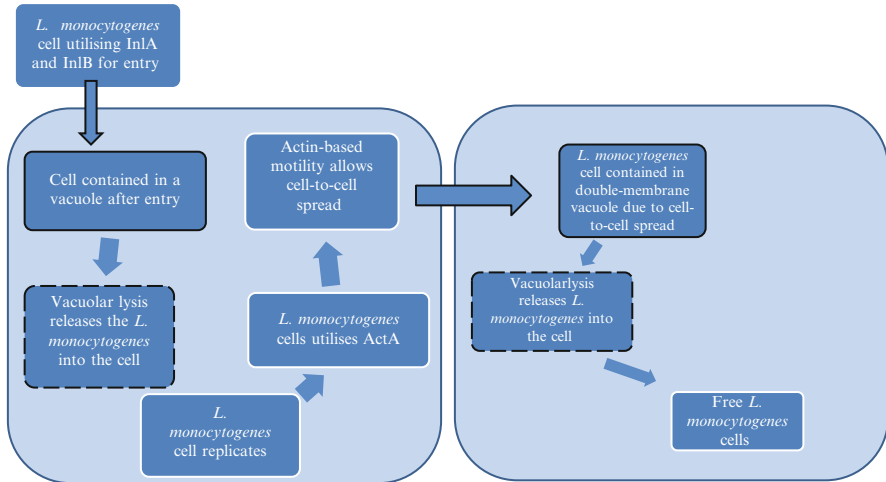


Fig. 2.3 Invasion of cells by *L. monocytogenes*

bind to the hepatocyte growth factor receptor (c-Met) with glycosaminoglycans (Pizarro-Cerdá et al. 2004). InlB appears to act as a co-factor in bacterial invasion. It may increase availability of E-Cadherin to InlA among other functions including increasing cell signalling and cell scattering (Pizarro-Cerdá et al. 2004). The InlA and InlB genes are encoded on the same operon and are co-expressed and co-regulated. Other internalins and their roles in establishing infection are currently poorly understood.

Listeriolysin O (LLO) is a haemolysin which is a major virulence factor in *L. monocytogenes* infection. Following invasion of the epithelial cells, the bacteria are enclosed in vacuoles in the cell. LLO lyses the vacuole to allow escape of the bacteria into the cytosol where it can multiply and cause further infection. The precise method of this lysis is not fully understood, although it is thought that the action of LLO is triggered by the low pH in the vacuole (Meyer-Morse et al. 2010). LLO is a pore-forming toxin which is cholesterol dependent as it has certain structural features and functions in common with host cholesterol-dependent cytolysins (Gekara et al. 2010). LLO has also been seen to suppress the immune response to the infection. LLO suppresses T cell activation by blocking T cell signalling (Gekara et al. 2010). LLO also appears to repress the innate immune response by altering the micro RNA (miRNA) activity in infected macrophages. Schnitger et al. (2011) have shown that LLO alters the miRNA signatures in infected macrophages. miRNAs are essential for post-translation alteration in the cell so miRNA interference would disrupt normal cell immune responses and so promote infection (Stavru et al. 2011). LLO also induces mitochondrial fragmentation to further alter the cell's natural behaviour (Stavru et al. 2011).

Listeriolysin S (LLS) has been identified relatively recently; previously it was thought that LLO was the only haemolysin of *L. monocytogenes*. LLS is only associated with lineage 1 evolutionary line, serotypes 1/2b and 4b, and confers increased virulence to *L. monocytogenes* infections (Cotter et al. 2008). Serotype 4b is the serotype most commonly associated with listeriosis infection and testing strains for virulence potential based on presence or absence of LLS is a possibility for the future differentiation of strains with different disease causing potential (Clayton et al. 2011).

The gene *actA* encodes for the product ActA which is a virulence determinant. At temperatures below 30 °C, *L. monocytogenes* is motile by flagella. However, at mammalian body temperature, ActA re-arranges the host actin by polymerisation for movement (Jacquet et al. 2002). This actin rearrangement allows the bacteria to spread from cell to cell and allows intracellular movement (Travier et al. 2013). ActA has also been shown to be important in adhesion as direct ActA-ActA interactions help bacterial aggregation, which help the bacteria to persist in the intestine (Travier et al. 2013).

L. monocytogenes is adapted to invade and thrive in the mammalian body. The low pH and high osmolarity of the stomach and the presence of bile salts in the gastrointestinal tract are usually major barriers to bacterial infection. *L. monocytogenes*, however, is able to survive these stresses to invade host cells and cause infection. SigB is a regulatory factor (see Sect. 2.3.2) which has been seen to be vital in surviving stress, especially bile stress tolerance (Zhang et al. 2011). SigB and PrfA have been shown to have some overlap in function. PrfA is a major regulatory factor which affects the gene expression of *L. monocytogenes* in response to its environment. PrfA seems to be mainly responsible for the regulation of virulence genes in a mammalian host environment (Bruno and Freitag 2010). The *hly* gene, responsible for LLO production, and the *actA* gene which encodes for ActA are physically linked in a listeria pathogenicity island (LIPI-1) (Dewamitta et al. 2010) which is regulated by PrfA (Jacquet et al. 2002).

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