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Listeria monocytogenes in the Food Processing Environment

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Preface

Listeriosis, a food-borne disease caused by *Listeria monocytogenes*, is a major concern for public health authorities. In addition, addressing issues relating to *L. monocytogenes* is a major economic burden on industry. Awareness of its ubiquitous nature and understanding its physiology and survival are important aspects of its control in the food processing environment and the reduction of the public health concern. *L. monocytogenes* can survive and even grow at refrigeration temperatures and high salt concentrations, conditions normally used to control bacteria. It can also survive various other stresses encountered in food processing, for example acid stress. In addition, it can form biofilm which facilitates its survival in processing environments. Appropriate methodologies are required for its detection and isolation. Characterisation of strains by pulsed field gel electrophoresis (PFGE) and other genotypic methods can facilitate identification of putative contamination routes, while gene manipulation can lead to an understanding of its survival mechanisms. Whole genome sequencing (WGS) of outbreak strains is becoming a part of outbreak investigation. Such WGS will lead to a greater understanding of the physiology of the organism as well as contributing to understanding epidemiology and pathogenicity. However, despite the advances of WGS, the best mechanism of public health protection is prevention. Awareness of its presence and control by conventional hygiene methods or by novel biocontrol methods such as bacteriocins and bacteriophage will help prevent cross-contamination of food from the environment and therefore reduce the public health burden. Listeria monitoring programmes such as those in Austria and Ireland can verify the success of control strategies.

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Contents

Chapter 1
Introduction

Despite extensive research in recent years, *Listeria monocytogenes* continues to be a threat to public health and a challenge to the food industry, particularly the ready-to-eat food industry. As consumer demand for less processed, less preserved, longer shelf-life ready-to-eat food increases, the threat of *L. monocytogenes* to public health and the food industry also increases. In the genus *Listeria* there are now 15 different species. However, *L. monocytogenes*, and to a lesser extent *L. ivanovii*, currently remain as the only pathogenic species. *L. monocytogenes* is pathogenic to humans, causing listeriosis, and *L. ivanovii* is mainly pathogenic to animals, although a few cases of human infection have been reported (Guillet et al. 2010; Snapir et al. 2006).

L. monocytogenes is a Gram positive, non-spore-forming, motile foodborne pathogen that is widely dispersed in the environment, being found in soil, water, and plant material. It can grow at refrigeration temperature, at pH 4.7 and at 10 % salt. It can persist in the harsh conditions of the food processing environment from which it can contaminate food. *L. monocytogenes* can also be carried asymptomatically by humans and animals. With the lack of a cooking or other anti-bacterial step, *L. monocytogenes* can persist in ready-to-eat (RTE) food, and if conditions become favourable, it can grow to numbers high enough to cause infection.

Listeriosis, infection with *L. monocytogenes*, can be a mild illness but the ability of the pathogen to cross the epithelial barrier of the intestinal tract, the blood brain barrier and the feto-placental barrier can also result in more severe illness like bacteremia, meningitis or spontaneous miscarriage. Although relatively rare, infection with *L. monocytogenes* can have a mortality rate of up to 30 %, resulting in a serious hazard, particularly for the high risk groups of the elderly and immunocompromised individuals.

Current knowledge suggests that cases of listeriosis are almost exclusively through foodborne infection. However, this critical transmission vector only became clear during the 1980s, principally as a result of a series of high-profile disease outbreaks, particularly the Canadian outbreak of 1981, linked to contaminated coleslaw. With many foodborne outbreaks every year since then, including the cantaloupe

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outbreak in the United States in 2011/2012, which was the most severe foodborne outbreak attributed to a bacterial pathogen, *L. monocytogenes* has been a driving force in the development of disease surveillance and control strategies. This includes global surveillance networks such as PulseNet, which allows international comparison of different strains of *L. monocytogenes* in order to facilitate early detection of outbreaks.

There has been an extensive amount of research on *L. monocytogenes* in recent years, more than could be addressed in a *Springer Brief*. This book addresses recent knowledge on *L. monocytogenes* from a practical viewpoint, addressing isolation of the organism, characterisation of isolates, occurrence/prevalence in food and food processing environments and the consequences and control of that occurrence. The focus of this *Springer Brief* is on practical aspects of occurrence and control of *L. monocytogenes*; other aspects such as mechanisms of infection or stress adaptation and survival, although important, are only briefly summarised.

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 (intrauterine 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

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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
United States of America					
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	Linnan 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)

(continued)

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)
Europe					
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	Lyytikainen 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)
Canada					
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)

(continued)

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)
Australia/New Zealand					
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)
Japan					
2001	Japan	38	Washed-type	1/2b	Makino et al. (2005)
Chile					
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 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*Listeria* spp.); 0.9 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*L. monocytogenes*)		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' L. Mono™ Agar	4 % (*L. monocytogenes*—RTE foods)	*L. monocytogenes*: 6 % (filled baguettes); 3 % (assorted salads)	Christison et al. (2008)

(continued)

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 (*Listeria* enrichment broth and modified Fraser broth). Plating onto Palcam agar	56 % (*Listeria* spp.); 38 % (*L. monocyto-genes*)	*L. monocytogenes*: 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 % (*Listeria* spp.); 8.7 % (*L. monocytogenes*)		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 % (*Listeria* spp.); 5.3 % (*L. monocytogenes*)	15.8 % of positive samples showed *L. monocytogenes* counts >100 CFU/g	Awaisheh (2009)
Latvia	RTE vacuum-packaged meat products (211 samples)	EN/ISO 11290-1 and EN/ISO 11290-2	18 % (*L. mono-cytogenes*)	*L. monocytogenes*: 42 % (cold-smoked, sliced, vacuum-packed beef and pork products); 0.8 % (cooked, sliced, vacuum-packaged meat products) 16 % of positive samples showed *L. monocytogenes* counts >100 CFU/g	Berzins 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 % (*L. mono-cytogenes*)	*L. monocytogenes*: 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%(*L.monocytogenes*)	*L. monocytogenes*: 8 % (milk products); 27.5 % (raw meat); 18 % (meat products); 30 % (fish); 0 % (salads)	Filiousis et al. (2009)
Ireland	Environmental samples in farm milking facilities (298 samples)	FDA method	19 % (*L. monocytogenes*)		Fox et al. (2009)
Spain	Deli meat products, smoked fish and paté (783 samples)	EN/ISO 11290-1 and EN/ISO 11290-2	12.7 % (*Listeria* spp.); 6.2 % (*L. monocytogenes*)	*L. monocytogenes*: 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 paté) 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 % (*Listeria* spp.); 2.3 % (*L. monocytogenes*)	*L. monocytogenes*: 7.0 % (sandwiches); 4.2 % (sliced meats) *L. monocytogenes* 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 % (*Listeria* spp.); 9.5 % (*L. monocytogenes*)	*L. monocytogenes*: 12 % (smoked fish); 4.76 % (cooked marinated products); 20 % (meat products), 2 % (vegetable salads)	Meloni et al. (2009)

(continued)

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 % (*Listeria* spp.); 4.8 % (*L. monocytogenes*)	*L. monocytogenes*: 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 Listeria Selective Agar	4.3 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*L. monocytogenes*—rtPCR); 4 % (*L. monocytogenes*—immunoassay and culturing)	*L. monocytogenes* at >100 CFU/g: 12 % (vacuum-packed RTE products)	Netschajew et al. (2009)
Ireland	Cheese (351 samples)	FDA method	6 % (*L. monocytogenes*)		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 % (*L. monocyto genes*)	*L. monocytogenes*: 6.7 % (mayonnaise-based deli-salads); 1.1 % (cooked meat products); 27.8 % (smoked fish) Positive samples with *L. monocytogenes* at >100 CFU/g: 16 % (smoked fish)	Uyttendaele et al. (2009)

United Arab Emirates	RTE salads	EN/ISO 11290-1	0 % (*L. monocyto-genes*)		Almualla et al. (2010)
Brazil	Minimally processed leafy vegetables from retail market (162 samples)	Immunoassay Listeria Rapid Test	3.7 % (*Listeria* spp.); 1.2 % (*L. monocytogenes*)		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 % (*L. monocytogenes*)	*L. monocytogenes*: 19.2 % (beef meat products); 15 % (poultry products)	Awaisheh (2010)
Finland	RTE cold-smoked pork products and plant environment of a single processing plant (183 samples)	EN/ISO 11290-1	21 % (*L. monocyto-genes*)	*L. monocytogenes*: 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)	Berzins et al. (2010)
Switzerland	Ingredients, final product and environmental samples from a single sandwich-producing plant (2,245 samples)	EN/ISO 11290-1	7.4 % (*L. monocyto-genes*—ingredients and sandwiches)	3.5 % (*L. monocytogenes*—environmental swabs)	Blatter et al. (2010)
Italy	RTE salads from retail stores (1,158 samples)	Immuno-enzymatic method mini-VIDAS SLM	0 % (*L. monocyto-genes*)		Caponigro et al. (2010)
Italy	Traditional Chinese food from restaurants and take-away establishments (118 samples)	EN/ISO 11290-1	0 % (*L. monocyto-genes*)		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 *Listeria* enrichment broth. Plating onto *Listeria* selective agar (Oxoid)	0.7 % (*L. monocyto-genes*)		Chung et al. (2010)

(continued)

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 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*L. monocytogenes*)	*L. monocytogenes* 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 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*L. monocytogenes*)		Maklon et al. (2010)

United Kingdom	RTE foods (5,840 samples)	Enrichment in Fraser broth; plating onto *Listeria* selective agar (Oxford formulation)	4.45 % (*Listeria* spp.); 2.55 % (*L. monocytogenes*)	*L. monocytogenes*: 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 *L. monocytogenes* 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 % (*L. monocytogenes*)	*L. monocytogenes*: 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% (*L. monocytogenes*)		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 % (*Listeria* spp.); 7.35 % (*L. monocytogenes*)		Perez-Rodriguez et al. (2010)
Italy	Raw meat and retail RTE products (1,268 samples)	EN/ISO 11290-1	11.7 % (*Listeria* spp.); 3.2 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*Listeria* spp.); 4.7 % (*L. monocytogenes*)	*L. monocytogenes*: 15.6 % (meat and meat products); 0 % (dairy and dairy products); 3.3 % (fresh vegetables); 0 % (fresh seafood); 1.1 % (RTE foods)	Stonsaovapak and Boonyaratanakornkit (2010)
Israel	Salads/dips, dairy, fish, poultry and meat products (10,413 samples)	USDA Laboratory Guidebook	12.1 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*L. monocytogenes*—VIDAS); up to 22.2 % (*L. monocytogenes*—PCR); up to 19.1 % (*L. monocytogenes*—qPCR)		Elizaquível et al. (2011)

Egypt	Street-vended RTE foods, including sandwiches and dishes of traditional food (576 samples)	Enrichment in *Listeria* Selective Broth; plating onto Oxford agar	24 % (*Listeria* spp.); 14 % (*L. monocytogenes*)	*L. monocytogenes*: 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 environment and external environmental samples from 16 farmhouse cheese manufacturing facilities (1,591 samples)	EN/ISO 11290-1	15.7 % (*L. monocytogenes*)	*L. monocytogenes*: 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 *L. monocytogenes* (LMO) II assay; plating onto modified Oxford media	11.4 % (*L. monocytogenes*)	*L. monocytogenes*: 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), panned 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 % (*Listeria* spp.); 1.92 % (*L. monocytogenes*)		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 % (*Listeria* spp.); 3.5 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*Listeria* spp.); 2 % (*L. monocytogenes*)		Mottin et al. (2011)
Italy	RTE salads (48 samples)	USFDA Bacteriological Analytical Manual and/or ISO protocol 11290-1	31.2 % (*Listeria* spp.); 6 % (*L. monocytogenes*)		Nabi et al. (2011)
Jordan	Traditional foods and dairy products (120 samples)	–		Four *Listeria* 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 % (*Listeria* spp.); 18.2 % (*L. monocytogenes*)	*L. monocytogenes*: 9.4 % (fresh broiler chicken); 13.3 % (chicken-shawirma); 76.7 % (chicken burguers); 30 % (chicken sausages); 0 % (mortadela)	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 % (*Listeria* spp.); 8.1 % (*L. monocytogenes*)		Syne et al. (2011)

USA	Environmental samples from small or very small RTE meat processing plants (688 samples)	USDA-FSIS procedure	9.5 % (*Listeria* spp.); 6.1 % (*L. monocytogenes*)		Williams et al. (2011)
Switzerland	RTE lettuce, fresh-cut fruit, and sprouts (233 samples)	EN/ISO 11290-1	2.1 % (*L. monocytogenes*)	*L. monocytogenes*: 3.5 % (RTE lettuce); 0 % (fresh-cut fruit and sprouts)	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 % (*Listeria* spp.); 2.6 % (*L. monocytogenes*)	*L. monocytogenes*: 5.1 % (soft cheese); 2.8 % (whey); 14.2 % (sandwiches); 1.2 % (cooked meat dishes); not determined in other products	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 % (*Listeria* spp.); 12.9 % (*L. monocytogenes*)	*L. monocytogenes*: 14.1 % (raw products); 12.2 % (ready-to-cook products); 11.4 % (RTE products)	Fallah et al. (2012)
Italy	RTE seafood products (38 samples)	EN/ISO 11290-2	23.68 % (*L. monocytogenes*)	*L. monocytogenes* at concentration >100 cfu/g: 7.9 %	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 % (*Listeria* spp.); 2.5 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*Listeria* spp.); 6.7 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*L. monocytogenes*)	*L. monocytogenes*: 0.4 % (cheese); 1.2 % (meat products); 12 % (fish products); 14 % (gravad and cold-smoked fish); 2 % (hot-smoked fish) *L. monocytogenes* 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 % (*L. monocytogenes*)		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 % (*L. monocytogenes*)	*L. monocytogenes*: 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	*L. monocytogenes* 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 % (*Listeria* spp.); 3 % (*L. monocytogenes*)	*L. monocytogenes* 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 % (*L. monocytogenes*—by culture); 10.47 % (*L. monocytogenes*—by multiplex-PCR); 32.98 % (*L. monocytogenes*—by DVC-FISH)		Moreno et al. (2012)
Japan	Imported cheese and non-cooked meat products (150 samples)	Bacteriological Analytical Manual	4.1 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*Listeria* spp.); 1.9 % (*L. monocytogenes*)		Rahimi et al. (2012)
Brazil	Packaged RTE vegetables (512 samples)	EN/ISO 11290-1	3.1 % (*L. monocytogenes*)		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 % (*L. monocytogenes*)		Taban (2012)
Iran	Raw/fresh, frozen, and RTE seafood products (245 samples)	Multiplex-PCR		*L. monocytogenes*: 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 % (*L. monocytogenes*)	*L. monocytogenes*: 10.3 % (hot or cold smoked or gravid fish); 2.07 % (packaged heat-treated meat products); 0.47 % (soft and semi-soft cheese) *L. monocytogenes* 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 % (*L. monocytogenes*)	*L. monocytogenes*: 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 % (*L. monocytogenes*—raw and RTE foods); 17 % (*L. monocytogenes*—processing environments)	*L. monocytogenes*: 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 % (*L. monocytogenes*)	*L. monocytogenes*: 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 streetside hawker stalls (396 samples)	EN/ISO 11290-1. Different selective agar were used, CHROMagar-Listeria, Listeria selective agar and PALCAM agar	17.9 % (*Listeria* spp.); 11.4 % (*L. monocytogenes*)	*L. monocytogenes*: 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 *Listeria* Enrichment broth. Plating on Palcam agar. Confirmation by API *Listeria* strip	6.8 % (*L. monocytogenes*)	*L. monocytogenes*: 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 *Listeria* enrichment broth. Plating on Palcam agar Real-time PCR	10.4 % (*L. monocytogenes*—by culture); 11.0 % (*L. monocytogenes*—by RT-PCR)	*L. monocytogenes*: 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 % (*Listeria* spp.) and 1 % (*L. monocytogenes*)		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 % (*L. monocytogenes*)	*L. monocytogenes*: 2 % (RTE meat); 0.3 % (RTE dairy); 5.4 % (fish); 2.2 % (smoked meat); 3.6 % (mixed meat products) *L. monocytogenes* 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 % (*L. monocytogenes*)		Manguiat and Fang (2013)
Spain	Plant-based foods (cereals, legumes, fruits and vegetables) (781 samples)	EN/ISO 11290-1	0 % (*L. monocytogenes*)		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 % (*Listeria* spp.); 1.6 % (*L. monocytogenes*)		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.

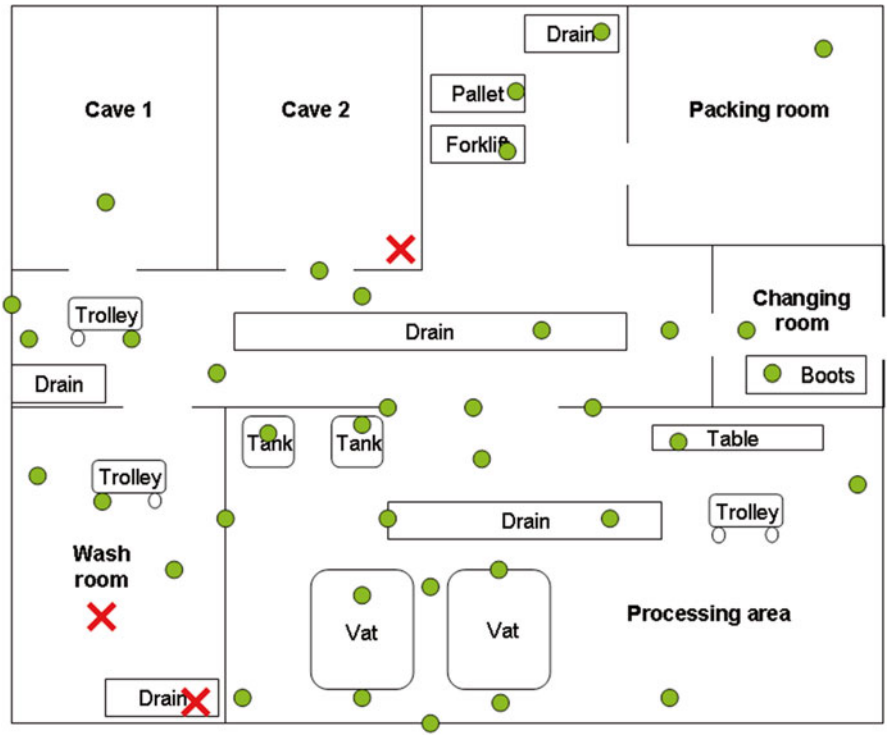

Fig. 2.1 A factory map (rough drawing; not to scale) showing potential sampling points. (**a**) green circle indicates negative sampling sites and, (**b**) red 'x' indicates positive sampling sites

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/microbiollimits/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 *Lm* 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 *L. monocytogenes*: 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 *L. monocytogenes* (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 *L. monocytogenes* 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

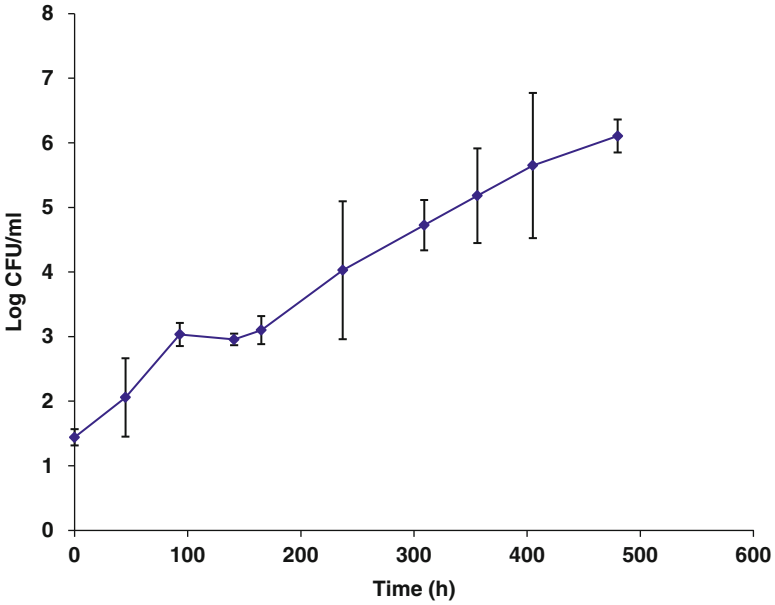

Fig. 2.2 Growth of *L. monocytogenes* on the surface of smoked salmon artificially inoculated with a cocktail of three strains and incubated at 8°C for 7 days and 12°C for 14 days

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

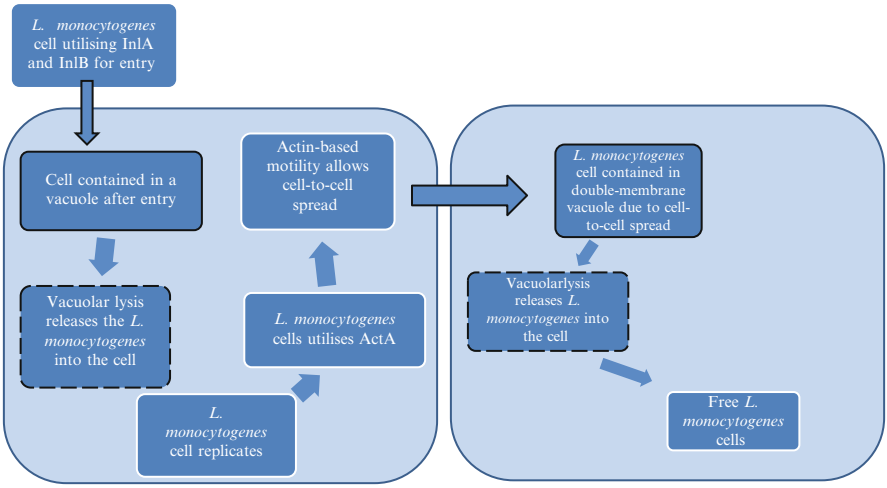

Fig. 2.3 Invasion of cells by *L. monocytogenes*

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).

Chapter 3
Gene Manipulation

In order to study gene function, the ability to manipulate chromosomally encoded genes is important. Several strategies have been developed to achieve this, including gene deletion and gene mutation studies.

3.1 Gene Deletion

There are several methods that can be used for gene deletion. In particular, the splicing overlap extension PCR (SOEing-PCR) method (Horton et al. 1990) is useful. The method involves generation of a spliced amplicon from the regions flanking the gene targeted for deletion. Different plasmid-based approaches can be used to facilitate the introduction of this spliced amplicon into the chromosome in place of the targeted gene. These approaches use temperature-sensitive cross-over events, with antibiotic resistance genes on the plasmid being used for selection of the recombinant strains. Studies with the recombinant strains can be used to demonstrate phenotypic changes as a result of the deleted gene (Abram et al. 2008; Muller et al. 2014). Using the SOEing-PCR method (Muller et al. 2014), showed that deletion of the gene *qacH* (which encodes is a small multidrug resistance transporter putatively associated with export of the quaternary ammonium compound benzalkonium chloride) resulted in reduced resistance to benzalkonium chloride. Strategies of gene deletion have been widely used in other organisms and are beginning to be used to study survival and resistance mechanisms in *L. monocytogenes*, which are important in developing effective strategies to control *L. monocytogenes* in the processing environment and during infection of the host.

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3.2 Mutant Libraries

Construction and screening of mutant libraries for altered phenotypic traits is a valuable tool in identifying genes responsible for particular phenotypes. Transposons, such as TN*916* and Tn*917* (Kathariou et al. 1987) or a *Tc1/mariner*-based system (Cummins et al. 2013) are often used for such library construction in *L. monocytogenes*. The principle behind such mutant libraries is insertional mutagenesis, where the transposon inserts into a gene thus disrupting the function of that gene. The position of the transposon can be determined by sequencing and the phenotypic effect of such gene disruption studied. Furthermore, the gene function can be complemented by insertion of a plasmid expressing the gene, leading to restoration of the wild-type phenotype. Using this method, Cummins et al. (2013) identified transposon insertion mutants of *L. monocytogenes* that are compromised for infection via the oral route. In addition, insertions into known virulence-related genes as well as into genes which encode another internalin and a transcriptional regulator were also identified.

Chapter 4
Sampling and Laboratory Analysis

Due to the ubiquitous nature of *L. monocytogenes*, contamination of food processing facilities is common. Although transient contamination may be somewhat unavoidable, *L. monocytogenes* strains can persist for long periods of time and cause more serious problems, as described earlier. They often occur in the processing environment with little competition for space/ nutrients etc. from other bacteria. Sampling for *L. monocytogenes* in a processing environment is an important tool for identifying not only if *L. monocytogenes* contamination is present, but also if more than one *L. monocytogenes* strain is present and if the contamination is persistent. The EURL *Lm* has produced a guidance document on sampling premises for *L. monocytogenes* (EU 2012). Sampling of a food processing environment is best performed using a pre-moistened sponge swab stick for testing surfaces (Fig. 4.1) (both food contact surfaces and non-food contact surfaces) and sterile dippers for liquids. Swab samples should be taken from a surface of approximately 1 m^2 if possible, in a zigzag pattern, and the swab then returned to its sterile bag for transport to the laboratory. Although it is important to focus on sampling areas which come into direct contact with food, other areas in which *L. monocytogenes* contamination would be expected, such as pooled water on the floor, drains, the under-side of shelves and trollies etc. should also be sampled as areas such as these often harbour strains which can spread contamination to food contact areas and subsequently to the food itself.

Current E.U. regulations specify a number of food samples which need to be tested by producers of RTE food, but the number of environmental samples, the frequency of sampling, or the time of sampling are not specified. This allows FBOs to design their own sampling plan in terms of environmental samples. The EURL Lm guidance document on sampling can help with this process. However, the numbers of samples taken is often negatively influenced by the high price of *L. monocytogenes* sample analysis and the fear of regulatory interference which may accompany positive samples found. As such, it can be difficult to accurately monitor rates of *L. monocytogenes* in the food processing environment as the FBOs who will voluntarily take part in surveys or sampling projects are likely to have fewer positive results than those who decide not to participate in such projects.

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Fig. 4.1 The type of swab that can be used for sampling for *L. monocytogenes*—a good surface area can be sampled

Samples should be taken in a manner which increases the chances of finding positives (EU 2012). Unfortunately, FBOs who have not been properly educated concerning *L. monocytogenes* contamination may sample with an aim to obtain negative samples to appease the authorities and prevent recall issues. Sampling programmes should ensure to sample in areas in which contamination would be expected to occur, for example, drains, pooled water etc. Additionally, samples should not be taken directly after cleaning/disinfection as sampling in this manner would severely decrease the chances of finding *L. monocytogenes* positive samples. In order to obtain the most accurate representation of the contamination present, samplings plans must attempt to obtain as many positive samples as possible.

4.1 Isolation Methods for *L. monocytogenes*

As *L. monocytogenes* contamination generally occurs in very low numbers, samples cannot be directly plated and counted as this would invariably give many false negative results. Enrichment steps (usually two) are needed in order to increase the population of *L. monocytogenes* to detectable numbers. Microorganisms in food are also known to often be stressed and/or injured so the use of a pre-enrichment step allows for the recovery of these cells. An enrichment medium should allow for the recovery of stressed/injured cells as well as the increase in *L. monocytogenes* cell numbers over competing microflora. *L. monocytogenes* enrichment broths generally contain several selective agents such as acriflavine, lithium chloride, polymixin B and cephalosporins in order to prevent the growth of other bacteria and fungi while encouraging the growth of *L. monocytogenes*.

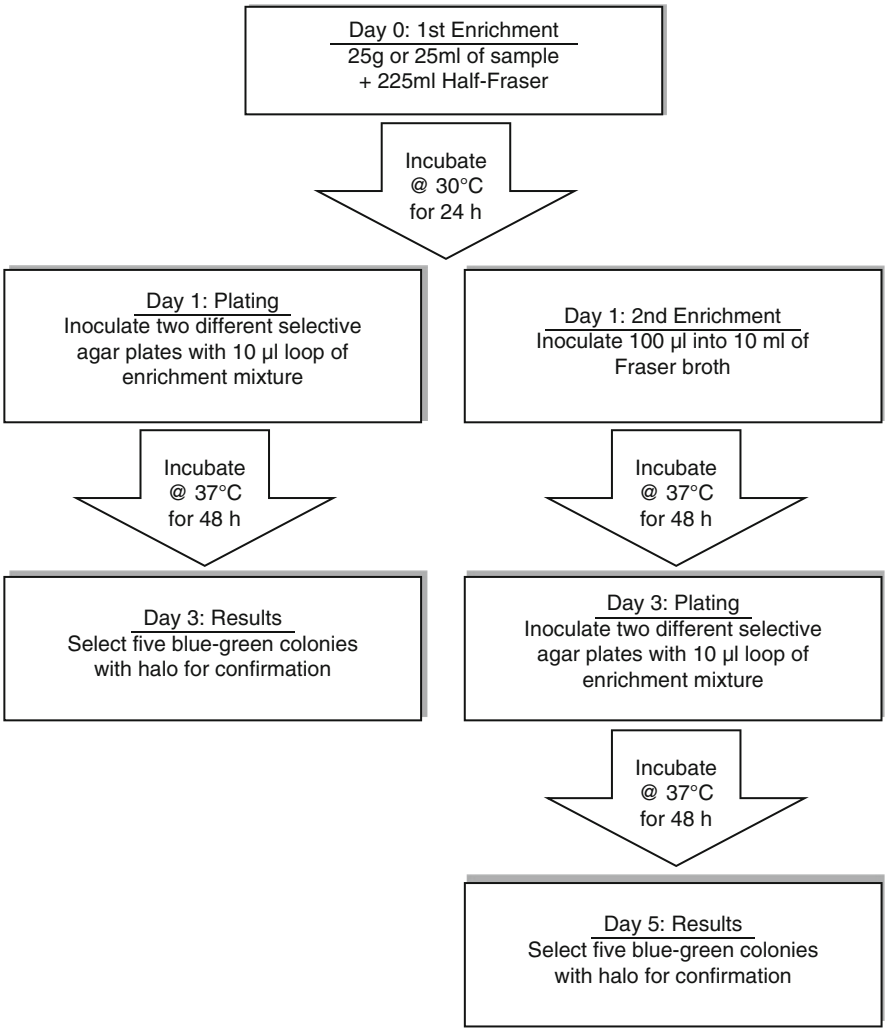


Fig. 4.2 Flowchart for the ISO analysis method

The international standard method ISO-11290 is one of the most commonly used methods for the detection/quantification of *L. monocytogenes* (Fig. 4.2). It involves two enrichment steps. First, for both liquids and foods, a 25 g/25 mL sample is added to a 1:10 dilution in half-Fraser broth (containing half the concentration of antibiotics) which is incubated at 30 °C for 24 h. This is followed by a 100 µl inoculation into 10 ml full Fraser broth incubated at 37 °C for 48 h. Both enriched cultures are plated on two Listeria selective agars, including a chromogenic agar, if possible. For sponge swab samples, 100 ml half-Fraser is added to the swab in a sterile container and the remainder of the method is identical to that used for food/liquids. Other species of Listeria can be mistakenly identified as *L. monocytogenes*

due to similar reactions on the agar plates, so further confirmation is needed. Several colonies (five are generally recommended) should be taken from each agar plate which indicates a positive *L. monocytogenes* sample. Each of the five colonies should undergo further confirmation testing to help ensure that the colonies isolated are not limited to false negative results. ISO 11290 specifies the use of biochemical tests for confirmation, so positive results can take up to 7 days to obtain. In many cases, tests involving the polymerase chain reaction (PCR) are used in place of biochemical tests, although this is generally confined to research studies. In a recently published study, Dalmasso et al. (2014) used PCR to analyse the enriched culture of 852 samples obtained from different processing environments and from different foods for the presence of *L. monocytogenes*, comparing the numbers of positive results obtained by plating and PCR. PCR of the second enrichment resulted in an increased number of positive results, indicating that this was a valid method of analysis (Dalmasso et al. 2014). PCR is a detection and confirmation assay, so confirmed results could be obtained in 3 days. Similar results were obtained during the recent outbreak involving quargel cheese (Rossmanith et al. 2010). Methods combining PCR with standard plating methods (where the results from PCR have been shown to be equal to or better than plating) offer a realistic alternative in front line testing as initial results may pre-date traditional ISO results by several days. There is the advantage that when the ISO method is continued, isolates can be obtained. This can be of vital importance in outbreak investigations. However, the high price of real-time PCR reagents may preclude this method from being used in routine sampling.

Following plating, more than one *L. monocytogenes* strain (identified by PFGE) can be present in the same sample so the isolation of more than one colony from each positive plate ensures a better chance of isolating all strains present in that sample. Both enrichment broths, and chromogenic agars contribute to the high price of *L. monocytogenes* sampling which may discourage food processors from testing a sufficient number of samples to adequately monitor *L. monocytogenes*.

A two-step enrichment procedure for the isolation of beta-haemolytic Listeria from raw meat/poultry was also developed which involves highly selective lithium chloride phenylethanol moxalactam (LPM) agar as well as a thin layer horse blood agar plate for the detection of beta-haemolysis. This method gives positive results in 3–4 days (McClain and Lee 1988).

University of Vermont medium (UVM) can also be used for the detection of *L. monocytogenes* as in the U.S. Department of Agriculture (USDA) method (Fig. 4.3). Similar to the ISO method which uses Fraser broth, two-steps of enrichment are used in the USDA method. Two enrichment broths, UVM I and UVM II, are used and differ mainly in their acriflavine concentrations. In the first enrichment step, a 25 g or 25 ml sample is added to 225 ml UVM I. The second enrichment step involves the addition of 0.1 ml of the UVM I/sample mixture (after 24 h incubation at 30 °C) to 10 ml UVM II and incubation for 24 h at 30 °C. Both the first and second enrichments are plated on *L. monocytogenes* selective agar. This method gives a detection limit of 0.04 CFU/g (Zhang et al. 2007).

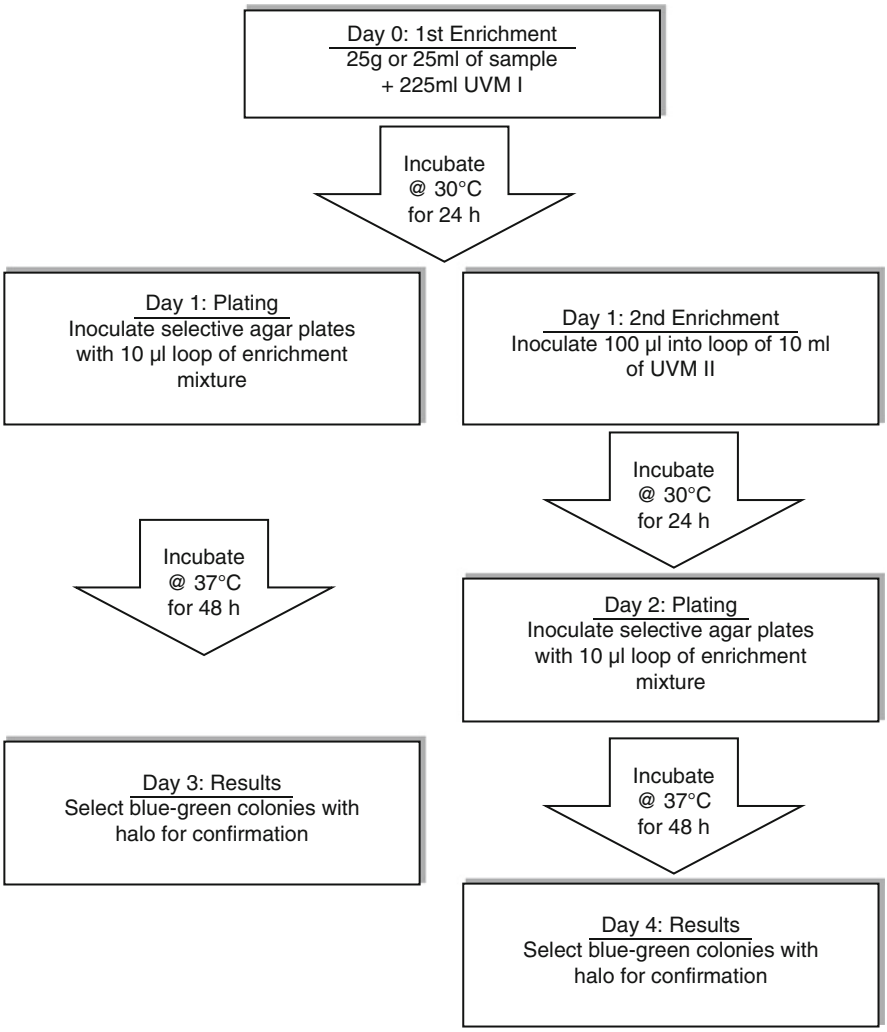


Fig. 4.3 Flowchart for the USDA analysis method

ONE broth-Listeria, which is approved by Association Française de Normalisation (AFNOR), has been advertised as giving similar results to the ISO method but with a significantly reduced incubation time of only 24 h at 30 °C and using only this single enrichment broth step, followed by plating on Listeria selective agar similar to the other methods mentioned previously (Fig. 4.4). ONE broth-Listeria contains a mixture of peptones, carbohydrates and salts to optimise *Listeria* spp. recovery and growth, however, the formula is not published. It has been shown that the limit of detection may not be as low as that obtained with other methods (Gómez et al. 2013), probably due to the shorter incubation time.

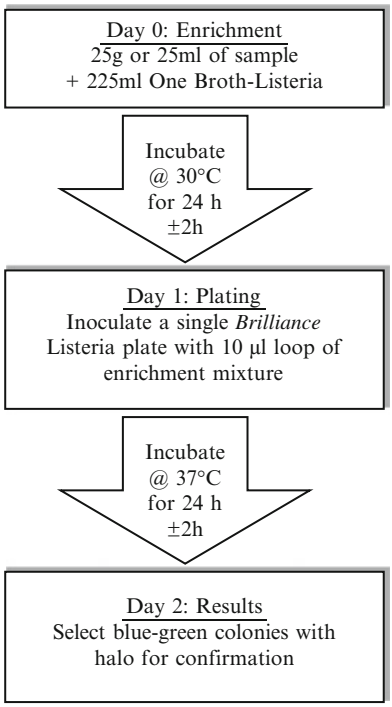


Fig. 4.4 Flowchart for the ONE-Broth analysis method

4.1.1 Confirmation of Isolates as L. monocytogenes

The use of selective agar in *L. monocytogenes* isolation gives an initial result that is presumptive positive, but confirmation of the *L. monocytogenes* isolates is needed as false positives can and do occur. Polymixin Acriflavine Lithium Chloride Ceftazidime Aesculin Mannitol (PALCAM) and Listeria selective agar (Oxford formulation) are both recommended for use in the ISO method although other *L. monocytogenes* selective agars give similar and sometimes even better results. Listeria selective agar (Oxford formulation), utilises several inhibitory components as well as the hydrolysation of aesculin and ferrous iron to differentiate *L. monocytogenes*. However, some strains of *Enterococci* can also grow on this medium and may exhibit a weak aesculin reaction. PALCAM agar often gives many false negatives as *L. innocua*, which can have higher growth rates than *L. monocytogenes* during enrichment, appears similar to *L. monocytogenes* on this agar. Other *L. monocytogenes* selective agars, including the chromogenic agars *Agar* Listeria Ottavani & Agosti (ALOA) and Brilliance Listeria Agar (BLA), are based on the phospholipase C activity and β-glucosidase activity of *L. monocytogenes* (Fig. 4.5). Some *L. ivanovii* strains can also display similar activity and appear analogous to *L. monocytogenes* (Becker et al. 2006). Other listeria species can display similar growth to *L. monocytogenes* on ALOA and BLA, e.g. round, smooth blue/green colonies.

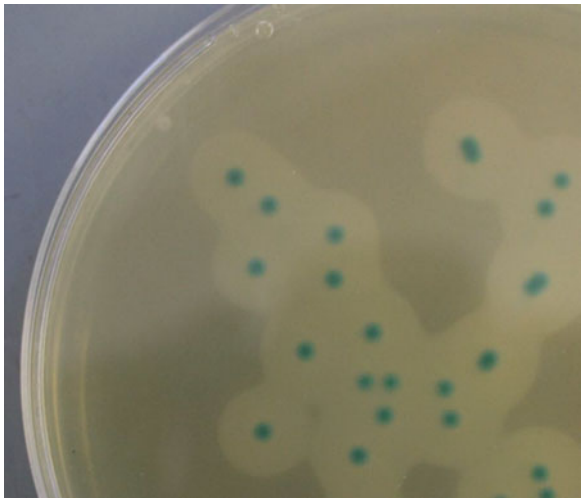


Fig. 4.5 Section of an ALOA agar plate showing blue-green colonies of *L. monocytogenes* with a halo

Often, the only visual difference between *L. monocytogenes* and other Listeria species on ALOA or BLA is whether or not a halo is produced. This can be misinterpreted if a nearby *L. monocytogenes* colony produces a halo close to another non-halo producing Listeria species on the plate. Rapid' *L. mono* uses the phospholipase activity combined with the fermentation of xylose to differentiate *L. monocytogenes* from other Listeria species and results can be obtained for *L. monocytogenes* within 24 h as opposed to BLA and ALOA where 48 h of incubation is usually needed. Overall, although the use of selective agar to identify *L. monocytogenes* is generally quite accurate, confirmation by other means should always be performed before any conclusions are drawn.

Common methods of confirmation of *L. monocytogenes* include confirmation by PCR, API kits and sequencing, which is becoming increasingly popular as costs of sequencing are reduced.

PCR is a relatively simple assay which involves the amplification of a DNA fragment. PCR methods for *L. monocytogenes* confirmation generally focus on virulence genes of *L. monocytogenes* as *L. innocua* and *L. monocytogenes* share a similar genome with the exception of certain key, virulence clusters. With conventional PCR, the DNA fragments need to be amplified, dyes added and the fragments are then run at the end-point on an agarose gel and separated by gel electrophoresis. From start to finish, conventional PCR may take several hours.

Alternatively, real-time PCR adds a fluorescent probe to the DNA fragments during replication which allows the results to be viewed during the amplification of the DNA fragments which reduces considerably the time taken to view the results (Fig. 4.6). The *hly* gene is commonly used in both conventional (Gawade et al. 2010) and real-time PCR (Rodriguez-Lazaro et al. 2004). Although real-time PCR is more expensive and requires more expertise than conventional PCR, it offers a distinct advantage as conventional PCR can only give a positive/negative result whereas with real-time PCR, *L. monocytogenes* can be measured quantitatively and not just qualitatively.

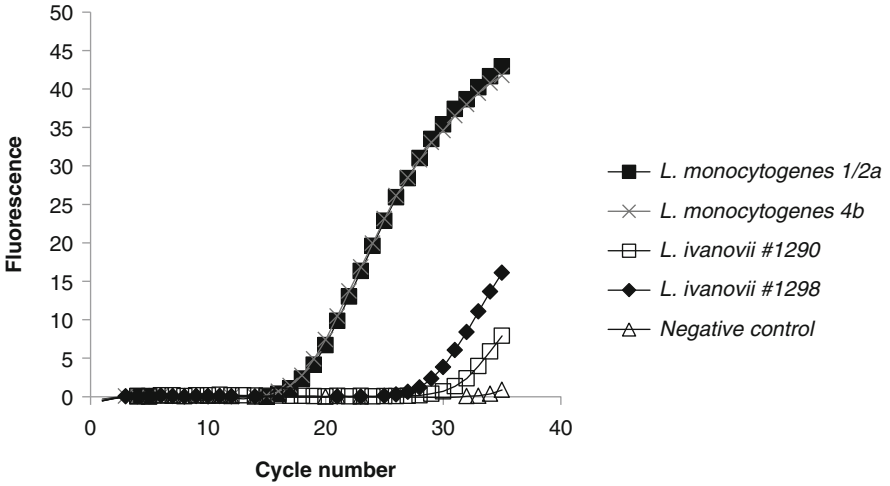


Fig. 4.6 Amplification plot for *hly* in *L. monocytogenes* and *L. ivanovii* following the rt-PCR methodology described by Rodriguez-Lazaro et al. (2004)

Quantitative PCR (qPCR) has been shown to be effective in, for example, analysis of *L. monocytogenes* in raw whole milk where detection of as low as 1 CFU/ml and quantitative analysis values of between 10 and 1,000 CFU/ml were obtained in less than 3 h (Paul et al. 2014). Real-time PCR also offers an advantage over conventional PCR as the closed-tube format helps to prevent any contamination which may occur during conventional PCR. Real-time PCR can also amplify a number of different DNA fragments which can be visualised at the same time by adding different coloured fluorescent probes to each different DNA sequence.

Various physiological methods of confirmation are available commercially in the form of kits which are easy to use and generally reliable, but can take several days to obtain results. API kits, available from Biomerieux, differentiate different species by examining their growth in the presence of various biochemical compounds. Colour change in up to 20 different biochemical reactions create a numerical pattern which can be used to indicate the bacterial species being tested. API kits are economical and easy to use but the confidence in the result is not as high as with genotypic methods. *L. monocytogenes* have been seen to be identified by API with a confidence of 97.7 % (Bille et al. 1992).

A similar bacterial identification test, Vitek 2, also available from Biomerieux, consists of a card containing 64 microwells which each contain a different reaction substrate or antibiotic. The colorimetric reaction in each well gives a bacterial identification, as well as the antibiotic susceptibility of the bacteria tested. The Vitek 2 system has been generally shown to be reliable. However, it was recently shown to be inaccurate in 1.4 % of cases seen due to atypical *L. monocytogenes* isolates which were negative for a phospholipase C reaction. According to the Vitek 2 method, these isolates were identified as *L. innocua* but were later confirmed as *L. monocytogenes* by PCR identification methods (De Lappe et al. 2014).

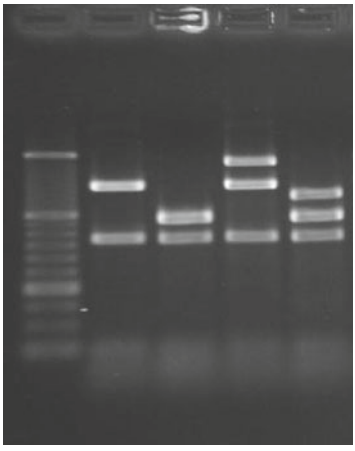
Differentiation of *L. monocytogenes* as distinct from other *Listeria* species has also been seen to be possible through phage typing. As different bacterial species are susceptible to different phages, the use of a specific phage which has a known, predictable species target can identify *L. monocytogenes* (Morton et al. 2013).

4.2 Methods for Characterisation of *L. monocytogenes*

4.2.1 Serotyping

Serotyping is historically, and continues to be, the first and foremost method of typing *L. monocytogenes*. It is based on variations in the somatic (O) and flagellar (H) antigens. Currently, 13 serotypes exist, although using commercially available methods it is very difficult to distinguish between serotypes 4b and 4e (Fig. 4.7). One significance of serotyping is the fact that 90 % of listeriosis outbreaks are caused by 1/2b and 4b/4e serotypes, while sporadic outbreaks are often caused by 1/2a and 1/2c serotypes. In food processing environments, serotype 1/2a is generally found in highest abundance, although 4b/4e, 1/2b and 1/2c are also commonly found but with less frequency (Nucera et al. 2010; Shen et al. 2013; Leong et al. 2014). However, serotyping gives relatively little information in terms of variations in strains and further characterisation is needed to identify if, for example, two strains can be considered the same.

a



b

Serovar	O-antigens	H-antigens
1/2a	I, II	A, B
1/2b	I,II	A, B, C
1/2c	I, II	B, D
3a	II, IV	A, B
3b	II, IV, (XII), (XIII)	A, B, C
3c	II, IV, (XII), (XIII)	B, D
4a	(V), VII, IX	A, B, C
4ab	V, VI, VII, IX, X	A, B, C
4b	V, VI	A, B, C
4c	V, VII	A, B, C
4d	(V), VI, VIII	A, B, C
4e	V, VI, (VIII), (IX)	A, B, C
7	XII, XIII	A, B, C

Fig. 4.7 (**a**) Agarose gel electrophoresis of DNA fragments generated by multiplex PCR to determine *L. monocytogenes* serogroup as in Doumith et al. (2004). *Lane 1*: O' RangeRuler 50 bp (Biolabs, England); *Lane 2*: 1/2a, 3a serogroup; *Lane 3*: 1/2b, 3b, 7 serogroup; *Lane 4*: 1/2c, 3c serogroup; *Lane 5*: 4b, 4d, 4e serogroup. (**b**) 13 Serovars of *L. monocytogenes* and their antigen reactions. Antigens in parentheses result in variable reactions. O-antigen III has been omitted from this table as the reaction to O-antigen III is variable for every serovar

Serotyping is traditionally performed with agglutination reactions using antisera available commercially, or specifically produced. However, this method can be costly and difficult to interpret. It relies on the visual interpretation of an agglutination reaction which can be weak and can appear similar to a non-reaction. Therefore, trained staff are needed to perform this assay. PCR is now commonly used, and this allows the identification of a strain as belonging to a certain serogroup (Doumith et al. 2004). Agglutination reactions are still needed to characterise a strain as belonging to a particular serotype within that group. This combination of PCR and serotyping can identify all serotypes, with the exception of differentiating between serotypes 4b and 4e, as serotype 4e strains can have variable agglutination reactions. While serotype 4b strains are far more common than serotype 4e strains, a method to definitively distinguish serotypes 4b and 4e is required.

4.2.2 Lineages

Based on serotype, *Listeria monocytogenes* is sub-divided into four evolutionary lineages (I, II, III, and IV) which have different but overlapping sources of origin, for review see Orsi et al. (2011). Most *L. monocytogenes* isolates belong to lineages I and II, which harbour the serotypes more commonly associated with human clinical cases. Lineage II isolates (which include serotype 1/2a) are common in foods, widespread in natural and farm environments, and are commonly isolated from animal listeriosis cases and sporadic human clinical cases. Lineage I isolates (which include serotypes 1/2b and 4b) are associated with the majority of human listeriosis outbreaks. Lineage III and IV strains are generally rare, although some serotype 4b strains can be from lineage IV, and are predominantly isolated from animal sources. Attempts to identify phenotypic traits specific to lineages have been for the most part unsuccessful. However, some generalisations on phenotypic traits of lineages can be made. Lineage II isolates generally have more plasmids and seem to be more resistant to bacteriocins than lineage I isolates. They also frequently have a premature stop codon in *inlA* leading to a truncated protein, and mutations in *prfA*. Lineage I isolates, on the other hand, can carry listeriolysin S.

4.2.3 Pulsed Field Gel Electrophoresis

Generally, PFGE is considered the "gold standard" for *L. monocytogenes* characterisation, however, with the technological advances and the progressively reducing cost of genome sequencing, this may not be the case in the near future. Although PFGE gives better differentiation than many other methods, including many mentioned here, the process is lengthy, uses expensive equipment and requires highly trained staff. Comparison between laboratories can also be difficult to achieve accurately, unless there is strict standardisation. On the other hand, sequencing

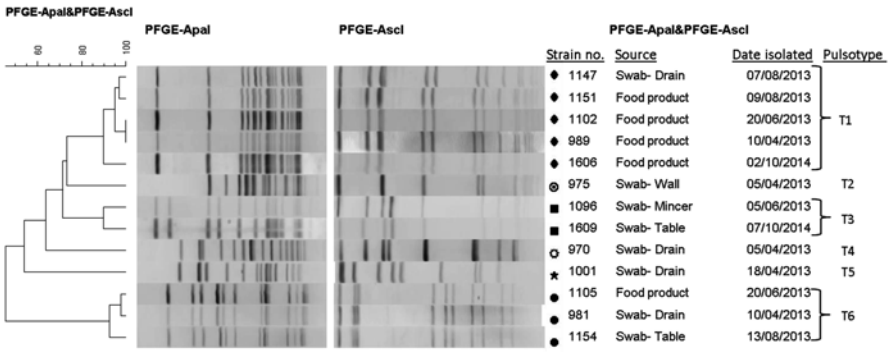


Fig. 4.8 PFGE profiles (digested with two restriction enzymes: ApaI and AscI) of *L. monocytogenes* isolated from a single food business facility. Isolate similarity dendrogram was generated using BioNumerics version 5.10 software (Applied Maths), using the unweighted pair group method with arithmetic mean (UPGMA) and the Dice coefficient with tolerance and optimization settings of 1 %. Pulsotype T1: Persistent pulsotype seen in both food and environmental swabs; Pulsotype T3: Persistent pulsotype isolated from environmental swabs 16 months apart; Pulsotype T6: Evidence of transfer between food and environment; Pulsotypes T2, T4 and T5: Sporadic contamination of the facility with various pulsotypes

techniques have much better inter-laboratory comparison as they are not subject to interpretation as PFGE profiles can be. PFGE is based on a bacterial strain being suspended in agarose, lysed to release the DNA which is then cut with specific restriction enzymes. The fragments are then separated by gel electrophoresis with alternating direction of current over a long period of time, generally for 21 h (PulseNetUSA 2009). This long run time, combined with the periodic change in the direction of current, termed pulsed-field, allows for the separation of very large, 1,000 kbp, fragments of DNA which is not possible with conventional electrophoretic separation, where the limit is usually 40–50 kb. This gives a pattern of bands, a PFGE profile (Fig. 4.8), which is then analysed using Bionumerics or similar software and added to a database to allow computer analysis of the strain profile, including the percentage similarity between strains. This computational analysis and creation of PFGE profile databases allows for the comparison of bacterial strains worldwide, if standardised methods are used. Two different restriction enzymes should be used (in two separate runs) and the resulting patterns combined as the use of only a single restriction enzyme can miss large sections of DNA which may contain variations between strains. Thus, PFGE performed with only a single enzyme can often mistakenly identify two distinct strains as identical (Hurley et al. 2014). Using two restriction enzymes gives a better level of differentiation (Fox et al. 2012) and, used in this way, PFGE analysis is a more exacting method than MLST, MLVA or ribotyping (Borucki et al. 2004).

Although it can be difficult to compare PFGE patterns between labs, the PulseNet International network has devised a general PFGE protocol in order to standardise the procedure and result in comparable PFGE profiles (PulseNetUSA 2009). Several conditions need to be met in order to meet the conditions set forth by PulseNet

including the use of *Salmonella* Braenderup cut by the *Xba*I restriction enzyme as a standard rather than the use of a traditional molecular weight ladder. In this way, large studies involving the comparison of PFGE profiles from various labs have been performed. For example, a study which compared *L. monocytogenes* PFGE profiles in both the Republic of Ireland and Northern Ireland found several PFGE profiles which were found in both countries (Hurley et al. 2014).

4.2.4 Multilocus Sequence Typing

Multilocus sequence typing (MLST) is based on sequencing a set of alleles of housekeeping genes and analysis of the differences in these sequences. A major advantage of MLST is that comparison of MLST results between laboratories is extremely accurate, as it is based on sequences. It can also reconstruct evolutionary linkages unlike PFGE or ribotyping. However, with the advances in genome sequencing, which gives an even higher level of differentiation, MLST may not be as useful as was previously hoped. One problem associated with MLST is that no set of genes has been agreed upon globally and in the past individual laboratories have often chosen different sets of genes to sequence which has made comparison between laboratories difficult.

A global database of MLST sequences, www.pasteur.fr/mlst, does however exist and allows comparison of MLST sequences with previously acquired MLST sequences. In this way, identification of common MLST types and comparison of MLST types across geographically distinct locations can occur which can shed some light on a strain's prevalence, adaptability to a particular niche etc. For example, certain *L. monocytogenes* MLST types (sequence type [ST] 9 and ST121) have been seen to be common in food contamination and seem to have adapted to the meat processing environment (Martin et al. 2014). In a recent publication which isolated and characterised 33 *L. monocytogenes* strains from RTE foods in China, 11 MLST types were found and 7 of these were shown to be previously established MLST types isolated in China for the first time (Wang et al. 2015). MLST is less discriminatory than PFGE. However, by examining the number of mutations in the sequences, the evolutionary distance between strains can be determined by MLST in a way which cannot be performed with PFGE analysis of results (Ragon et al. 2008; Wang et al. 2012).

4.2.5 Multi-Virulence-Locus Sequence Typing

Multi-virulence-locus sequence typing (MVLST) is very similar to MLST but differs in the genes sequenced. While MLST is based on sequencing housekeeping genes as they are common to all *L. monocytogenes* strains, MVLST instead sequences virulence genes. Although not all strains will have all virulence genes, in

general the greater variations associated with virulence genes allow MVLST to offer greater discriminatory power than MLST. Virulence genes evolve faster than housekeeping genes and similar strains with variations in their virulence profiles can be significantly different in their potential to cause listeriosis outbreaks and epidemics (Chen et al. 2007; Doijad et al. 2014). This makes MVLST a better candidate than MLST for investigating the epidemiological evolution of *L. monocytogenes* strains.

4.2.6 Multiple-Locus Variable Tandem Repeat Analysis

Multiple-Locus Variable Tandem Repeat Analysis (MLVA) uses tandem repeat sequences in regions of genomic DNA to differentiate between strains. Regions of tandem repeat sequences occur at different frequencies in various regions of DNA and by analysing these, bacterial strains can be typed. Although found to have less discriminatory power than PFGE, certain advantages including high-throughput, rapidity, ease of analysis and inter-laboratory comparison are associated with MLVA (Sperry et al. 2008). Depending on the method used, the time taken to complete MLVA analysis can be counted in hours, as opposed to the 5 days needed for PFGE analysis (Murphy et al. 2007). It was seen to have similar discriminatory power to MLST and, due to its speed and high reproducibility in particular, can be considered a viable first-line subtyping method in outbreak and listeriosis investigations (Chenal-Francisque et al. 2013).

4.2.7 Ribotyping

Conceptually, ribotyping is based on similar principles to PFGE: both are based on cleavage of total genomic DNA by restriction enzymes followed by electrophoretic separation. However, ribotyping analysis targets conserved chromosomal genetic elements and involves southern blot transfer and hybridisation of labelled rRNA (ribosomal RNA) with a radiolabelled ribosomal operon probe. Strain differentiation is based on the differences in location and number of rRNA gene sequences. Although the discriminatory power of ribotyping has been generally found to be weaker than PFGE or MLST, the ability of ribotyping to be automated offers a distinct advantage over other methods. Automated ribotyping (AR) has been successfully developed and is considered a method of choice for many large scale studies due to high reproducibility and the lower level of expertise needed in comparison to PFGE (Fig. 4.9). For use in outbreak investigation, automated ribotyping may be of use in gathering initial results but the data would need to be confirmed by a more discriminatory method such as PFGE or genome sequencing (Aarnisalo et al. 2003).

Ribotyping has allowed the identification of a *L. monocytogenes* strain which was persistent in a smoked fish processing facility for at least 11 years (Vongkamjan et al. 2013).

EcoRI Pattern #	Source/Site	RiboPrinter Pattern	DuPont -ID
1	Cheese		DUP-1038
2	Ice Cream		DUP-1045
3	Washing Water/ Dairy Plant		DUP-18598
4	Equipment Swab		DUP-1039
5	Farm		DUP-1042
6	Floor/Dairy Plant		DUP-19170

Fig. 4.9 The RiboPrinter Patterns and the DuPont—ID for *L. monocytogenes* from different sources using *EcoRI* enzyme

The higher discriminatory power of PFGE over ribotyping was reported by Fonnesbech Vogel et al. (2004) who found that ribotyping had a discriminatory index of 0.874, compared to a discriminatory index of PFGE of 0.969. Similarly, several other studies have shown the higher discriminatory power of PFGE (Aarnisalo et al. 2003; Grif et al. 2006; Louie et al. 1996; Kerouanton et al. 1998; Lukinmaa et al. 2004). Nevertheless, although ribotyping is not as discriminatory as PFGE, it is rapid, simple to conduct, highly standardized and labour-saving, and may therefore be a good alternative for a rapid epidemiology study of *L. monocytogenes* isolates. However, on occasions when a high discriminatory power is needed other subtyping techniques such as PFGE may be necessary.

4.2.8 Physiological Methods

Contrasting with the other methods listed here, physiological methods are not molecular based but instead measure the phenotypic behaviour of bacterial strains under various conditions. Physiological methods are commonly used in combination with molecular methods as an important tool to elucidate gene function. Traditionally, phenotypic arrays were very time-consuming and labour intensive which limited them greatly. More recently, high-throughput technologies have been developed which vastly reduce both time and labour needed for phenotypic arrays. Examples of high-throughput phenotypic arrays include the API system from Biomerieux, which use a combination of up to 20 biochemical tests for the identification and/or differentiation of Gram-positive and Gram-negative bacteria and the OmniLog phenotype Microarray analysis, which uses a redox dye to measure cell metabolism simultaneously under up to 1,920 different growth conditions.

Fourier-Transformed Infrared Spectroscopy (FTIR) is a subtyping method based on the fact that the infrared spectra are a reflection of the overall molecular

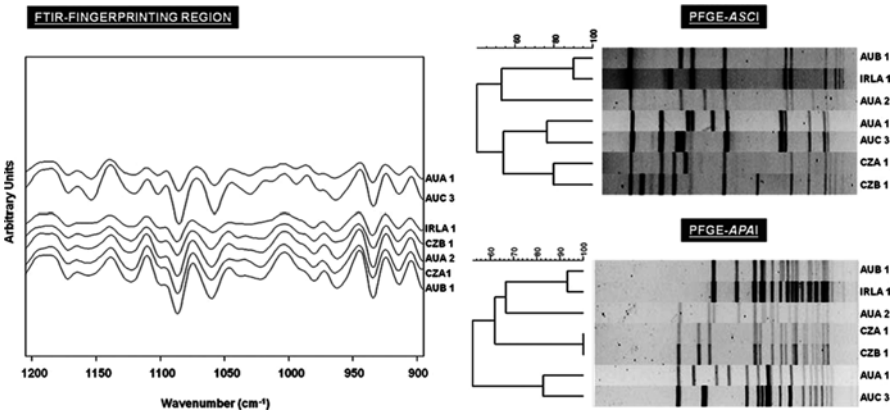


Fig. 4.10 Seven representative persistent *Listeria monocytogenes* pulsed-field gel electrophoresis (PFGE) types from six European dairies including a comparison of the Fourier-transform infrared (FTIR) fingerprinting region (900–1,200/cm) and the PFGE cluster analysis (restriction enzymes AscI and ApaI). Abbreviations for representative *L. monocytogenes* persistent PFGE types are located to the right of each figure part: *AU* Austria, *IRL* Ireland, *CZ* Czech Republic

composition (Fig. 4.10). Strains that differ in their molecular make-up will therefore show distinct vibrational spectra (Alvarez-Ordóñez and Prieto 2012). In FTIR, analysis of the spectra related to organic functional groups, including proteins, lipids, carbohydrates and nucleic acids, of an isolate is examined by spectroscopy. The potential discriminatory power of FTIR was seen when, combined with multivariate analysis, FTIR was able to discriminate between strains with similar PFGE patterns (Davis and Mauer 2011). Stessl et al. (2014) showed that metabolic fingerprinting by FTIR spectroscopy could be used as a rapid analytical tool to facilitate prescreening of potentially persistent *L. monocytogenes* contaminants.

4.2.9 Whole Genome Sequencing as a Tool for Outbreak Investigation

Genome sequencing offers an indisputable advantage over any other molecular method as the entire genome is sequenced and used for comparison. This means that advantages offered by MLST, MVLST, MLVA and ribotyping are all already inbuilt in genome sequencing. Up to recently, the main drawback of genome sequencing has been the cost involved. However, it is now being increasingly used as advances in the technology allow faster and cheaper generation of sequences. For example, the 6 year persistence of two *L. monocytogenes* strains in two different fish processing facilities was evidenced by genome sequencing (Holch et al. 2013).

Other methods listed, including PFGE, MLST, and ribotyping, often miss large areas of difference between strains; however, as genome sequencing uses the entire

DNA sequence, strain analysis with genome sequencing is much more accurate. The use of whole genome sequencing in outbreak investigation is becoming increasingly popular, as described in Sect. 2.1.3, since the confidence in strain characterisation is much higher than with other methods.

Many of the *L. monocytogenes* isolates involved in human illness have been shown by whole genome sequencing to be quite stable, with variations occurring in relatively few areas of the genome. Although this may mean that much of the sequence generated with whole genome sequencing is identical, the fact that all of the genome is available for analysis with this method eliminates the danger that differences between strains could be missed by other methods including PFGE, MLST etc.

In the Quargel cheese outbreak which occurred in Europe in 2009 and 2010, genome sequencing allowed the identification of two distinct strains which were responsible for the outbreak. Both strains belonged to the 1/2a serotype but differed in several important characteristics including their invasive capacity. In this case, whole genome sequencing allowed for the examination of the differences in invasive capacity of the two strains which could then also be compared with invasive assays in Caco-2 cells. The strain QOC1 was shown to have a higher invasive capacity than strain QOC2 which could be attributed to extra internalin genes contained in the QOC1 genome. As shown in that case, whole genome sequencing allows for some evaluation of the virulence of a strain even without invasion assays, which are not always accurate in respect to the real behaviour of a strain in a pathogenic scenario.

Strain QOC1 was also shown to have a stress survival islet not found in QOC2 and, in physiological tests, was shown to resist acidic, alkaline and gastric stress better than QOC2. Whole genome sequencing results for these strains allowed complex analysis of the strains and suggested that they did not evolve from a common ancestor and that contamination was more likely due to two separate contamination events which overlapped to form one major outbreak (Rychli et al. 2014).

Another advantage of whole genome sequencing is that, as the entire genome sequence is obtained, previous MLST and MLVA data can be compared to sequences obtained through whole genome sequencing by which the relatedness of the strains can be analysed.

One of the major challenges in whole genome sequencing which has emerged in recent years is the challenge involved in storing the large amounts of data generated. Despite this problem, the use of whole genome sequencing, especially in outbreak investigations, seems to be constantly increasing.

Chapter 5
Reducing the Occurrence of *L. monocytogenes*

As *L. monocytogenes* is ubiquitous in the environment it is not reasonable to aim for total elimination of the organism. Therefore, control of its occurrence is essential.

5.1 Control of *L. monocytogenes* During Food Processing

As the majority of listeriosis is foodborne, reduction of *L. monocytogenes* in food will contribute to a reduction in the disease burden. Reduction of *L. monocytogenes* in the food processing environment will result in a reduced risk of cross-contamination of food, and should therefore result in reduced occurrence in food. Such process control is a vital part of *L. monocytogenes* control, and an important part of this is creating awareness of *L. monocytogenes* occurrence.

5.1.1 Awareness

RTE food producers need to be especially vigilant regarding *L. monocytogenes* as their products will be directly consumed and will not necessarily undergo a heat step, or any other microbial inactivation step, which would destroy any *L. monocytogenes* present, such as cooking, after leaving the processing environment. One of the biggest hindrances to the prevention of *L. monocytogenes* contamination in food is the lack of awareness. The fear that positive samples will have a negative impact on a facility can outweigh the fear of more serious problems which a contamination issue would create. This fear of positives can discourage food processors from taking an adequate number of samples and can cause food processors to negatively influence sampling by, for example, sampling in areas which have recently been disinfected, in order to ensure that samples taken do not test positive for *L. monocytogenes*. This improper sampling or insufficient number of samples taken is especially seen in industry sectors which are not technically included in the RTE category

K. Jordan et al., *Listeria monocytogenes in the Food Processing Environment*,
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but which may produce RTE foods, for example, uncut vegetable producers. RTE food processors are required to perform regular sampling for *L. monocytogenes* whereas primary production, the category under which uncut vegetable production falls, is not subject to these regulations. This gap in the regulations creates a grey area in which certain sectors of food production can choose to ignore the need for *L. monocytogenes* monitoring (Luber et al. 2011).

Certain sectors of the food processing industry seem to be more aware than others of *L. monocytogenes* due partly to historical outbreaks seen in the sector. Irish farmhouse cheese-makers have been involved in several *L. monocytogenes* occurrence surveys in recent years and have seen their *L. monocytogenes* prevalence in environmental samples drop from 13.1 % in environmental samples in 2011 to 3.9 % in combined environmental and food samples in 2013/2014 (Fox et al. 2011a; Leong et al. 2014). This fall in positive samples can be partly attributed to their heightened awareness of *L. monocytogenes* and their involvement in *L. monocytogenes* analysis through interactions such as these surveys.

The education of food processing workers in regard to *L. monocytogenes* is also of vital importance. Unless fully aware of the dangers of *L. monocytogenes*, as well as the steps which can help to prevent the spread of contamination, staff can be an important source and/or carrier of *L. monocytogenes* contamination.

Subtyping of *L. monocytogenes* isolates can also be important in identifying possible sources of contamination (Sperry et al. 2008). Numerous varying subtypes of *L. monocytogenes* strains isolated from the food processing environment generally indicate, not only that the hygiene practices in place are not sufficient to prevent contamination, but also that contamination is frequently occurring from an outside source which could include raw materials, contamination from an adjoining farm etc. Persistent contamination of a food processing facility with a single strain (or low number of strains), in contrast, indicates that a more specific problem may be present within the processing facility. This could include the presence of harbourage sites, formation of biofilms, insufficient cleaning regime etc. The monitoring and reduction of environmental contamination is vital as evidence that *L. monocytogenes* strains from the processing plant enter the food has been seen in several studies in which strains from the environment and from food have been typed and found to be indistinguishable (Lambertz et al. 2013; Klaeboe et al. 2006; López et al. 2008).

Awareness about *L. monocytogenes* for RTE food businesses is also vital as raw material suppliers do not have regulations in place requiring their products to be *L. monocytogenes* free. It is, therefore, the responsibility of the food processor to ensure that any raw materials entering their food chain are *L. monocytogenes* free. This absence of regulations for raw material suppliers is due to the fact that *L. monocytogenes* is generally destroyed during food processing, usually during a heat step. However, certain foods, even RTE foods, may not undergo sufficient treatment to eliminate and prevent the further growth of any *L. monocytogenes* present. In this case, the *L. monocytogenes* status of the raw material should be monitored and the raw materials kept *L. monocytogenes* free. Raw materials have been shown to be a major source of *L. monocytogenes* contamination in finished products (Miettinen and Wirtanen 2006).

Vendors also need to be aware of the dangers associated with *L. monocytogenes* in RTE foods as the temperature at which foods are stored can be vital in ensuring

that *L. monocytogenes* numbers, if present even in very low numbers, remain low enough so as not to cause a health risk (González et al. 2013).

Third party food preparation such as hotels and catering companies can also be a source of *L. monocytogenes* infections if the correct precautions to prevent *L. monocytogenes* are not taken. HACCP points, the sufficient separation of raw and cooked foods, the proper training of staff, etc. are all important in preventing *L. monocytogenes.* However, these systems can sometimes breakdown, for example in the coleslaw outbreak in Nova Scotia in 1981 in which *L. monocytogenes* was originally identified as the cause of foodborne listeriosis (Kathariou 2002).

Consumers represent the final step in the food chain and they are a vital step in preventing listeriosis infections. As the reporting of mild listeriosis is low and it is impossible to really know how common contamination in the home is, the importance of the consumer in preventing listeriosis can often be underplayed. As non-RTE foods do not have any impetus to be *L. monocytogenes* free, contaminated foods are often present in the home and cross-contamination can occur. Education of the consumer in the importance of separating raw and cooked foods and in keeping foods at the correct temperatures is vital. Products are often also consumed in the home after the end of shelf-life has passed so, even if all possible precautions were taken by the food processor, the consumer may still ingest a contaminated food due to their disregard of the best before date.

5.1.2 Attention to Detail in Hygiene

The ubiquitous nature of *L. monocytogenes* in soil, water, faeces etc. means that contamination of food processing facilities can occur from many different sources. Although this can make identification of the contamination source difficult, typing methods such as PFGE can help to identify similar strains and therefore make assumptions about their source. The transfer of *L. monocytogenes* from the processing environment to the food, where it can cause a significant health risk, has been well documented (Chaitiemwong et al. 2014). Therefore, the removal of *L. monocytogenes* from the processing environment is vital in preventing the spread of *L. monocytogenes* to the food being produced and/or processed.

A major factor in keeping a facility free of *L. monocytogenes* is the design of the facility itself. Non-purpose designed facilities are common especially in industries such as farmhouse cheese making where converted farm buildings may house the food processing facility. These facilities may not be correctly designed or equipped to prevent contamination and redesign of the building itself is often necessary in these cases. The separation of raw materials and finished products as well as the presence of boot wash areas, hand washing areas etc. are vital in preventing the spread of *L. monocytogenes* in a facility. The design of a facility should also allow clean-in-place (CIP), where all areas of the facility and all equipment therein can be completely cleaned without having to remove/dismantle equipment and with little or no manual input from the operator (Bremer et al. 2006).

The existence of harbourage sites, areas where disinfectants/sanitizers cannot properly reach, are a frequent source of *L. monocytogenes* contamination.

Harbourage sites may be due to ill design, unsuitable materials/equipment or even to damaged materials. Due to the inaccessible nature of these sites, disinfectants/sanitizers may not be able to reach properly or may only reach in lower concentrations than would be needed to inhibit the bacteria therein. One theory suggests that constant low level of disinfectant in harbourage sites such as this may allow bacterial strains to evolve tolerances against certain chemicals being used. If bacteria then proliferate out from this site, the strain may have increased tolerance against the chemical even used in its intended concentration (Lundén et al. 2003). However, this theory is not strongly supported; general correlations between persistent strains and sanitizer resistant strains are not often seen (Heir et al. 2004). Any facility designed specifically for food processing should attempt to be free of harbourage sites and food processors should make every effort to remove any harbourage sites which may exist in a non-purpose designed facility. Unfortunately this can be extremely difficult and generally, harbourage sites remain an inherent danger in terms of contamination in food processing facilities (Carpentier and Cerf 2011).

A facility's staff can also be a major factor in *L. monocytogenes* contamination. A recent study in Chile found that 38 % of samples collected from the hands and fingernails of workers in a cured meat processing plant tested positive for *L. monocytogenes* (Saludes et al. 2014). Personal protective equipment including coats, gloves, hairnets and boot wash being in proper use as well as the separation of staff working with raw materials and finished products are all important factors in preventing contamination.

5.1.3 The Ten Commandments of Listeria *Vigilance*

End-product control is currently the primary mechanism for analysis of food before its release onto the market. An alternative strategy that can be even more beneficial is a good processing environment control strategy. Basically, if *L. monocytogenes* is not in the processing environment, then the risk of cross-contamination to food is reduced. The following ten points summarise a good processing environment control strategy.

5.1.3.1 Commandment 1: Understand the Nature of *L. monocytogenes* Contamination

Listeria monocytogenes can grow at refrigeration temperature, is resistant to various stresses and food is an important route of transmission via the food processing environments (FPE). Most FPEs are contaminated to some extent.

5.1.3.2 Commandment 2: Take FPE Contamination Serious

Article 5 of the EU Directive 2073/2005 should be taken seriously and a FPE monitoring concept developed as a core activity of Good Hygiene Practices (GHP).

5.1.3.3 Commandment 3: Choose the Right Sampling Sites and Methodology

Sample the processing environment with a view of finding the organism. The most informative sampling sites can vary depending on the food commodity produced. Consider the difference in information that will be achieved from sampling of food contact materials versus non-food contact materials. Sampling is the most critical procedural step in any circumstances and, if done inappropriately is of little benefit. Use swabs that have enough contact surface to sample the 900 cm^2 mentioned in many guidelines. Choose sampling sites from manufacturing or handling steps that are applied on most of the products produced (e.g. conveyor belts before packaging, slicer blades).

5.1.3.4 Commandment 4: Choose the Right Sampling Frequency

Recommendations on sampling frequency can only be expressed in general terms. If a FPE is being sampled the first time, use a broad sampling approach. If the contamination status is already known, test a restricted number of sampling sites frequently rather than a lot of sampling sites only once. Sampling frequency can be reduced if negative results are shown, but should be increased again if positive results are detected or if there are changes to the processing environment or manufacturing process. Sampling frequency should be dynamic.

5.1.3.5 Commandment 5: Establish Critical Control Areas

To facilitate prioritisation of counter-measures, clearly define critical control areas (CCA) where FPE contamination is not acceptable under any circumstances. It makes a difference whether a *L. monocytogenes* positive drain is located in a general processing area or if it is located where food is handled prior to packing. Critical control areas should be clearly marked (e.g. by marks on floors, in construction maps) and hygiene barriers should prevent CCAs being visited or trespassed by unqualified personnel. The high hygiene standard that should exist in CCAs can only be monitored by taking an appropriate number of FPE samples.

5.1.3.6 Commandment 6: Trace the Route of Transmission of Isolates Most Importantly in CCAs

To combat contamination it is vital to keep all isolates at a safe and appropriate place (e.g. a contract laboratory). Use molecular typing to identify the putative routes of transmission of a pathogen in the facility. To reduce the costs, start with combating contamination in a CCA where the risk for contamination of the food commodity is the highest.

5.1.3.7 Commandment 7: Be Aware of Phases of Reconstruction

During building work, hygiene measures are usually difficult to maintain at a food processing facility. On the one hand, craftsmen of various occupations with no training in hygiene need to have access to the FPE. Recommending the use of hygiene protection (overshoes, overcoats) to craftsmen is frequently in vain because it limits their maneuverability. Building material, often stored before use outdoors, needs to be carried around. Insects and rodents can get access to the FPE. On the other hand, the FBO frequently needs to produce food in processing rooms adjacent to the reconstruction area. Be aware of the increased risk of cross contamination during such reconstruction periods, and construct physical barriers between food production and construction. Try to prevent access of craftsmen to production areas as much as possible. Observe careful and intensified sanitation programmes in the processing areas during the reconstruction phase, and sanitise the entire FPE after completion of the reconstruction phase. Verify the success of this process by subsequent sampling of the FPE.

5.1.3.8 Commandment 8: In Cases of Widespread Contamination, Critically Review the Floor Sanitation Procedures Applied

If FPE monitoring demonstrates a widespread contamination of a genetically indistinguishable *L. monocytogenes* strain, re-consider your sanitation procedure (Who is in the sanitation team? Sanitiser used? Concentration used appropriately? All areas covered? Are the surfaces allowed to dry off before food production begins again?) and the workflow pattern. Use drain water sampling to control the efficiency of sanitation. Don't forget to sample the biofilms in drains.

5.1.3.9 Commandment 9: Structure Your Data

After all the experience gained, we are confident that safe food production is possible even if there is contamination of a FPE. However, the following criteria must be met:

- The extent of contamination must be known (implies intensified sampling)
- Contamination was never detected in the food commodity produced
- FPE contamination is infrequent (reported only irregularly)
- Contamination is detectable only in compartments where a risk for cross-contamination is low
- The food produced does not support growth of *L. monocytogenes* on its surface

To demonstrate that the FBO has met these requirements, organization of the self-control data is necessary and must be put into a structured decision making process. We recommend to seek the advice of experts that help to facilitate the decision making process.

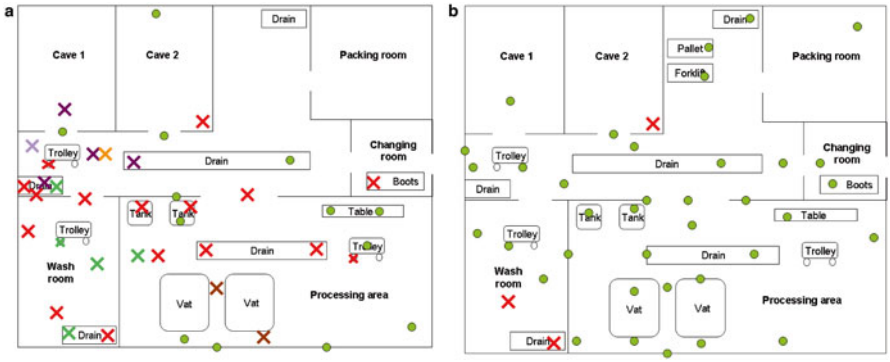

Fig. 5.1 Sampling plan results mapped for a food processing facility before (**a**) and after (**b**) corrective action was implemented to control *L. monocytogenes* (Dalmasso and Jordan 2014). *Green spot—L. monocytogenes* negative sample; *coloured X*—different colours indicate strains with a different Pulsed Field Gel Electrophoresis profile. The sampling times were approximately one month apart (reprinted with permission)

5.1.3.10 Commandment 10: Carefully Document Your Progress and Efforts

Documentation is a pillar in any FBO communication process, either within an operation or with regulators or specialists from the outside. Documentation of ingredients and raw materials used as well as any contamination patterns is essential. A map of the facility can help with this (Fig. 5.1).

5.2 Bacteriocins

Bacteriocins have the potential to inhibit other bacteria, including pathogens, in many cases resulting in cell death. Therefore, they have potential as a mechanism to control *L. monocytogenes*. Bacteriocins are ribosomally-synthesised peptides that are pore-forming agents, which act by disrupting the integrity of the target cell membrane. The spectrum of activity can be broad, where a wide variety of unrelated species are inactivated, or narrow, where only closely related species are inactivated. Lactic acid bacteria (LAB), in particular, are known to produce a variety of broad and narrow spectrum bacteriocins possessing characteristics that make them ideal for use in pathogen inactivation. These characteristics include their host range, their pH- and heat-tolerance, and the fact that they are non-toxic. In addition, they are inactivated by host proteases, resulting in little or no effect on the host microflora (Gálvez et al. 2007). Bacteriocins may have several different modes of action, such as inhibition of cell wall synthesis, inhibition of both DNAse and RNAse activity, and more commonly, formation of pores in the membrane of the target cell (Cleveland et al. 2001).

Lactic acid bacteria are ideal candidates for bacteriocin production in biocontrol of *L. monocytogenes*, as they also have a long history of safe use (Bourdichon et al. 2012). They are also an integral part of the gut microflora of humans and animals. The best studied bacteriocin which exhibits anti-listerial activity is nisin; a class-I bacteriocin produced by the LAB, *Lactococcus lactis* (Rogers 1928). Most research has focused on higher risk pathogens such as *E. coli* O157:H7 that are more frequently associated with disease outbreaks. Some studies have focused on combination treatments, for example nisin in combination with acids and EDTA (Bari et al. 2005).

Other bacteriocins, such as pediocin and enterocin, which are not currently used commercially, have been shown to have potential for application as food preservatives. Pediocin is a class-IIa bacteriocin, which differs from nisin in that its host-range is considerably narrower; however, it is highly specific in its activity against *L. monocytogenes*. Enterocin AS-48 has a similar effect on *L. monocytogenes* growth on food (Cobo Molinos et al. 2005). In that study, an immersion treatment with 25 μg/ml of the enterocin AS-48 resulted in a significant reduction of *L. monocytogenes* counts of up to 2.3 log CFU/ml when compared to an untreated control. Mundticin, a bacteriocin produced by *Enterococcus mundtii*, was found to have potential in biocontrol of modified atmosphere packaged and stored mungbean sprouts when used in a washing step or as a coating (Bennik et al. 1999). BACTIBASE is a specific online database dedicated to bacteriocins (http://bactibase.pfba-lab-tun.org/main.php). While the producer and target organisms are listed, there is little information relating to food substrates or efficacy in food systems.

To date, insufficient data has been generated to obtain a complete picture of the potential use for many bacteriocins. The current regulatory situation dictates against the use of bacteriocins as biocontrol agents as in many cases there is currently insufficient supporting data to assure the regulatory authorities of their efficacy and safety. Nisin, discovered in the 1940s, is the only bacteriocin to have been granted a license for use in food to date (Gálvez et al. 2007). Considering the fact that the efficacy of bacteriocins is dependent largely on surrounding environmental factors, there is much further research required in this area in order to ascertain the optimal environmental conditions under which each particular bacteriocin can be applied.

5.3 Bacteriophage

In 1919, Felix d'Herelle discovered bacteriophage (Fig. 5.2), (D'Herelle 1922) and was the first to propose their use as biocontrol agents. However, the fact that early clinical trials involving phage therapy were deemed unreliable, coupled with the discovery of antibiotics, has meant that interest in studying phage as biocontrol agents seemed unnecessary. It has only been in recent times, with the observed increase in antibiotic resistance in pathogens, that the focus of research has shifted back to development of phage-based treatments.

Bacteriophages are logical candidates for biocontrol of *Listeria monocytogenes* in food. They are numerically the most abundant organisms on earth, outnumbering

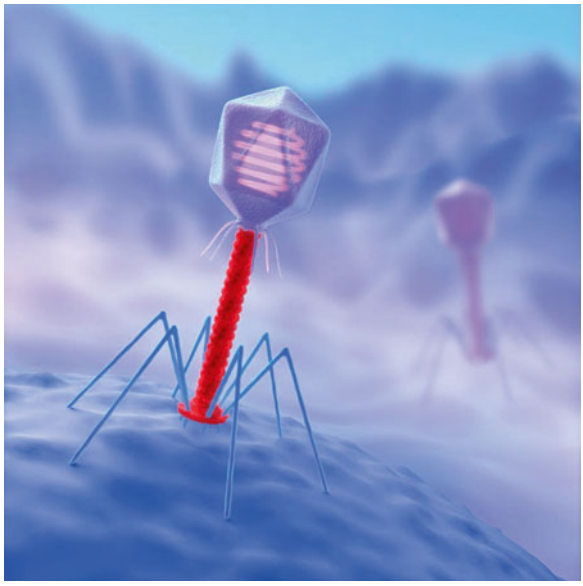

Fig. 5.2 Image of a bacteriophage attacking a bacteria

bacteria in many environments by a factor of 10 (Brüssow et al. 2004). They exhibit a high degree of specificity towards their target host bacterium, and as a result, are safe for use in food processing, considering they will have no detrimental effect on the microflora of the eventual consumer, nor will they have an effect on any other desired bacteria in the food. In addition, they are composed entirely of proteins and nucleic acids; their eventual breakdown will be equally as harmless to the host. Moreover, bacteriophage also possess other desirable attributes, including a relative stability during storage, and the ability to self-perpetuate (Carlton et al. 2005). Of particular importance in terms of suitability for biocontrol of *L. monocytogenes* is finding a virulent phage that is strictly lytic (Fig. 5.3), rather than a lysogenic phage which can be genetically unstable. Lytic phage are genetically stable, will always kill infected cells, and cannot therefore integrate its genome into that of the bacterial chromosome to form a lysogen (Guenther et al. 2009). In addition, it is preferable to choose phage that are incapable of transduction, and thus incapable of facilitating phage-mediated horizontal gene transfer (Brüssow et al. 2004). It is also of critical importance that the full genome sequence of such phage is known, and that any phage applied to food does not encode any virulence factors or toxins which may be harmful (Hagens and Loessner 2010).

The consensus among microbiologists is that bacteriophages do not have any known adverse effects on humans, animals, or the environment. For this reason, many scientists and food safety experts predict that bacteriophages could become a useful tool in the reduction of dangerous pathogens in the food chain. However, there are concerns that limited safety data testing has been undertaken, although bacteriophages have been widely used for treatment of human diseases in the former Soviet Union (Chanishivili and Sharpe 2010).

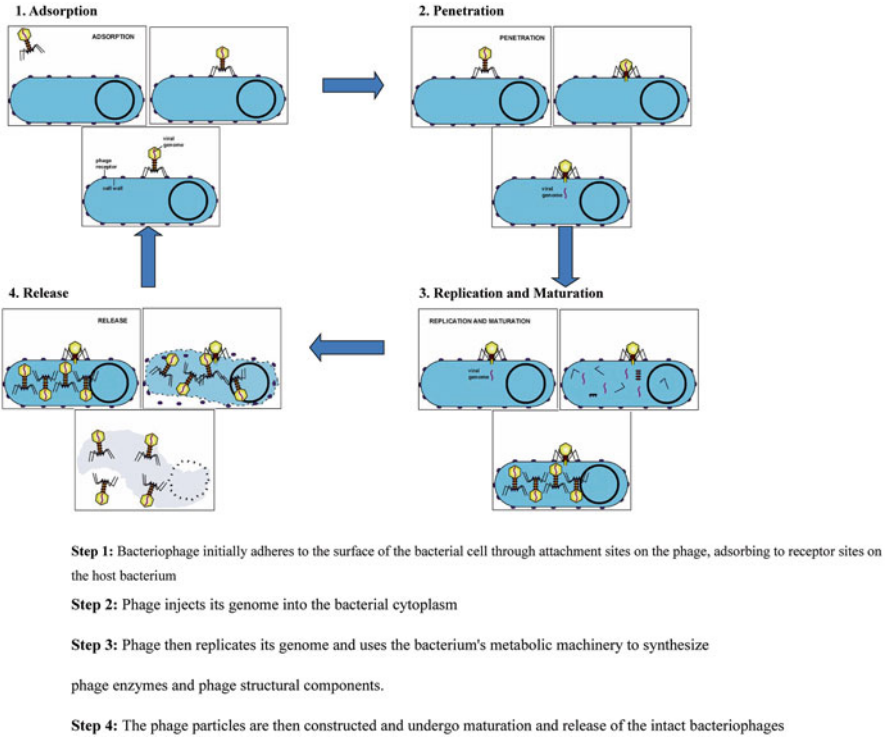

Fig. 5.3 Life cycle of a lytic bacteriophage (Jordan and McAullife 2012; reprinted with permission). *Step 1*: Bacteriophage initially adheres to the surface of the bacterial cell through attachment sites on the phage, adsorbing to receptor sites on the host bacterium. *Step 2*: Phage injects its genome into the bacterial cytoplasm. *Step 3*: Phage then replicates its genome and uses the bacterium's metabolic machinery to synthesize phage enzymes and phage structural components. *Step 4*: The phage particles are then constructed and undergo maturation and release of the intact bacteriophages

The renewed interest into use of bacteriophage as biocontrol agents has resulted in the development of several commercial products designed for this purpose. In 2006, the first such product, namely the LMP-102 phage preparation (now more commonly known as ListShield™), was granted GRAS (Generally Recognised As Safe) status by the FDA for use as a food additive in the control of *L. monocytogenes* in ready-to-eat foods (Lang 2006). This preparation consists of six different phage, which have been shown to be an effective cocktail against 170 bacterial strains (Bren 2007). Another phage preparation known as Listex™ was also granted GRAS status by the USDA's food safety and inspection service in 2006 for use on foods to control *L. monocytogenes* (Lang 2006). This approval means that Listex™ no longer has to be listed as a food additive when used in the processing of food. In addition to this, a more recent alteration to the GRAS status of Listex™ identifies the phage product as a processing aid (Goodridge and Bisha 2011). With regard to specificity for pathogenic *L. monocytogenes* serovars, the Listex™ P100 phage preparation has been demonstrated to be able to infect up to 95 % of isolates belonging to the serovar groups 1/2a,b and 4b (Klumpp et al. 2008; Carlton et al. 2005).

The efficacy of the Listex™ P100 phage, and indeed all phage products used in food processing, depends on many extrinsic and intrinsic parameters. Such parameters include concentration of the phage applied, environmental conditions (such as the type of food, consistency, and specific matrix), ionic strength and pH of the bacterial surface (both of which are critical to the effectiveness at which phage bind to their ligands), and storage conditions (Guenther et al. 2009).

A review of bacteriophage-based biocontrol strategies against foodborne pathogens (Goodridge and Bisha 2011) summarised the applications of such strategies. More specifically, Leverentz et al. (2003, 2006) studied the application of lytic bacteriophage for biocontrol of *L. monocytogenes* (Leverentz et al. 2003, 2006). They showed that the phage reduced *L. monocytogenes* concentrations on honey dew melons by 2.0–4.6 logs as compared with the control, but on fresh cut apples, the phage cocktail only reduced the *L. monocytogenes* by <0.4 log units. It is possible that the reduced efficacy of the phage on the apple slices was due to the low pH (pH 4.4) on the cut surface of the apples. In combination with the bacteriocin nisin, a greater reduction in *L. monocytogenes* was seen (Leverentz et al. 2003). A similar result was found by Dykes and Moorhead (2002), although in laboratory media, not in a practical application. In a further study with honey dew melons, Leverentz et al. (2006) showed that a cocktail of six phages resulted in a larger reduction in *L. monocytogenes w*hen the phages were applied at higher concentrations (Leverentz et al. 2006). This highlights the importance of phage dose during practical application.

A further study (Guenther et al. 2009) demonstrated the efficacy of two broad host range phages (A511 and P100) for control of *L. monocytogenes* on lettuce leaves and cabbage. The vegetables were artificially contaminated with bacteria (10^3 CFU/g), followed by addition of phage at concentrations of 3×10^6–3×10^8 PFU/g, with storage at 6 °C for 6 d. The results indicated that the phages were able to reduce the concentrations of the *L. monocytogenes* strains by more than 2 logs in both the lettuce and cabbage.

These results demonstrate the effectiveness of using phages to control pathogens on food. In addition to the bacterial reductions, the studies are noteworthy because they show that phage treatments can control *L. monocytogenes* at refrigeration temperatures. However, further research on applications of bacteriophage, and combinations of bacteriophage and bacteriocins, is required. In particular, organic farming practices dictate that only natural antimicrobials be used in production, which makes phages an obvious choice as a biocontrol approach in such establishments.

5.4 Antagonistic Cultures

Many different cultures are used to inhibit pathogen growth on food. In general, the efficacy of the antagonism has been studied, but not the mode of action. This lack of characterisation of the compounds involved means that these antagonistic cultures are further from practical application than other, more characterised mechanisms of inhibition like bacteriocins and bacteriophages.

Chapter 6
National Listeria Monitoring Programmes

Monitoring the occurrence of *L. monocytogenes* in the food processing environment, especially small RTE food processing environments, is important and can have an impact on reducing such occurrence. There are many surveys of *L. monocytogenes* occurrence (Table 2.2), and these are valuable in creating awareness of *L. monocytogenes* occurrence in particular places. However, even more valuable are National surveys that continually monitor food processing environments on a regular basis. Such programmes facilitate awareness of *L. monocytogenes* occurrence, and also facilitate comparative analysis of occurrence over time.

6.1 Austrian Listeria Monitoring Programme

In an attempt to control *L. monocytogenes*, the Austrian cheese industry has instigated a voluntary sampling programme aimed at early detection of *L. monocytogenes* followed by targeted intervention strategies. The Austrian *Listeria* monitoring programme comprises various levels of investigation; Level 1 deals with the routine monitoring of samples, Level 2 is an intervention phase if positive results are detected, Level 3 is an intensive sanitation phase and requires confirmation of successful control.

Level 1 Routine monitoring. Samples associated with cheese processing (such as smear, brine or wash water) are analysed at least every month. Smear liquid can be used to spread on the surface of cheese and is a good matrix to monitor cross contamination. Where smear is not used, brine or wash water (used to clean trolleys or trays) can be used. Alternatively, drain water can be a good sample matrix for detection of processing facility contamination. Negative results are certified and used by the company management to document the status of safety. If *L. innocua* or other non-pathogenic Listeria are detected, an inappropriate status of hygiene is recorded as it is possible that pathogenic *L. monocytogenes* are introduced by the same route as the non-pathogenic species. Reconsideration of hygiene measures are recommended to the company management.

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Level 2 Intervention. If *L. monocytogenes* is detected, an intervention phase is initiated. An increased number of samples are collected by the factory personnel from sources which have shown contamination and from additional sources (tanks, racks, conveyor belts, etc.). The intervention examination is intended to clarify the extent of the contamination scenario. It should also help the manufacturer to decide whether a risk for cross-contamination to processed food has arisen. Isolates from food contact materials are treated as if those would have been isolated from the food commodity itself. In parallel, investigations of cheese samples according to the legal requirements determine whether a FPE contamination has already reached the food batch. If yes, and a test indicates that a food batch does not comply with the legal requirements then the batch should not be delivered or should be recalled from the market (internal recall).

Level 3 If the intervention examination confirms the monitoring result, a scrupulous sanitation of the FPE in addition to routine procedures is strongly recommended (Level 3). The sanitation usually cannot be performed without advice from external experts. The sanitation should be systematic, including a crucial survey of all factors that might drive the contamination scenario. This in particular includes a critical review of hygiene barriers, internal traffic management, the maintenance of buildings and rooms, and the cleaning and disinfection procedures applied. Typing of in-house strains supports the sanitation specialist to trace the contamination to hotspots from where *L. monocytogenes* might re-contaminate. A heavily contaminated FPE is difficult to sanitize. In most cases the goal is to control the contamination to spots from where a food batch contamination can be excluded. This status of a co-existence of FPE contamination with pending food processing is a fragile reality in many food processing enterprises and should be monitored carefully.

6.2 Irish Listeria Monitoring Programme

As part of an initiative by the Food Institutional Research Measure (funded by the Department of Agriculture, Food and the Marine), a project on Listeria monitoring in food processing environments in Ireland commenced in March 2013. Sixty-seven FBOs take part in the project and can be categorised into several industry sectors: dairy, meat, fish, vegetable and miscellaneous. The majority of FBOs produce at least one RTE food and are located throughout the Republic of Ireland.

Every 2 months, each FBO sends samples to a participating laboratory to be analysed by the ISO 11290 method. A sample set generally consists of six environmental swab samples and two food samples, although liquid samples can also be sent if FBOs have brine, water etc. to be tested. Environmental swabs are analysed immediately while food samples are stored and analysed following the best before date, in order to prevent recall issues which would discourage FBOs from taking part in this voluntary programme.

FBOs are informed of presumptive results immediately and positive isolates are stored and further characterised. Confirmatory PCR, serotyping and PFGE are performed on all isolates obtained. PFGE allows the identification of persistent strains and FBOs are offered advice especially if particular contamination issues (such as persistence) are identified.

Through this programme, a pattern of contamination in Irish food processing facilities can be seen and a general *L. monocytogenes* contamination level of 4.6 % was found in the first year of the programme with similar percentage of positives found in food and environmental samples (Leong et al. 2014).

A similar programme has just commenced in Northern Ireland.

Chapter 7
Conclusions

Despite extensive research, outbreaks related to *L. monocytogenes* continue and issues like host factors effecting pathogenicity and virulence factors are not fully resolved. As *L. monocytogenes* is ubiquitous in the general environment, elimination of the organism is an unreasonable objective. Therefore, control of *L. monocytogenes* is vital in addressing prevention of listeriosis. Awareness of the prevalence of *L. monocytogenes* in food processing facilities and use of appropriate control measures are important tools in the efforts for such control. Process control sampling and analysis are an important aspect of control measures. Reducing occurrence in the food processing environment reduces the risk of cross-contamination to food, and therefore has an impact on public health.

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References

Aarnisalo K, Autio T, Sjoberg AM, Lunden J, Korkeala H, Suihko ML. Typing of *Listeria monocytogenes* isolates originating from the food processing industry with automated ribotyping and pulsed-field gel electrophoresis. J Food Prot. 2003;66(2):249–55.

Aase B, Sundheim G, Langsrud S, Rørvik LM. Occurrence of and a possible mechanism for resistance to a quaternary ammonium compound in *Listeria monocytogenes*. Int J Food Microbiol. 2000;62(1–2):57–63. http://dx.doi.org/10.1016/S0168-1605(00)00357-3.

Abadias M, Usall J, Anguera M, Solsona C, Vinas I. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. Int J Food Microbiol. 2008;123(1–2):121–9. doi:10.1016/j.ijfoodmicro.2007.12.013.

Abram F, Starr E, Karatzas KAG, Matlawska-Wasowska K, Boyd A, Wiedmann M, et al. Identification of components of the Sigma B Regulon in *Listeria monocytogenes* that contribute to acid and salt tolerance. Appl Environ Microbiol. 2008;74(22):6848–58. doi:10.1128/AEM.00442-08.

Allerberger F, Guggenbichler JP. Listeriosis in Austria—report of an outbreak in 1986. Acta Microbiol Hung. 1989;36(2–3):149–52.

Almualla NA, Laleye LC, Abushelaibi AA, Al-Qassemi RA, Wasesa AA, Baboucarr J. Aspects of the microbiological quality and safety of ready-to-eat foods in Sharjah supermarkets in the United Arab Emirates. J Food Prot. 2010;73(7):1328–31.

Althaus D, Hofer E, Corti S, Julmi A, Stephan R. Bacteriological survey of ready-to-eat lettuce, fresh-cut fruit, and sprouts collected from the Swiss market. J Food Prot. 2012;75(7):1338–41. doi:10.4315/0362-028X.JFP-12-022.

Alvarez-Ordóñez A, Prieto M. Fourier transform infrared spectroscopy in food microbiology. Springer briefs in food, health, and nutrition. 2012.

Anonymous. Virgin Blue airline chicken wrap 2008. http://www.outbreakdatabase.com/details/virgin-blue-airline-chicken-wrap-2008 (2008). Accessed 8 Apr 2012.

Anonymous. Listeriosis outbreak in Santiago de Chile. http://en.mercopress.com/2009/04/09/listeriosis-outbreak-in-santiago-de-chile (2009a). Accessed 8 Apr 2012.

Anonymous. OZFOODNET quarterly report, 1 July to 30 September 2009. http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3304f.htm (2009b). Accessed 16 Jan 2015.

Anonymous. SanGar Produce & Processing Company Chopped Celery 2010. http://www.outbreakdatabase.com/details/sangar-produce-processing-company-chopped-celery-2010 (2010). Accessed 8 Apr 2012.

Aparecida de Oliveira M, Abeid Ribeiro EG, Morato Bergamini AM, Pereira De Martinis EC. Quantification of *Listeria monocytogenes* in minimally processed leafy vegetables using a combined method based on enrichment and 16S rRNA real-time PCR. Food Microbiol. 2010;27(1):19–23. doi:10.1016/j.fm.2009.07.003.

K. Jordan et al., *Listeria Monocytogenes in the Food Processing Environment*,
SpringerBriefs in Food, Health, and Nutrition, DOI 10.1007/978-3-319-16286-7

Aureli P, Fiorucci GC, Caroli D, Marchiaro G, Novara O, Leone L, et al. An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria monocytogenes*. N Engl J Med. 2000;342(17):1236–41. doi:10.1056/nejm200004273421702.

Awaisheh SS. Survey of *Listeria monocytogenes* and other *Listeria* spp. contamination in different common ready-to-eat food products in Jordan. Pak J Biol Sci. 2009;12(23):1491–7.

Awaisheh SS. Incidence and contamination level of *Listeria monocytogenes* and other *Listeria* spp. in ready-to-eat meat products in Jordan. J Food Prot. 2010;73(3):535–40.

Badosa E, Trias R, Parés D, Pla M, Montesinos E. Microbiological quality of fresh fruit and vegetable products in Catalonia (Spain) using normalised plate-counting methods and real time polymerase chain reaction (QPCR). J Sci Food Agric. 2008;88(4):605–11. doi:10.1002/jsfa.3124.

Bari ML, Ukuku DO, Kawasaki T, Inatsu Y, Isshiki K, Kawamoto S. Combined efficacy of nisin and pediocin with sodium lactate, citric acid, phytic acid, and potassium sorbate and EDTA in reducing the *Listeria monocytogenes* population of inoculated fresh-cut produce. J Food Prot. 2005;68(7):1381–7.

Beaufort A. The determination of ready-to-eat foods into *Listeria monocytogenes* growth and no growth categories by challenge tests. Food Control. 2011;22(9):1498–502. http://dx.doi.org/10.1016/j.foodcont.2010.07.014.

Becker B, Schuler S, Lohneis M, Sabrowski A, Curtis GD, Holzapfel WH. Comparison of two chromogenic media for the detection of *Listeria monocytogenes* with the plating media recommended by EN/DIN 11290-1. Int J Food Microbiol. 2006;109(1–2):127–31. doi:10.1016/j.ijfoodmicro.2006.01.030.

Bennik MH, van Overbeek W, Smid EJ, Gorris LG. Biopreservation in modified atmosphere stored mungbean sprouts: the use of vegetable-associated bacteriocinogenic lactic acid bacteria to control the growth of *Listeria monocytogenes*. Lett Appl Microbiol. 1999;28(3):226–32.

Bergmann S, Beard PM, Pasche B, Lienenklaus S, Weiss S, Gahan CG, et al. Influence of internalin A murinisation on host resistance to orally acquired listeriosis in mice. BMC Microbiol. 2013;13:90. doi:10.1186/1471-2180-13-90.

Berzins A, Terentjeva M, Korkeala H. Prevalence and genetic diversity of *Listeria monocytogenes* in vacuum-packaged ready-to-eat meat products at retail markets in Latvia. J Food Prot. 2009;72(6):1283–7.

Berzins A, Hellstrom S, Silins I, Korkeala H. Contamination patterns of *Listeria monocytogenes* in cold-smoked pork processing. J Food Prot. 2010;73(11):2103–9.

Bille J, Catimel B, Bannerman E, Jacquet C, Yersin MN, Caniaux I, et al. API *Listeria*, a new and promising one-day system to identify *Listeria* isolates. Appl Environ Microbiol. 1992;58(6):1857–60.

Bille J, Blanc DS, Schmid H, Boubaker K, Baumgartner A, Siegrist HH, et al. Outbreak of human listeriosis associated with tomme cheese in northwest Switzerland, 2005. Euro Surveill. 2006;11(6):91–3.

Blatter S, Giezendanner N, Stephan R, Zweifel C. Phenotypic and molecular typing of *Listeria monocytogenes* isolated from the processing environment and products of a sandwich-producing plant. Food Control. 2010;21(11):1519–23. doi:10.1016/j.foodcont.2010.04.025.

Borucki MK, Peppin JD, White D, Loge F, Call DR. Variation in biofilm formation among strains of *Listeria monocytogenes*. Appl Environ Microbiol. 2003;69(12):7336–42.

Borucki MK, Kim SH, Call DR, Smole SC, Pagotto F. Selective discrimination of *Listeria monocytogenes* epidemic strains by a mixed-genome DNA microarray compared to discrimination by pulsed-field gel electrophoresis, ribotyping, and multilocus sequence typing. J Clin Microbiol. 2004;42(11):5270–6. doi:10.1128/jcm.42.11.5270-5276.2004.

Bouayad L, Hamdi T-M. Prevalence of *Listeria* spp. in ready to eat foods (RTE) from Algiers (Algeria). Food Control. 2012;23(2):397–9. doi:10.1016/j.foodcont.2011.08.006.

Bourdichon F, Casaregola S, Farrokh C, Frisvad JC, Gerds ML, Hammes WP, et al. Food fermentations: microorganisms with technological beneficial use. Int J Food Microbiol. 2012;154(3):87–97. doi:http://dx.doi.org/10.1016/j.ijfoodmicro.2011.12.030.

Bremer PJ, Fillery S, McQuillan AJ. Laboratory scale clean-in-place (CIP) studies on the effectiveness of different caustic and acid wash steps on the removal of dairy biofilms. Int J Food Microbiol. 2006;106(3):254–62. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.07.004.

Bren L. Bacteria-eating virus approved as food additive. FDA Consum. 2007;41:20–2.
Brett MSY, Short P, McLauchlin J. A small outbreak of listeriosis associated with smoked mussels. Int J Food Microbiol. 1998;43(3):223–9. http://dx.doi.org/10.1016/S0168-1605(98)00116-0.
Bruno Jr JC, Freitag NE. Constitutive activation of PrfA tilts the balance of *Listeria monocytogenes* fitness towards life within the host versus environmental survival. PLoS One. 2010;5(12):e15138. doi:10.1371/journal.pone.0015138.
Brüssow H, Canchaya C, Hardt W-D. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiol Mol Biol Rev. 2004;68(3): 560–602. doi:10.1128/mmbr.68.3.560-602.2004.
Bula CJ, Bille J, Glauser MP. An epidemic of food-borne listeriosis in western Switzerland: description of 57 cases involving adults. Clin Infect Dis. 1995;20(1):66–72.
Cabedo L, Picart i Barrot L, Teixido i Canelles A. Prevalence of *Listeria monocytogenes* and *Salmonella* in ready-to-eat food in Catalonia, Spain. J Food Prot. 2008;71(4):855–9.
Camejo A, Carvalho F, Reis O, Leitao E, Sousa S, Cabanes D. The arsenal of virulence factors deployed by *Listeria monocytogenes* to promote its cell infection cycle. Virulence. 2011;2(5):379–94. doi:10.4161/viru.2.5.17703.
Caponigro V, Ventura M, Chiancone I, Amato L, Parente E, Piro F. Variation of microbial load and visual quality of ready-to-eat salads by vegetable type, season, processor and retailer. Food Microbiol. 2010;27(8):1071–7. doi:10.1016/j.fm.2010.07.011.
Carlton RM, Noordman WH, Biswas B, de Meester ED, Loessner MJ. Bacteriophage P100 for control of *Listeria monocytogenes* in foods: Genome sequence, bioinformatic analyses, oral toxicity study, and application. Regul Toxicol Pharmacol. 2005;43(3):301–12. http://dx.doi.org/10.1016/j.yrtph.2005.08.005.
Carpentier B, Cerf O. Review — persistence of *Listeria monocytogenes* in food industry equipment and premises. Int J Food Microbiol. 2011;145(1):1–8. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.01.005.
Carrique-Mas JJ, Hokeberg I, Andersson Y, Arneborn M, Tham W, Danielsson-Tham ML, et al. Febrile gastroenteritis after eating on-farm manufactured fresh cheese—an outbreak of listeriosis? Epidemiol Infect. 2003;130(1):79–86.
Carter, M. Final report: investigation of outbreak 99-372 (unpublished data). Baltimore, MD; 2000.
Catellani P, Alberghini L, Feletti S, Boldrin V. Survey on the microbiological quality of Chinese food preparations. Vet Res Commun. 2010;34 Suppl 1:S183–7. doi:10.1007/s11259-010-9372-7.
CDC. Update: multistate outbreak of listeriosis—United States, 1998–1999. MMWR Morb Mortal Wkly Rep. 1999;47(51–52):1117–8.
CDC. Outbreak of listeriosis associated with homemade Mexican-style cheese—North Carolina, October 2000–January 2001. MMWR Morb Mortal Wkly Rep. 2001;50(26):560–2.
CDC. Foodborne outbreak online database (2006). http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx. Accessed 30 Mar 2015.
CDC. Multistate outbreak of Listeriosis linked to whole cantaloupes from Jensen farms, Colorado, final report. http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/120811/index.html (2011). Accessed 8 Apr 2012.
CDC. Multistate outbreak of Listeriosis linked to imported Frescolina Marte Brand Ricotta Salata cheese. http://www.cdc.gov/listeria/outbreaks/cheese-09-12/index.html (2012). Accessed 16 Jan 2015.
CDC. Multistate outbreak of Listeriosis linked to Crave brothers farmstead cheeses. http://www.cdc.gov/listeria/outbreaks/cheese-07-13/index.html (2013). Accessed 16 Jan 15.
CDC. Multistate outbreak of Listeriosis linked to commercially produced, prepackaged caramel apples. http://www.cdc.gov/listeria/outbreaks/caramel-apples-12-14/index.html (2014a). Accessed 16 Jan 2014.
CDC. Multistate outbreak of Listeriosis linked to Roos foods dairy products. http://www.cdc.gov/listeria/outbreaks/cheese-02-14/index.html (2014b). Accessed 16 Jan 2015.

Chae MS, Schraft H, Truelstrup Hansen L, Mackereth R. Effects of physicochemical surface characteristics of *Listeria monocytogenes* strains on attachment to glass. Food Microbiol. 2006;23(3):250–9. doi:10.1016/j.fm.2005.04.004.

Chaitiemwong N, Hazeleger WC, Beumer RR, Zwietering MH. Quantification of transfer of *Listeria monocytogenes* between cooked ham and slicing machine surfaces. Food Control. 2014;44:177–84. http://dx.doi.org/10.1016/j.foodcont.2014.03.056.

Chanishivili N, Sharpe R. A literature review of the practical application of bacteriophage research. 2010.

Chen Y, Zhang W, Knabel SJ. Multi-virulence-locus sequence typing identifies single nucleotide polymorphisms which differentiate epidemic clones and outbreak strains of *Listeria monocytogenes*. J Clin Microbiol. 2007;45(3):835–46. doi:10.1128/jcm.01575-06.

Chen B-Y, Pyla R, Kim T-J, Silva JL, Jung Y-S. Prevalence and contamination patterns of *Listeria monocytogenes* in catfish processing environment and fresh fillets. Food Microbiol. 2010;27(5):645–52. http://dx.doi.org/10.1016/j.fm.2010.02.007.

Chen Y, Ross WH, Whiting RC, Van Stelten A, Nightingale KK, Wiedmann M, et al. Variation in *Listeria monocytogenes* dose responses in relation to subtypes encoding a full-length or truncated internalin A. Appl Environ Microbiol. 2011;77(4):1171–80. doi:10.1128/aem.01564-10.

Chenal-Francisque V, Diancourt L, Cantinelli T, Passet V, Tran-Hykes C, Bracq-Dieye H, et al. Optimized multilocus variable-number tandem-repeat analysis assay and its complementarity with pulsed-field gel electrophoresis and multilocus sequence typing for *Listeria monocytogenes* clone identification and surveillance. J Clin Microbiol. 2013;51(6):1868–80. doi:10.1128/jcm.00606-13.

Cho KM, Kambiranda DM, Kim SW, Math RK, Lim WJ, Hong SY, Yun HD. Simultaneous detection of food-borne pathogenic bacteria in ready-to-eat Kimbab using multiplex PCR method. Food Sci Biotechnol. 2008;17(6):1240–5.

Christison CA, Lindsay D, von Holy A. Microbiological survey of ready-to-eat foods and associated preparation surfaces in retail delicatessens, Johannesburg. S Afr Food Control. 2008;19(7):727–33. doi:10.1016/j.foodcont.2007.07.004.

Chung M-S, Kim C-M, Ha S-D. Detection and enumeration of microorganisms in ready-to-eat foods, ready-to-cook foods and fresh-cut produce in Korea. J Food Saf. 2010;30(2):480–9. doi:10.1111/j.1745-4565.2010.00221.x.

Clayton EM, Hill C, Cotter PD, Ross RP. Real-time PCR assay to differentiate Listeriolysin S-positive and -negative strains of *Listeria monocytogenes*. Appl Environ Microbiol. 2011;77(1):163–71. doi:10.1128/aem.01673-10.

Cleveland J, Montville TJ, Nes IF, Chikindas ML. Bacteriocins: safe, natural antimicrobials for food preservation. Int J Food Microbiol. 2001;71(1):1–20. doi:10.1016/s0168-1605(01)00560-8.

Cobo Molinos A, Abriouel H, Ben Omar N, Valdivia E, Lucas López R, Maqueda M, et al. Effect of immersion solutions containing enterocin AS-48 on *Listeria monocytogenes* in vegetable foods. Appl Environ Microbiol. 2005;71(12):7781–7. doi:10.1128/aem.71.12.7781-7787.2005.

Cordano AM, Jacquet C. *Listeria monocytogenes* isolated from vegetable salads sold at supermarkets in Santiago, Chile: prevalence and strain characterization. Int J Food Microbiol. 2009;132(2–3):176–9. doi:10.1016/j.ijfoodmicro.2009.04.008.

Cotter PD, Draper LA, Lawton EM, Daly KM, Groeger DS, Casey PG, et al. Listeriolysin S, a novel peptide haemolysin associated with a subset of lineage I *Listeria monocytogenes*. PLoS Pathog. 2008;4(9):e1000144. doi:10.1371/journal.ppat.1000144.

Craig AS. Book review. N Engl J Med. 2007;356(9):970–1. doi:10.1056/NEJMbkrev57428.

Cruz CD, Fletcher GC. Prevalence and biofilm-forming ability of *Listeria monocytogenes* in New Zealand mussel (Perna canaliculus) processing plants. Food Microbiol. 2011;28(7):1387–93. http://dx.doi.org/10.1016/j.fm.2011.06.014.

Cruz CD, Silvestre FA, Kinoshita EM, Landgraf M, Franco BDGM, Destro MT. Epidemiological survey of *Listeria monocytogenes* in a gravlax salmon processing line. Braz J Microbiol. 2008;39:375–83.

Cumming M, Kludt P, Matyas B, DeMaria A, Stiles T, Han L, Gilchrist M, Neves P, Fitzgibbons E, Condon S. Outbreak of *Listeria monocytogenes* infections associated with pasteurized milk from a local dairy—Massachusetts, 2007. MMWR Morb Mortal Wkly Rep. 2008;57(40):1097–100.

Cummins J, Casey PG, Joyce SA, Gahan CGM. A mariner transposon-based signature-tagged mutagenesis system for the analysis of oral infection by *Listeria monocytogenes*. PLoS One. 2013;8(9):e75437. doi:10.1371/journal.pone.0075437.

Dalmasso M, Jordan K. Absence of growth of *Listeria monocytogenes* in naturally contaminated Cheddar cheese. J Dairy Res. 2014;81:46–53.

Dalmasso M, Bolocan AS, Hernandez M, Kapetanakou AE, Kuchta T, Manios SG, et al. Comparison of polymerase chain reaction methods and plating for analysis of enriched cultures of *Listeria monocytogenes* when using the ISO11290-1 method. J Microbiol Methods. 2014;98:8–14. http://dx.doi.org/10.1016/j.mimet.2013.12.018.

Dalton CB, Austin CC, Sobel J, Hayes PS, Bibb WF, Graves LM, et al. An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. N Engl J Med. 1997;336(2):100–5. doi:10.1056/nejm199701093360204.

Davis R, Mauer LJ. Subtyping of *Listeria monocytogenes* at the haplotype level by Fourier transform infrared (FT-IR) spectroscopy and multivariate statistical analysis. Int J Food Microbiol. 2011;150(2–3):140–9. doi:10.1016/j.ijfoodmicro.2011.07.024.

Dawson SJ, Evans MR, Willby D, Bardwell J, Chamberlain N, Lewis DA. Listeria outbreak associated with sandwich consumption from a hospital retail shop, United Kingdom. Euro Surveill. 2006;11(6):89–91.

de Castro V, Escudero J, Rodriguez J, Muniozguren N, Uribarri J, Saez D, et al. Listeriosis outbreak caused by Latin-style fresh cheese, Bizkaia, Spain, August 2012. Euro Surveill. 2012;17(42).

De Giusti M, Aurigemma C, Marinelli L, Tufi D, De Medici D, Di Pasquale S, et al. The evaluation of the microbial safety of fresh ready-to-eat vegetables produced by different technologies in Italy. J Appl Microbiol. 2010;109(3):996–1006. doi:10.1111/j.1365-2672.2010.04727.x.

De Lappe N, Lee C, O'Connor J, Cormican M. Misidentification of *Listeria monocytogenes* by the Vitek 2 system. J Clin Microbiol. 2014;52(9):3494–5. doi:10.1128/jcm.01725-14.

de Valk H, Vaillant V, Jacquet C, Rocourt J, Le Querrec F, Stainer F, et al. Two consecutive nationwide outbreaks of Listeriosis in France, October 1999–February 2000. Am J Epidemiol. 2001;154(10):944–50.

Dewamitta SR, Nomura T, Kawamura I, Hara H, Tsuchiya K, Kurenuma T, et al. Listeriolysin O-dependent bacterial entry into the cytoplasm is required for calpain activation and interleukin-1 alpha secretion in macrophages infected with *Listeria monocytogenes*. Infect Immun. 2010;78(5):1884–94. doi:10.1128/iai.01143-09.

D'Herelle F. The bacteriophage: its role in immunity. Baltimore: Williams and Wilkins Co. Waverly Press; 1922.

Disson O, Grayo S, Huillet E, Nikitas G, Langa-Vives F, Dussurget O, et al. Conjugated action of two species-specific invasion proteins for fetoplacental listeriosis. Nature. 2008;455(7216):1114–8. doi:10.1038/nature07303.

Djordjevic D, Wiedmann M, McLandsborough LA. Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. Appl Environ Microbiol. 2002;68(6):2950–8.

Doijad S, Lomonaco S, Poharkar K, Garg S, Knabel S, Barbuddhe S, et al. Multi-virulence-locus sequence typing of 4b *Listeria monocytogenes* isolates obtained from different sources in India over a 10-year period. Foodborne Pathog Dis. 2014;11(7):511–6. doi:10.1089/fpd.2013.1716.

D'Orazio SEF. Animal models for oral transmission of *Listeria monocytogenes*. Front Cell Infect Microbiol. 2014;4:15. doi:10.3389/fcimb.2014.00015.

Dorozynski A. Seven die in French listeria outbreak. Br Med J. 2000;320(7235):601.

Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J Clin Microbiol. 2004;42(8):3819–22. doi:10.1128/jcm.42.8.3819-3822.2004.

Dykes GA, Moorhead SM. Combined antimicrobial effect of nisin and a listeriophage against *Listeria monocytogenes* in broth but not in buffer or on raw beef. Int J Food Microbiol. 2002;73(1):71–81. http://dx.doi.org/10.1016/S0168-1605(01)00710-3.

EC. COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union. 2005;L338(3).

EC. Technical Guidance Document on shelf-life studies for *Listeria monocytogenes* in ready-to eat foods. http://ec.europa.eu/food/food/biosafety/salmonella/docs/technical_guidance_listeria_en.pdf (2008). Accessed 19 Jan 2015.

EC. EURL Lm TECHNICAL GUIDANCE DOCUMENT for conducting shelf-life studies on *Listeria monocytogenes* in ready-to-eat foods. https://sites.anses.fr/en/system/files/EURL%20Lm_Technical%20Guidance%20Document%20Lm%20shelf-life%20studies_V3_2014-06-06.pdf (2014). Accessed 19 Jan 2015.

EFSA. Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods in the EU, 2010–2011 part A: *Listeria monocytogenes* prevalence estimates. EFSA J. 2013;11(6):3241.

EFSA. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. EFSA J. 2014;12(3):3547,312. doi:10.2903/j.efsa.2014.3547.

Eglezos S. The bacteriological quality of retail-level peanut, almond, cashew, hazelnut, Brazil, and mixed nut kernels produced in two Australian nut-processing facilities over a period of 3 years. Foodborne Pathog Dis. 2010;7(7):863–6. doi:10.1089/fpd.2009.0471.

Eilertz I, Danielsson-Tham ML, Hammarberg KE, Reeves MW, Rocourt J, Seeliger HP, et al. Isolation of *Listeria monocytogenes* from goat cheese associated with a case of listeriosis in goat. Acta Vet Scand. 1993;34(2):145–9.

Elizaquível P, Gabaldón JA, Aznar R. Quantification of *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157:H7 in non-spiked food products and evaluation of real-time PCR as a diagnostic tool in routine food analysis. Food Control. 2011;22(2):158–64. doi:10.1016/j.foodcont.2010.05.018.

Elmali M, Oner S, Yaman H. Detection of *Listeria monocytogenes* by using PCR method in refrigerated ready-to-eat food consumed in Turkey. Medycyna Weterynaryina. 2013;69:36–9.

El-Shenawy M, Manes J, Soriano JM. *Listeria* spp. in street-vended ready-to-eat foods. Interdiscip Perspect Infect Dis. 2011;2011:968031. doi:10.1155/2011/968031.

Endrikat S, Gallagher D, Pouillot R, Hicks Quesenberry H, Labarre D, Schroeder CM, et al. A comparative risk assessment for *Listeria monocytogenes* in prepackaged versus retail-sliced deli meat. J Food Prot. 2010;73(4):612–9.

Ericsson H, Eklow A, Danielsson-Tham ML, Loncarevic S, Mentzing LO, Persson I, et al. An outbreak of listeriosis suspected to have been caused by rainbow trout. J Clin Microbiol. 1997;35(11):2904–7.

EU. Guidelines on sampling the food processing area and equipment for the detection of *Listeria monocytogenes*. http://ec.europa.eu/food/food/biosafety/salmonella/docs/guidelines_sampling_en.pdf (2012). Accessed 19 Jan 2015.

Fallah AA, Saei-Dehkordi SS, Rahnama M, Tahmasby H, Mahzounieh M. Prevalence and antimicrobial resistance patterns of *Listeria* species isolated from poultry products marketed in Iran. Food Control. 2012;28(2):327–32. doi:10.1016/j.foodcont.2012.05.014.

Fallah AA, Saei-Dehkordi SS, Mahzounieh M. Occurrence and antibiotic resistance profiles of *Listeria monocytogenes* isolated from seafood products and market and processing environments in Iran. Food Control. 2013;34(2):630–6. doi:10.1016/j.foodcont.2013.06.015.

Farber JM, Daley EM, MacKie MT, Limerick B. A small outbreak of listeriosis potentially linked to the consumption of imitation crab meat. Lett Appl Microbiol. 2000;31(2):100–4.

FDA. *Listeria monocytogenes* risk assessment: II. Hazard identification. http://www.fda.gov/food/scienceresearch/researchareas/riskassessmentsafetyassessment/ucm183981.html (2003). Accessed 8 Jan 2012.

Filiousis G, Johansson A, Frey J, Perreten V. Prevalence, genetic diversity and antimicrobial susceptibility of *Listeria monocytogenes* isolated from open-air food markets in Greece. Food Control. 2009;20(3):314–7. doi:10.1016/j.foodcont.2008.05.018.

Fleming DW, Cochi SL, MacDonald KL, Brondum J, Hayes PS, Plikaytis BD, et al. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N Engl J Med. 1985;312(7):404–7. doi:10.1056/nejm198502143120704.

Flint JA, Van Duynhoven YT, Angulo FJ, DeLong SM, Braun P, Kirk M, et al. Estimating the burden of acute gastroenteritis, foodborne disease, and pathogens commonly transmitted by food: an international review. Clin Infect Dis. 2005;41(5):698–704. doi:10.1086/432064.

Fonnesbech Vogel B, Huss HH, Ojeniyi B, Ahrens P, Gram L. Elucidation of *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. Appl Environ Microbiol. 2001;67(6):2586–95. doi:10.1128/aem.67.6.2586-2595.2001.

Fonnesbech Vogel B, Fussing V, Ojeniyi B, Gram L, Ahrens P. High-resolution genotyping of *Listeria monocytogenes* by fluorescent amplified fragment length polymorphism analysis compared to pulsed-field gel electrophoresis, random amplified polymorphic DNA analysis, ribotyping, and PCR-restriction fragment length polymorphism analysis. J Food Prot. 2004;67(8): 1656–65.

Food Safety News (2014). http://www.foodsafetynews.com/2014/09/danish-listeria-outbreak-claims-15-victims/#.VRkamXxSjsI. Accessed 30 Mar 2015.

Fox E, O'Mahony T, Clancy M, Dempsey R, O'Brien M, Jordan K. *Listeria monocytogenes* in the Irish dairy farm environment. J Food Prot. 2009;72(7):1450–6.

Fox E, Hunt K, O'Brien M, Jordan K. *Listeria monocytogenes* in Irish farmhouse cheese processing environments. Int J Food Microbiol. 2011a;145 Supplement 1(0):S39–45. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.10.012.

Fox EM, Leonard N, Jordan K. Physiological and transcriptional characterization of persistent and nonpersistent *Listeria monocytogenes* isolates. Appl Environ Microbiol. 2011b;77(18): 6559–69. doi:10.1128/aem.05529-11.

Fox E, Hunt K, O'Brien M, Jordan K. *Listeria monocytogenes* in Irish farmhouse cheese processing environments. Int J Food Microbiol. 2011c;145, Supplement 1(0):S39–45. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.10.012.

Fox EM, deLappe N, Garvey P, McKeown P, Cormican M, Leonard N, et al. PFGE analysis of *Listeria monocytogenes* isolates of clinical, animal, food and environmental origin from Ireland. J Med Microbiol. 2012;61(Pt 4):540–7. doi:10.1099/jmm.0.036764-0.

Fretz R, Pichler J, Sagel U, Much P, Ruppitsch W, Pietzka AT, et al. Update: multinational listeriosis outbreak due to 'Quargel', a sour milk curd cheese, caused by two different *L. monocytogenes* serotype 1/2a strains, 2009–2010. Euro Surveill. 2010;15(16).

Frye DM, Zweig R, Sturgeon J, Tormey M, LeCavalier M, Lee I, et al. An outbreak of febrile gastroenteritis associated with delicatessen meat contaminated with *Listeria monocytogenes*. Clin Infect Dis. 2002;35(8):943–9. doi:10.1086/342582.

FSANZ. How to determine the shelf life of food: a guidance document (Food Standards Agency New Zealand). http://www.foodsafety.govt.nz/elibrary/industry/determine-shelf-life-of-food/how-to-determine-the-shelf-life-of-food-revision.pdf (2014). Accessed 6 Oct 2014.

Gahan CGM, Hill C. *Listeria monocytogenes*: survival and adaptation in the gastrointestinal tract. Front Cell Infect Microbiol. 2014;4:9. doi:10.3389/fcimb.2014.00009.

Galvão NN, Chiarini E, Destro MT, de Aguiar Ferreira M, Nero LA. PFGE characterisation and adhesion ability of *Listeria monocytogenes* isolates obtained from bovine carcasses and beef processing facilities. Meat Sci. 2012;92(4):635–43. http://dx.doi.org/10.1016/j.meatsci.2012.06.011.

Gálvez A, Abriouel H, López RL, Omar NB. Bacteriocin-based strategies for food biopreservation. Int J Food Microbiol. 2007;120(1–2):51–70. doi:10.1016/j.ijfoodmicro.2007.06.001.

Gambarin P, Magnabosco C, Losio MN, Pavoni E, Gattuso A, Arcangeli G, et al. *Listeria monocytogenes* in ready-to-eat seafood and potential hazards for the consumers. Int J Microbiol. 2012;2012:497635. doi:10.1155/2012/497635.

Gandhi M, Chikindas ML. Listeria: a foodborne pathogen that knows how to survive. Int J Food Microbiol. 2007;113(1):1–15. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.07.008.

Garrido V, Vitas AI, García-Jalón I. Survey of *Listeria monocytogenes* in ready-to-eat products: prevalence by brands and retail establishments for exposure assessment of listeriosis in Northern Spain. Food Control. 2009;20(11):986–91. http://dx.doi.org/10.1016/j.foodcont.2008.11.013.

Gaulin C, Ramsay D, Ringuette L, Ismail J. First documented outbreak of *Listeria monocytogenes* in Quebec, 2002. Can Commun Dis Rep. 2003;29(21):181–6.

Gawade L, Barbuddhe SB, Bhosle S. Isolation and confirmation of *Listeria* species from seafood off Goa region by polymerase chain reaction. Indian J Microbiol. 2010;50(4):385–9. doi:10.1007/s12088-011-0064-y.

Gekara NO, Zietara N, Geffers R, Weiss S. *Listeria monocytogenes* induces T cell receptor unresponsiveness through pore-forming toxin listeriolysin O. J Infect Dis. 2010;202(11):1698–707. doi:10.1086/657145.

Gilmour MW, Graham M, Van Domselaar G, Tyler S, Kent H, Trout-Yakel KM, et al. High-throughput genome sequencing of two *Listeria monocytogenes* clinical isolates during a large foodborne outbreak. BMC Genomics. 2010;11:120. doi:10.1186/1471-2164-11-120.

Giovannacci I, Ragimbeau C, Queguiner S, Salvat G, Vendeuvre JL, Carlier V, et al. *Listeria monocytogenes* in pork slaughtering and cutting plants. Use of RAPD, PFGE and PCR-REA for tracing and molecular epidemiology. Int J Food Microbiol. 1999;53(2–3):127–40.

Gombas DE, Chen Y, Clavero RS, Scott VN. Survey of *Listeria monocytogenes* in ready-to-eat foods. J Food Prot. 2003;66(4):559–69.

Gómez D, McGuinness S, Reddington K, O'Grady J, Yangüela J, Barry T. Evaluation of a novel *Listeria* enrichment broth combined with a real-time PCR diagnostics assay for the specific detection of *Listeria monocytogenes* in RTE pork products. Int J Food Sci Technol. 2013;48(5):1103–8. doi:10.1111/ijfs.12053.

González D, Vitas AI, Díez-Leturia M, García-Jalón I. *Listeria monocytogenes* and ready-to-eat seafood in Spain: study of prevalence and temperatures at retail. Food Microbiol. 2013;36(2):374–8. http://dx.doi.org/10.1016/j.fm.2013.06.023.

Goodridge LD, Bisha B. Phage-based biocontrol strategies to reduce foodborne pathogens in foods. Bacteriophage. 2011;1(3):130–7. doi:10.4161/bact.1.3.17629.

Gormley FJ, Little CL, Grant KA, de Pinna E, McLauchlin J. The microbiological safety of ready-to-eat specialty meats from markets and specialty food shops: a UK wide study with a focus on *Salmonella* and *Listeria monocytogenes*. Food Microbiol. 2010;27(2):243–9. doi:10.1016/j.fm.2009.10.009.

Gottlieb SL, Newbern EC, Griffin PM, Graves LM, Hoekstra RM, Baker NL, et al. Multistate outbreak of Listeriosis linked to turkey deli meat and subsequent changes in US regulatory policy. Clin Infect Dis. 2006;42(1):29–36. doi:10.1086/498113.

Goulet V, Jacquet C, Vaillant V, Rebiere I, Mouret E, Lorente C, et al. Listeriosis from consumption of raw-milk cheese. Lancet. 1995;345(8964):1581–2.

Goulet V, Rocourt J, Rebiere I, Jacquet C, Moyse C, Dehaumont P, et al. Listeriosis outbreak associated with the consumption of rillettes in France in 1993. J Infect Dis. 1998;177(1):155–60.

Goulet V, Hebert M, Hedberg C, Laurent E, Vaillant V, De Valk H, et al. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. Clin Infect Dis. 2012;54(5):652–60. doi:10.1093/cid/cir902.

Grif K, Heller I, Wagner M, Dierich M, Wurzner R. A comparison of *Listeria monocytogenes* serovar 4b isolates of clinical and food origin in Austria by automated ribotyping and pulsed-field gel electrophoresis. Foodborne Pathog Dis. 2006;3(1):138–41. doi:10.1089/fpd.2006.3.138.

Guenther S, Huwyler D, Richard S, Loessner MJ. Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. Appl Environ Microbiol. 2009;75(1):93–100. doi:10.1128/AEM.01711-08.

Guillet C, Join-Lambert O, Le Monnier A, Leclercq A, Mechaï F, Mamzer-Bruneel M-F, et al. Human Listeriosis caused by *Listeria ivanovii*. Emerg Infect Dis. 2010;16(1):136–8. doi:10.3201/eid1601.091155.

Hagens S, Loessner MJ. Bacteriophage for biocontrol of foodborne pathogens: calculations and considerations. Curr Pharm Biotechnol. 2010;11(1):58–68.

Hall RSD, Lim I, Murphy F, Davos D, Lanser J. A cluster of listeriosis cases in South Australia. Commun Dis Intell. 1996;20:465.

Harvey J, Keenan KP, Gilmour A. Assessing biofilm formation by *Listeria monocytogenes* strains. Food Microbiol. 2007;24(4):380–92. http://dx.doi.org/10.1016/j.fm.2006.06.006.

Health-Canada. *Listeria monocytogenes* challenge testing of refrigerated ready-to-eat foods. http://www.hc-sc.gc.ca/fnan/alt_formats/pdf/legislation/pol/listeria_monocytogenes-test-eng.pdf (2012). Accessed 19 Jan 2015.

Hein I, Klinger S, Dooms M, Flekna G, Stessl B, Leclercq A, et al. Stress survival islet 1 (SSI-1) survey in *Listeria monocytogenes* reveals an insert common to *Listeria innocua* in sequence

type 121 *L. monocytogenes* strains. Appl Environ Microbiol. 2011;77(6):2169–73. doi:10.1128/aem.02159-10.

Heir E, Lindstedt B-A, Røtterud O-J, Vardund T, Kapperud G, Nesbakken T. Molecular epidemiology and disinfectant susceptibility of *Listeria monocytogenes* from meat processing plants and human infections. Int J Food Microbiol. 2004;96(1):85–96. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.014.

Ho JL, Shands KN, Friedland G, Eckind P, Fraser DW. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Arch Intern Med. 1986;146(3):520–4.

Hoelzer K, Sauders BD, Sanchez MD, Olsen PT, Pickett MM, Mangione KJ, et al. Prevalence, distribution, and diversity of *Listeria monocytogenes* in retail environments, focusing on small establishments and establishments with a history of failed inspections. J Food Prot. 2011;74(7):1083–95. doi:10.4315/0362-028x.jfp-10-567.

Holah JT, Taylor JH, Dawson DJ, Hall KE. Biocide use in the food industry and the disinfectant resistance of persistent strains of *Listeria monocytogenes* and *Escherichia coli*. Soc Appl Microbiol Symp Ser. 2002;31:111s–20s.

Holch A, Webb K, Lukjancenko O, Ussery D, Rosenthal BM, Gram L. Genome sequencing identifies two nearly unchanged strains of persistent *Listeria monocytogenes* isolated at two different fish processing plants sampled 6 years apart. Appl Environ Microbiol. 2013;79(9):2944–51. doi:10.1128/aem.03715-12.

Horton RM, Cai ZL, Ho SN, Pease LR. Gene splicing by overlap extension: tailor-made genes using the polymerase chain reaction. Biotechniques. 1990;8(5):528–35.

Hurd S, Phan Q, Hadler J, Mackenzie B, Lance-Parker S, Blake P, Deasy M, Rankin J, Frye D, Lee I, Werner B, Bugia D, Bidol S, Stoltman G, Boulton M, Widemann M, Kornstein L, Reddy S, Mojica B, Guido F, Huang A, Vincent C, Bugenhagen A, Corby J, Carloni E, Holcomb M, Kondracki S, Woron R, Zansky S, Smith P, Dowdle G, Nichols C, Smith F, Gerber D, Jones T, Moore W, Ahrabi-Fard S, Davis J. Multistate outbreak of listeriosis—United States, 2000. MMWR Morb Mortal Wkly Rep. 2000;49(50):1129–30.

Hurley D, Luque-Sastre L, DeLappe N, Moore JE, Cormican M, Jordan KN, et al. Comparison of *Listeria monocytogenes* isolates across the island of Ireland. J Food Prot. 2014;77(8):1402–6. doi:10.4315/0362-028X.JFP-14-026.

Jackson KA, Biggerstaff M, Tobin-D'Angelo M, Sweat D, Klos R, Nosari J, et al. Multistate outbreak of *Listeria monocytogenes* associated with Mexican-style cheese made from pasteurized milk among pregnant, Hispanic women. J Food Prot. 2011;74(6):949–53. doi:10.4315/0362-028x.jfp-10-536.

Jacquet C, Catimel B, Brosch R, Buchrieser C, Dehaumont P, Goulet V, et al. Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992. Appl Environ Microbiol. 1995;61(6):2242–6.

Jacquet C, Saint-Cloment C, Brouille F, Catimel B, Rocourt J. La liste'riose humaine en France en 1997. Epidemiologique Hebdomadaire. 1998;33:142–3.

Jacquet C, Gouin E, Jeannel D, Cossart P, Rocourt J. Expression of ActA, Ami, InlB, and listeriolysin O in *Listeria monocytogenes* of human and food origin. Appl Environ Microbiol. 2002;68(2):616–22.

Jamali H, Chai LC, Thong KL. Detection and isolation of *Listeria* spp. and *Listeria monocytogenes* in ready-to-eat foods with various selective culture media. Food Control. 2013;32(1):19–24. doi:10.1016/j.foodcont.2012.11.033.

Jelfs P, Cheung K, Bansal J, Dalton C, Gilbert G. An investigation of an outbreak of listeriosis linked to consumption of contaminated fruit salad. Microbiol Aust. 2000;21:A90.

Jensen A, Frederiksen W, Gerner-Smidt P. Risk factors for listeriosis in Denmark, 1989–1990. Scand J Infect Dis. 1994;26(2):171–8.

Jordan K, McAullife O. Biotechnological approaches for control of *Listeria monocytogenes* in foods. Curr Biotechnol. 2012;1:267–80.

Kahraman T, Ozmen G, Ozinan B, Omer Goksoy E. Prevalence of *Salmonella* spp. and *Listeria monocytogenes* in different cheese types produced in Turkey. Br Food J. 2010;112(11):1230–6. doi:10.1108/00070701011088214.

Kastbjerg VG, Gram L. Model systems allowing quantification of sensitivity to disinfectants and comparison of disinfectant susceptibility of persistent and presumed nonpersistent *Listeria monocytogenes*. J Appl Microbiol. 2009;106(5):1667–81. doi:10.1111/j.1365-2672.2008.04134.x.

Kathariou S. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. J Food Prot. 2002;65(11):1811–29.

Kathariou S, Metz P, Hof H, Goebel W. Tn916-induced mutations in the hemolysin determinant affecting virulence of *Listeria monocytogenes*. J Bacteriol. 1987;169(3):1291–7.

Kazmierczak MJ, Wiedmann M, Boor KJ. Alternative sigma factors and their roles in bacterial virulence. Microbiol Mol Biol Rev. 2005;69(4):527–43. doi:10.1128/mmbr.69.4.527-543.2005.

Kernbauer E, Maier V, Rauch I, Müller M, Decker T. Route of infection determines the impact of type I interferons on innate immunity to *Listeria monocytogenes*. PLoS One. 2013;8(6):e65007. doi:10.1371/journal.pone.0065007.

Kerouanton A, Brisabois A, Denoyer E, Dilasser F, Grout J, Salvat G, et al. Comparison of five typing methods for the epidemiological study of *Listeria monocytogenes*. Int J Food Microbiol. 1998;43(1–2):61–71. http://dx.doi.org/10.1016/S0168-1605(98)00098-1.

Klaeboe H, Rosef O, Fortes E, Wiedmann M. Ribotype diversity of *Listeria monocytogenes* isolates from two salmon processing plants in Norway. Int J Environ Health Res. 2006;16(5):375–83. doi:10.1080/09603120600869406.

Klumpp J, Dorscht J, Lurz R, Bielmann R, Wieland M, Zimmer M, et al. The terminally redundant, nonpermuted genome of *Listeria bacteriophage* A511: a model for the SPO1-like myoviruses of gram-positive bacteria. J Bacteriol. 2008;190(17):5753–65. doi:10.1128/jb.00461-08.

Koch J, Dworak R, Prager R, Becker B, Brockmann S, Wicke A, et al. Large listeriosis outbreak linked to cheese made from pasteurized milk, Germany, 2006–2007. Foodborne Pathog Dis. 2010;7(12):1581–4. doi:10.1089/fpd.2010.0631.

Koser CU, Ellington MJ, Cartwright EJ, Gillespie SH, Brown NM, Farrington M, et al. Routine use of microbial whole genome sequencing in diagnostic and public health microbiology. PLoS Pathog. 2012;8(8):e1002824. doi:10.1371/journal.ppat.1002824.

Kotzekidou P. Microbiological examination of ready-to-eat foods and ready-to-bake frozen pastries from university canteens. Food Microbiol. 2013a;34(2):337–43. doi:10.1016/j.fm.2013.01.005.

Kotzekidou P. Survey of *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* O157:H7 in raw ingredients and ready-to-eat products by commercial real-time PCR kits. Food Microbiol. 2013b;35(2):86–91. doi:10.1016/j.fm.2013.03.007.

Kovacevic J, McIntyre LF, Henderson SB, Kosatsky T. Occurrence and distribution of listeria species in facilities producing ready-to-eat foods in British Columbia, Canada. J Food Prot. 2012a;75(2):216–24. doi:10.4315/0362-028X.JFP-11-300.

Kovacevic J, Mesak LR, Allen KJ. Occurrence and characterization of *Listeria* spp. in ready-to-eat retail foods from Vancouver, British Columbia. Food Microbiol. 2012b;30(2):372–8. doi:10.1016/j.fm.2011.12.015.

Kovacevic M, Burazin J, Pavlovic H, Kopjar M, Pilizota V. Prevalence and level of *Listeria monocytogenes* and other *Listeria* sp. in ready-to-eat minimally processed and refrigerated vegetables. World J Microbiol Biotechnol. 2013;29(4):707–12. doi:10.1007/s11274-012-1226-8.

Kramarenko T, Roasto M, Meremäe K, Kuningas M, Põltsama P, Elias T. *Listeria monocytogenes* prevalence and serotype diversity in various foods. Food Control. 2013;30(1):24–9. doi:10.1016/j.foodcont.2012.06.047.

Kushwaha K, Muriana PM. Analysis of tissue invasiveness of adherent strains of *Listeria monocytogenes* by in vivo mouse assay. Int J Food Microbiol. 2010a;141(1–2):104–9. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.03.004.

Kushwaha K, Muriana PM. Comparison of invasiveness among surface-adherent variants of *Listeria monocytogenes* in Caco-2 cell culture assays. Int J Food Microbiol. 2010b;138(1–2):166–71. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.11.024.

Kuzmanovic J, Asanin R, Baltic M, Misic D, Dimitrijevic M, Stojanovic M, et al. Presence of *Listeria* spp. in fish samples, fish products and sea products. Acta Vet. 2011;61(2–3):193–203. doi:10.2298/avb1103193k.

Lambertz ST, Nilsson C, Bradenmark A, Sylven S, Johansson A, Jansson LM, et al. Prevalence and level of *Listeria monocytogenes* in ready-to-eat foods in Sweden 2010. Int J Food Microbiol. 2012;160(1):24–31. doi:10.1016/j.ijfoodmicro.2012.09.010.

Lambertz ST, Ivarsson S, Lopez-Valladares G, Sidstedt M, Lindqvist R. Subtyping of *Listeria monocytogenes* isolates recovered from retail ready-to-eat foods, processing plants and listeriosis patients in Sweden 2010. Int J Food Microbiol. 2013;166(1):186–92. doi:10.1016/j.ijfoodmicro.2013.06.008.

Lang LH. FDA approves use of bacteriophages to be added to meat and poultry products. Gastroenterology. 2006;131(5):1370. doi:10.1053/j.gastro.2006.10.012.

Latorre AA, Van Kessel JS, Karns JS, Zurakowski MJ, Pradhan AK, Boor KJ, et al. Biofilm in milking equipment on a dairy farm as a potential source of bulk tank milk contamination with *Listeria monocytogenes*. J Dairy Sci. 2010;93(6):2792–802. doi:10.3168/jds.2009-2717.

Latorre AA, Van Kessel JAS, Karns JS, Zurakowski MJ, Pradhan AK, Boor KJ, et al. Increased in vitro adherence and on-farm persistence of predominant and persistent *Listeria monocytogenes* strains in the milking system. Appl Environ Microbiol. 2011;77(11):3676–84. doi:10.1128/AEM.02441-10.

Lawrence LM, Gilmour A. Characterization of *Listeria monocytogenes* isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. Appl Environ Microbiol. 1995;61(6):2139–44.

Lennon D, Lewis B, Mantell C, Becroft D, Dove B, Farmer K, et al. Epidemic perinatal listeriosis. Pediatr Infect Dis. 1984;3(1):30–4.

Leong D, Alvarez-Ordonez A, Jordan K. Monitoring occurrence and persistence of *Listeria monocytogenes* in foods and food processing environments in the Republic of Ireland. Front Microbiol. 2014;5. doi:10.3389/fmicb.2014.00436.

Leong D, Alvarez-Ordóñez A, Jordan K. A note on challenge trials to determine the growth of Listeria monocytogenes on mushrooms. Irish J Agric Food Res. 2015 (in press).

Leverentz B, Conway WS, Camp MJ, Janisiewicz WJ, Abuladze T, Yang M, et al. Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. Appl Environ Microbiol. 2003;69(8):4519–26.

Leverentz B, Conway WS, Janisiewicz W, Abadias M, Kurtzman CP, Camp MJ. Biocontrol of the food-borne pathogens *Listeria monocytogenes* and *Salmonella Enterica serovar Poona* on fresh-cut apples with naturally occurring bacterial and yeast antagonists. Appl Environ Microbiol. 2006;72(2):1135–40. doi:10.1128/AEM.72.2.1135-1140.2006.

Linnan MJ, Mascola L, Lou XD, Goulet V, May S, Salminen C, et al. Epidemic listeriosis associated with Mexican-style cheese. N Engl J Med. 1988;319(13):823–8. doi:10.1056/nejm198809293191303.

Little CL, Sagoo SK, Gillespie IA, Grant K, McLauchlin J. Prevalence and level of *Listeria monocytogenes* and other *Listeria* species in selected retail ready-to-eat foods in the United Kingdom. J Food Prot. 2009;72(9):1869–77.

Lomonaco S, Decastelli L, Nucera D, Gallina S, Manila Bianchi D, Civera T. *Listeria monocytogenes* in gorgonzola: subtypes, diversity and persistence over time. Int J Food Microbiol. 2009;128(3):516–20. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.10.009.

López V, Villatoro D, Ortiz S, López P, Navas J, Dávila JC, et al. Molecular tracking of *Listeria monocytogenes* in an Iberian pig abattoir and processing plant. Meat Sci. 2008;78(1–2):130–4. http://dx.doi.org/10.1016/j.meatsci.2007.05.002.

Louie M, Jayaratne P, Luchsinger I, Devenish J, Yao J, Schlech W, et al. Comparison of ribotyping, arbitrarily primed PCR, and pulsed-field gel electrophoresis for molecular typing of *Listeria monocytogenes*. J Clin Microbiol. 1996;34(1):15–9.

Lourenço A, Neves E, Brito L. Susceptibility of *Listeria monocytogenes* from traditional cheese-dairies to in-use sanitizers. Food Control. 2009;20(6):585–9. http://dx.doi.org/10.1016/j.foodcont.2008.08.009.

Luber P, Crerar S, Dufour C, Farber J, Datta A, Todd ECD. Controlling *Listeria monocytogenes* in ready-to-eat foods: working towards global scientific consensus and harmonization – recommendations for improved prevention and control. Food Control. 2011;22(9):1535–49.

Lukinmaa S, Aarnisalo K, Suihko ML, Siitonen A. Diversity of *Listeria monocytogenes* isolates of human and food origin studied by serotyping, automated ribotyping and pulsed-field gel electrophoresis. Clin Microbiol Infect. 2004;10(6):562–8. doi:10.1111/j.1469-0691.2004.00876.x.

Lunden JM, Miettinen MK, Autio TJ, Korkeala HJ. Persistent *Listeria monocytogenes* strains show enhanced adherence to food contact surface after short contact times. J Food Prot. 2000;63(9):1204–7.

Lundén J, Autio T, Markkula A, Hellström S, Korkeala H. Adaptive and cross-adaptive responses of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants. Int J Food Microbiol. 2003;82(3):265–72. http://dx.doi.org/10.1016/S0168-1605(02)00312-4.

Lunden JM, Autio TJ, Sjoberg AM, Korkeala HJ. Persistent and nonpersistent *Listeria monocytogenes* contamination in meat and poultry processing plants. J Food Prot. 2003;66(11):2062–9.

Lyytikainen O, Autio T, Maijala R, Ruutu P, Honkanen-Buzalski T, Miettinen M, et al. An outbreak of *Listeria monocytogenes* serotype 3a infections from butter in Finland. J Infect Dis. 2000;181(5):1838–41. doi:10.1086/315453.

MacDonald PD, Whitwam RE, Boggs JD, MacCormack JN, Anderson KL, Reardon JW, et al. Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produced Mexican-style cheese. Clin Infect Dis. 2005;40(5):677–82. doi:10.1086/427803.

Maistro LC, Miya NTN, Sant'Ana AS, Pereira JL. Microbiological quality and safety of minimally processed vegetables marketed in Campinas, SP – Brazil, as assessed by traditional and alternative methods. Food Control. 2012;28(2):258–64. doi:10.1016/j.foodcont.2012.05.021.

Makino SI, Kawamoto K, Takeshi K, Okada Y, Yamasaki M, Yamamoto S, et al. An outbreak of food-borne listeriosis due to cheese in Japan, during 2001. Int J Food Microbiol. 2005;104(2):189–96. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.02.009.

Maklon K, Minami A, Kusumoto A, Takeshi K, Nguyen TB, Makino S, et al. Isolation and characterization of *Listeria monocytogenes* from commercial asazuke (Japanese light pickles). Int J Food Microbiol. 2010;139(3):134–9. doi:10.1016/j.ijfoodmicro.2010.03.025.

Manguiat LS, Fang TJ. Microbiological quality of chicken- and pork-based street-vended foods from Taichung, Taiwan, and Laguna. Philippines Food Microbiol. 2013;36(1):57–62. doi:10.1016/j.fm.2013.04.005.

Marian MN, Sharifah Aminah SM, Zuraini MI, Son R, Maimunah M, Lee HY, et al. MPN-PCR detection and antimicrobial resistance of *Listeria monocytogenes* isolated from raw and ready-to-eat foods in Malaysia. Food Control. 2012;28(2):309–14. doi:10.1016/j.foodcont.2012.05.030.

Martin B, Perich A, Gomez D, Yanguela J, Rodriguez A, Garriga M, et al. Diversity and distribution of *Listeria monocytogenes* in meat processing plants. Food Microbiol. 2014;44:119–27. doi:10.1016/j.fm.2014.05.014.

Martins EA, Leal Germano PM. *Listeria monocytogenes* in ready-to-eat, sliced, cooked ham and salami products, marketed in the city of São Paulo, Brazil: occurrence, quantification, and serotyping. Food Control. 2011;22(2):297–302. doi:10.1016/j.foodcont.2010.07.026.

McClain D, Lee WH. Development of USDA-FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. J Assoc Off Anal Chem. 1988;71(3):660–4.

McLauchlin J, Hall SM, Velani SK, Gilbert RJ. Human listeriosis and pate: a possible association. BMJ. 1991;303(6805):773–5.

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, et al. Food-related illness and death in the United States. Emerg Infect Dis. 1999;5(5):607–25.

Mead PS, Dunne EF, Graves L, Wiedmann M, Patrick M, Hunter S, et al. Nationwide outbreak of listeriosis due to contaminated meat. Epidemiol Infect. 2006;134(4):744–51. doi:10.1017/S0950268805005376.

Meldrum RJ, Ellis PW, Mannion PT, Halstead D, Garside J. Prevalence of *Listeria monocytogenes* in ready-to-eat foods sampled from the point of sale in Wales, United Kingdom. J Food Prot. 2010;73(8):1515–8.

Meldrum RJ, Garside J, Mannion P, Charles D, Ellis P. Variation in the annual unsatisfactory rates of selected pathogens and indicators in ready-to-eat food sampled from the point of sale or service in Wales, United Kingdom. J Food Prot. 2012;75(12):2238–40. doi:10.4315/0362-028X.JFP-12-244.

Meloni D, Galluzzo P, Mureddu A, Piras F, Griffiths M, Mazzette R. *Listeria monocytogenes* in RTE foods marketed in Italy: prevalence and automated EcoRI ribotyping of the isolates. Int J Food Microbiol. 2009;129(2):166–73. doi:10.1016/j.ijfoodmicro.2008.11.014.

Mengesha D, Zewde BM, Toquin MT, Kleer J, Hildebrandt G, Gebreyes WA. Occurrence and distribution of *Listeria monocytogenes* and other *Listeria* species in ready-to-eat and raw meat products. Berl Munch Tierarztl Wochenschr. 2009;122(1–2):20–4.

Meyer C, Fredriksson-Ahomaa M, Kleta S, Ellerbroek L, Thiel S, Märtlbauer E. Occurrence of *L. monocytogenes* in ready-to-eat poultry products available on the German market. Food Res Int. 2012;48(2):944–7. doi:10.1016/j.foodres.2012.02.022.

Meyer-Morse N, Robbins JR, Rae CS, Mochegova SN, Swanson MS, Zhao Z, et al. Listeriolysin O is necessary and sufficient to induce autophagy during *Listeria monocytogenes* infection. PLoS One. 2010;5(1):e8610. doi:10.1371/journal.pone.0008610.

Miettinen H, Wirtanen G. Ecology of *Listeria* spp. in a fish farm and molecular typing of *Listeria monocytogenes* from fish farming and processing companies. Int J Food Microbiol. 2006;112(2):138–46. doi:10.1016/j.ijfoodmicro.2006.06.016.

Miettinen MK, Siitonen A, Heiskanen P, Haajanen H, Bjorkroth KJ, Korkeala HJ. Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold-smoked rainbow trout. J Clin Microbiol. 1999;37(7):2358–60.

Mitchell DL. A case cluster of listeriosis in Tasmania. Commun Dis Intell. 1991;15:427.

Miya S, Takahashi H, Ishikawa T, Fujii T, Kimura B. Risk of *Listeria monocytogenes* contamination of raw ready-to-eat seafood products available at retail outlets in Japan. Appl Environ Microbiol. 2010;76(10):3383–6. doi:10.1128/AEM.01456-09.

Moorhead SM, Dykes GA. Influence of the sigB gene on the cold stress survival and subsequent recovery of two *Listeria monocytogenes* serotypes. Int J Food Microbiol. 2004;91(1):63–72. http://dx.doi.org/10.1016/S0168-1605(03)00332-5.

Moreno Y, Sánchez-Contreras J, Montes RM, García-Hernández J, Ballesteros L, Ferrús MA. Detection and enumeration of viable *Listeria monocytogenes* cells from ready-to-eat and processed vegetable foods by culture and DVC-FISH. Food Control. 2012;27(2):374–9. doi:10.1016/j.foodcont.2012.04.017.

Morobe IC, Obi CL, Nyila MA, Gashe BA, Matsheka MI. Prevalence, antimicrobial resistance profiles of *Listeria monocytogenes* from various foods in Gaborone. Botsw Afr J Biotechnol. 2009;8(22):6383–7.

Morton J, Karoonuthaisiri N, Charlermroj R, Stewart LD, Elliott CT, Grant IR. Phage display-derived binders able to distinguish *Listeria monocytogenes* from other *Listeria* species. PLoS One. 2013;8(9):e74312. doi:10.1371/journal.pone.0074312.

Mottin V, Fisch E, Cardoso M. Microbial contamination of luncheon meat sliced and packaged at supermarkets in Porto Alegre. Braz Acta Sci Vet. 2011;39(1):940.

Muhterem-Uyar M, Dalmasso M, Bolocan AS, Hernandez M, Kapetanakou AE, Kuchta T, et al. Environmental sampling for *Listeria monocytogenes* control in food processing facilities reveals three contamination scenarios. Food Control. 2015;51:94–107. http://dx.doi.org/10.1016/j.foodcont.2014.10.042.

Mujahid S, Orsi R, Boor K, Wiedmann M. Protein level identification of the *Listeria monocytogenes* Sigma H, Sigma L, and Sigma C regulons. BMC Microbiol. 2013;13(1):156.

Muller A, Rychli K, Zaiser A, Wieser C, Wagner M, Schmitz-Esser S. The *Listeria monocytogenes* transposon Tn6188 provides increased tolerance to various quaternary ammonium compounds and ethidium bromide. FEMS Microbiol Lett. 2014;361(2):166–73. doi:10.1111/1574-6968.12626.

Murphy M, Corcoran D, Buckley JF, O'Mahony M, Whyte P, Fanning S. Development and application of multiple-locus variable number of tandem repeat analysis (MLVA) to subtype a collection of *Listeria monocytogenes*. Int J Food Microbiol. 2007;115(2):187–94. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.10.022.

Nabi AQ, Vernile A, Spano G, Lasalandra G, Beneduce L, Colelli G, et al. PCR-based quality control of *Listeria* spp. and *L. monocytogenes* in ready-to-eat salads sold in Italy. Curr Nutr Food Sci. 2011;7(1):57–62.

Nakari UM, Rantala L, Pihlajasaari A, Toikkanen S, Johansson T, Hellsten C, et al. Investigation of increased listeriosis revealed two fishery production plants with persistent *Listeria* contamination in Finland in 2010. Epidemiol Infect. 2014:1–9. doi:10.1017/s095026881300349x.

Nesbakken T, Kapperud G, Caugant DA. Pathways of *Listeria monocytogenes* contamination in the meat processing industry. Int J Food Microbiol. 1996;31(1–3):161–71.

Netschajew A, Fredriksson-Ahomaa M, Sperner B, Stolle A. Detection of *Listeria monocytogenes* in vacuum-packed meat products using real-time PCR, immunoassay and culturing. Arch Leb. 2009;60:12–7.

Niels le Souef P, Walters BN. Neonatal listeriosis: a summer outbreak. Med J Aust. 1981;2(4):188–91.

Nightingale KK, Windham K, Martin KE, Yeung M, Wiedmann M. Select *Listeria monocytogenes* subtypes commonly found in foods carry distinct nonsense mutations in inlA, leading to expression of truncated and secreted internalin A, and are associated with a reduced invasion phenotype for human intestinal epithelial cells. Appl Environ Microbiol. 2005;71(12): 8764–72. doi:10.1128/aem.71.12.8764-8772.2005.

Norwood DE, Gilmour A. Adherence of *Listeria monocytogenes* strains to stainless steel coupons. J Appl Microbiol. 1999;86(4):576–82.

Nucera D, Lomonaco S, Bianchi DM, Decastelli L, Grassi MA, Bottero MT, et al. A five year surveillance report on PFGE types of *Listeria monocytogenes* isolated in Italy from food and food related environments. Int J Food Microbiol. 2010;140(2–3):271–6. doi:10.1016/j.ijfoodmicro.2010.04.016.

O'Brien M, Hunt K, McSweeney S, Jordan K. Occurrence of foodborne pathogens in Irish farmhouse cheese. Food Microbiol. 2009;26(8):910–4. http://dx.doi.org/10.1016/j.fm.2009.06.009.

O'Byrne CP, Karatzas KA. The role of sigma B (sigma B) in the stress adaptations of *Listeria monocytogenes*: overlaps between stress adaptation and virulence. Adv Appl Microbiol. 2008;65:115–40. doi:10.1016/s0065-2164(08)00605-9.

Ojeniyi B, Christensen J, Bisgaard M. Comparative investigations of *Listeria monocytogenes* isolated from a turkey processing plant, turkey products, and from human cases of listeriosis in Denmark. Epidemiol Infect. 2000;125(2):303–8.

Okada Y, Monden S, Igimi S, Yamamoto S. The occurrence of *Listeria monocytogenes* in imported ready-to-eat foods in Japan. J Vet Med Sci. 2012;74(3):373–5. doi:10.1292/jvms.11-0262.

Omar SS, Dababneh BF, Qatatsheh A, Abu-Romman S, Hawari AD, Aladaileh S. The incidence of *Listeria* species and other indicator bacteria in some traditional foods sold in Karak city, Jordan. J Food Agric Environ. 2011;9:79–81.

Orsi RH, den Bakker HC, Wiedmann M. *Listeria monocytogenes* lineages: genomics, evolution, ecology, and phenotypic characteristics. Int J Med Microbiol. 2011;301(2):79–96. doi:10.1016/j.ijmm.2010.05.002.

Ortiz S, Lopez V, Villatoro D, Lopez P, Davila JC, Martinez-Suarez JN. A 3-year surveillance of the genetic diversity and persistence of *Listeria monocytogenes* in an Iberian pig slaughterhouse and processing plant. Foodborne Pathog Dis. 2010;7(10):1177–84. doi:10.1089/fpd.2010.0535.

Osaili TM, Alaboudi AR, Nesiar EA. Prevalence of *Listeria* spp. and antibiotic susceptibility of *Listeria monocytogenes* isolated from raw chicken and ready-to-eat chicken products in Jordan. Food Control. 2011;22(3–4):586–90. doi:10.1016/j.foodcont.2010.10.008.

Pagotto F, Ng LK, Clark C, Farber J. Canadian listeriosis reference service. Foodborne Pathog Dis. 2006;3(1):132–7. doi:10.1089/fpd.2006.3.132.

Parihar VS, Barbuddhe SB, Danielsson-Tham ML, Tham W. Isolation and characterization of *Listeria* species from tropical seafoods. Food Control. 2008;19(6):566–9. doi:10.1016/j.foodcont.2007.06.009.

Paul M, Baranzoni GM, Albonetti S, Brewster JD. Direct, quantitative detection of *Listeria monocytogenes* in fresh raw whole milk by qPCR. Int Dairy J. 2014(0). http://dx.doi.org/10.1016/j.idairyj.2014.09.008.

Perez-Rodriguez F, Castro R, Posada-Izquierdo GD, Valero A, Carrasco E, Garcia-Gimeno RM, et al. Evaluation of hygiene practices and microbiological quality of cooked meat products during slicing and handling at retail. Meat Sci. 2010;86(2):479–85. doi:10.1016/j.meatsci.2010.05.038.

Pesavento G, Ducci B, Nieri D, Comodo N, Lo Nostro A. Prevalence and antibiotic susceptibility of *Listeria* spp. isolated from raw meat and retail foods. Food Control. 2010;21(5):708–13. doi:10.1016/j.foodcont.2009.10.012.

Pichler J, Much P, Kasper S, Fretz R, Auer B, Kathan J, et al. An outbreak of febrile gastroenteritis associated with jellied pork contaminated with *Listeria monocytogenes*. Wien Klin Wochenschr. 2009;121(3–4):149–56. doi:10.1007/s00508-009-1137-3.

Pizarro-Cerdá J, Sousa S, Cossart P. Exploitation of host cell cytoskeleton and signalling during *Listeria monocytogenes* entry into mammalian cells. C R Biol. 2004;327(6):523–31. http://dx.doi.org/10.1016/j.crvi.2004.06.001.

Pradhan AK, Ivanek R, Grohn YT, Bukowski R, Geornaras I, Sofos JN, et al. Quantitative risk assessment of listeriosis-associated deaths due to *Listeria monocytogenes* contamination of deli meats originating from manufacture and retail. J Food Prot. 2010;73(4):620–30.

Pricope L, Nicolau A, Wagner M, Rychli K. The effect of sublethal concentrations of benzalkonium chloride on invasiveness and intracellular proliferation of *Listeria monocytogenes*. Food Control. 2013;31(1):230–5. http://dx.doi.org/10.1016/j.foodcont.2012.09.031.

Public Health Agency of Canada (PHAC). Lessons learned: Public Health Agency of Canada's response to the 2008 listeriosis outbreak. http://www.phac-aspc.gc.ca/fs-sa/listeria/2008-lessons-lecons-eng.php. Accessed 31 Mar 2015.

PulseNetUSA. International Standard PulseNet protocol. http://www.cdc.gov/pulsenet/pathogens/listeria.html (2009). Accessed 23 Apr 2014.

Ragon M, Wirth T, Hollandt F, Lavenir R, Lecuit M, Le Monnier A, et al. A new perspective on *Listeria monocytogenes* evolution. PLoS Pathog. 2008;4(9):e1000146. doi:10.1371/journal.ppat.1000146.

Rahimi E, Shakerian A, Raissy M. Prevalence of *Listeria* species in fresh and frozen fish and shrimp in Iran. Ann Microbiol. 2012;62(1):37–40. doi:10.1007/s13213-011-0222-9.

Reingold AL. Outbreak investigations—a perspective. Emerg Infect Dis. 1998;4(1):21–7.

Riedo FX, Pinner RW, Tosca ML, Cartter ML, Graves LM, Reeves MW, et al. A point-source foodborne listeriosis outbreak: documented incubation period and possible mild illness. J Infect Dis. 1994;170(3):693–6.

Rodriguez-Lazaro D, Hernandez M, Scortti M, Esteve T, Vazquez-Boland JA, Pla M. Quantitative detection of *Listeria monocytogenes* and *Listeria innocua* by real-time PCR: assessment of hly, iap, and lin02483 targets and AmpliFluor technology. Appl Environ Microbiol. 2004;70(3):1366–77.

Rogers LA. The inhibiting effect of *Streptococcus lactis* on *Lactobacillus bulgaricus*. J Bacteriol. 1928;16(5):321–5.

Rossmanith P, Mester P, Wagner M, Schoder D. Demonstration of the effective performance of a combined enrichment/real-time PCR method targeting the prfA gene of *Listeria monocytogenes* by testing fresh naturally contaminated acid curd cheese. Lett Appl Microbiol. 2010;51(4):480–4. doi:10.1111/j.1472-765X.2010.02925.x.

Ryan S, Begley M, Hill C, Gahan CG. A five-gene stress survival islet (SSI-1) that contributes to the growth of *Listeria monocytogenes* in suboptimal conditions. J Appl Microbiol. 2010;109(3):984–95. doi:10.1111/j.1365-2672.2010.04726.x.

Rychli K, Muller A, Zaiser A, Schoder D, Allerberger F, Wagner M, et al. Genome sequencing of *Listeria monocytogenes* "Quargel" listeriosis outbreak strains reveals two different strains with distinct in vitro virulence potential. PLoS One. 2014;9(2):e89964. doi:10.1371/journal.pone.0089964.

Sabat AJ, Budimir A, Nashev D, Sa-Leao R, van Dijl J, Laurent F, et al. Overview of molecular typing methods for outbreak detection and epidemiological surveillance. Euro Surveill. 2013;18(4):20380.

Salamina G, Dalle Donne E, Niccolini A, Poda G, Cesaroni D, Bucci M, et al. A foodborne outbreak of gastroenteritis involving *Listeria monocytogenes*. Epidemiol Infect. 1996;117(3):429–36.

Saludes M, Troncoso M, Figueroa G. Presence of *Listeria monocytogenes* in Chilean food matrices. Food Control. 2014(0). http://dx.doi.org/10.1016/j.foodcont.2014.08.008.

Sant'Ana AS, Igarashi MC, Landgraf M, Destro MT, Franco BD. Prevalence, populations and pheno- and genotypic characteristics of *Listeria monocytogenes* isolated from ready-to-eat vegetables marketed in Sao Paulo, Brazil. Int J Food Microbiol. 2012;155(1–2):1–9. doi:10.1016/j.ijfoodmicro.2011.12.036.

Sauders BD, Sanchez MD, Rice DH, Corby J, Stich S, Fortes ED, et al. Prevalence and molecular diversity of *Listeria monocytogenes* in retail establishments. J Food Prot. 2009;72(11):2337–49.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, et al. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis. 2011;17(1):7–15. doi:10.3201/eid1701.091101p1.

Scharff RL. Health-related costs from foodborne illness in the United States. http://publichealth.lacounty.gov/eh/docs/ReportPublication/HlthRelatedCostsFromFoodborneIllinessUS.pdf (2010). Accessed 19 Jan 2015.

Schlech 3rd WF, Lavigne PM, Bortolussi RA, Allen AC, Haldane EV, Wort AJ, et al. Epidemic listeriosis—evidence for transmission by food. N Engl J Med. 1983;308(4):203–6. doi:10.1056/nejm198301273080407.

Schnitger AK, Machova A, Mueller RU, Androulidaki A, Schermer B, Pasparakis M, et al. Listeria monocytogenes infection in macrophages induces vacuolar-dependent host miRNA response. PLoS One. 2011;6(11), e27435. doi:10.1371/journal.pone.0027435.

Schmid B, Klumpp J, Raimann E, Loessner MJ, Stephan R, Tasara T. Role of cold shock proteins in growth of *Listeria monocytogenes* under cold and osmotic stress conditions. Appl Environ Microbiol. 2009;75(6):1621–7. doi:10.1128/aem.02154-08.

Schubert W-D, Urbanke C, Ziehm T, Beier V, Machner MP, Domann E, et al. Structure of internalin, a major invasion protein of *Listeria monocytogenes*, in complex with its human receptor E-Cadherin. Cell. 2002;111(6):825–36. http://dx.doi.org/10.1016/S0092-8674(02)01136-4.

Schvartzman MS, Belessi X, Butler F, Skandamis P, Jordan K. Comparison of growth limits of *Listeria monocytogenes* in milk, broth and cheese. J Appl Microbiol. 2010;109(5):1790–9. doi:10.1111/j.1365-2672.2010.04807.x.

Schvartzman MS, Belessi C, Butler F, Skandamis PN, Jordan KN. Effect of pH and water activity on the growth limits of *Listeria monocytogenes* in a cheese matrix at two contamination levels. J Food Prot. 2011;74(11):1805–13. doi:10.4315/0362-028x.jfp-11-102.

Schwartz B, Hexter D, Broome CV, Hightower AW, Hirschhorn RB, Porter JD, et al. Investigation of an outbreak of listeriosis: new hypotheses for the etiology of epidemic *Listeria monocytogenes* infections. J Infect Dis. 1989;159(4):680–5.

Shen J, Rump L, Zhang Y, Chen Y, Wang X, Meng J. Molecular subtyping and virulence gene analysis of *Listeria monocytogenes* isolates from food. Food Microbiol. 2013;35(1):58–64. doi:10.1016/j.fm.2013.02.014.

Sim J, Hood D, Finnie L, Wilson M, Graham C, Brett M, et al. Series of incidents of *Listeria monocytogenes* non-invasive febrile gastroenteritis involving ready-to-eat meats. Lett Appl Microbiol. 2002;35(5):409–13.

Simpson DM. Microbiology and epidemiology in foodborne disease outbreaks: the whys and when nots. J Food Prot. 1996;59(1):93–5.

Smith B. Listeriosis in the Rio Grande Valley 2003–2008. http://www.borderhealth.org/files/res_591.pdf (2008). Accessed 2 Apr 2012.

Snapir YM, Vaisbein E, Nassar F. Low virulence but potentially fatal outcome—*Listeria ivanovii*. Eur J Intern Med. 2006;17(4):286–7. http://dx.doi.org/10.1016/j.ejim.2005.12.006.

Sospedra I, Rubert J, Soriano JM, Mañes J. Survey of microbial quality of plant-based foods served in restaurants. Food Control. 2013;30(2):418–22. doi:10.1016/j.foodcont.2012.08.004.

Sperry KE, Kathariou S, Edwards JS, Wolf LA. Multiple-locus variable-number tandem-repeat analysis as a tool for subtyping *Listeria monocytogenes* strains. J Clin Microbiol. 2008;46(4):1435–50. doi:10.1128/jcm.02207-07.

Stavru F, Bouillaud F, Sartori A, Ricquier D, Cossart P. *Listeria monocytogenes* transiently alters mitochondrial dynamics during infection. Proc Natl Acad Sci U S A. 2011;108(9):3612–7. doi:10.1073/pnas.1100126108.

Stessl B, Fricker M, Fox E, Karpiskova R, Demnerova K, Jordan K, et al. Collaborative survey on the colonization of different types of cheese-processing facilities with *Listeria monocytogenes*. Foodborne Pathog Dis. 2014;11(1):8–14. doi:10.1089/fpd.2013.1578.

Stonsaovapak S, Boonyaratanakornkit M. Prevalence and antimicrobial resistance of *Listeria* species in food products in Bangkok. Thailand J Food Saf. 2010;30(1):154–61. doi:10.1111/j.1745-4565.2009.00197.x.

Swaminathan B, Gerner-Smidt P. The epidemiology of human listeriosis. Microbes Infect. 2007;9(10):1236–43. doi:10.1016/j.micinf.2007.05.011.

Swaminathan B, Cabanes D, Zhang W, Cossart P. *Listeria monocytogenes*. In: Doyle MP, Beuchat IR, Montville TJ, editors. Food microbiology: fundamentals and frontiers. Washington, D.C.: ASM Press; 2007. p. 457–91.

Syne SM, Ramsubhag A, Adesiyun AA. Occurrence and genetic relatedness of *Listeria* spp. in two brands of locally processed ready-to-eat meats in Trinidad. Epidemiol Infect. 2011; 139(5):718–27. doi:10.1017/s095026881000172x.

Taban BM. *Listeria monocytogenes* in cig kofte without meat: a novel bulgur ball product. J Food Agric Environ. 2012;10:130–2.

Takahashi H, Kuramoto S, Miya S, Kimura B. Desiccation survival of *Listeria monocytogenes* and other potential foodborne pathogens on stainless steel surfaces is affected by different food soils. Food Control. 2011;22(3–4):633–7. http://dx.doi.org/10.1016/j.foodcont.2010.09.003.

Toledo-Arana A, Dussurget O, Nikitas G, Sesto N, Guet-Revillet H, Balestrino D, et al. The *Listeria* transcriptional landscape from saprophytism to virulence. Nature. 2009; 459(7249):950–6. doi:10.1038/nature08080.

Tompkin RB. Control of *Listeria monocytogenes* in the food-processing environment. J Food Prot. 2002;65:709–25.

Travier L, Guadagnini S, Gouin E, Dufour A, Chenal-Francisque V, Cossart P, et al. ActA promotes *Listeria monocytogenes* aggregation, intestinal colonization and carriage. PLoS Pathog. 2013;9(1):e1003131. doi:10.1371/journal.ppat.1003131.

Unnerstad H, Bannerman E, Bille J, Danielson-Tham ML, Waak E, Tham W. Prolonged contamination of a dairy with *Listeria monocytogenes*. Neth Milk Dairy J. 1996;50:493–9.

Uyttendaele M, Busschaert P, Valero A, Geeraerd AH, Vermeulen A, Jacxsens L, et al. Prevalence and challenge tests of *Listeria monocytogenes* in Belgian produced and retailed mayonnaise-based deli-salads, cooked meat products and smoked fish between 2005 and 2007. Int J Food Microbiol. 2009;133(1–2):94–104. doi:10.1016/j.ijfoodmicro.2009.05.002.

Vasilev V, Japheth R, Breuer R, Andorn N, Ben Abraham R, Yoni Y, et al. A survey of *Listeria monocytogenes* strains, isolated from ready-to-eat foods in Israel over a period of 10 years, 1998–2007. Food Control. 2010;21(8):1179–81. doi:10.1016/j.foodcont.2010.01.015.

Viswanath P, Murugesan L, Knabel SJ, Verghese B, Chikthimmah N, Laborde LF. Incidence of *Listeria monocytogenes* and *Listeria* spp. in a small-scale mushroom production facility. J Food Prot. 2013;76(4):608–15. doi:10.4315/0362-028x.jfp-12-292.

Vit M, Olejnik R, Dlhy J, Karpiskova R, Castkova J, Prikazsky V, et al. Outbreak of listeriosis in the Czech Republic, late 2006—preliminary report. Euro Surveill. 2007;12(2):E070208.1.

Vitas AI, VAeI G-J. Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain). Int J Food Microbiol. 2004;90(3):349–56. http://dx.doi.org/10.1016/S0168-1605(03)00314-3.

Vongkamjan K, Roof S, Stasiewicz MJ, Wiedmann M. Persistent *Listeria monocytogenes* subtypes isolated from a smoked fish processing facility included both phage susceptible and resistant isolates. Food Microbiol. 2013;35(1):38–48. doi:10.1016/j.fm.2013.02.012.

Vorst KL, Todd EC, Rysert ET. Transfer of *Listeria monocytogenes* during mechanical slicing of turkey breast, bologna, and salami. J Food Prot. 2006;69(3):619–26.

Wang Y, Zhao A, Zhu R, Lan R, Jin D, Cui Z, et al. Genetic diversity and molecular typing of *Listeria monocytogenes* in China. BMC Microbiol. 2012;12:119. doi:10.1186/1471-2180-12-119.

Wang G, Qian W, Zhang X, Wang H, Ye K, Bai Y, et al. Prevalence, genetic diversity and antimicrobial resistance of *Listeria monocytogenes* isolated from ready-to-eat meat products in Nanjing. China Food Control. 2015;50:202–8. http://dx.doi.org/10.1016/j.foodcont.2014.07.057.

Watson CaO K. Listeria outbreak in Western Australia. Commun Dis Intell. 1990;24:9–12.

Wiedmann M, Arvik TJ, Hurley RJ, Boor KJ. General stress transcription factor ϛB and its role in acid tolerance and virulence of *Listeria monocytogenes*. J Bacteriol. 1998;180(14):3650–6.

Williams SK, Roof S, Boyle EA, Burson D, Thippareddi H, Geornaras I, et al. Molecular ecology of *Listeria monocytogenes* and other *Listeria* species in small and very small ready-to-eat meat processing plants. J Food Prot. 2011;74(1):63–77. doi:10.4315/0362-028X.JFP-10-097.

Wulff G, Gram L, Ahrens P, Vogel BF. One group of genetically similar *Listeria monocytogenes* strains frequently dominates and persists in several fish slaughter- and smokehouses. Appl Environ Microbiol. 2006;72(6):4313–22. doi:10.1128/aem.02288-05.

Yde M, Genicot A. Use of PFGE to characterize clonal relationships among Belgian clinical isolates of *Listeria monocytogenes*. J Med Microbiol. 2004;53(Pt 5):399–402.

Zarei M, Maktabi S, Ghorbanpour M. Prevalence of *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, and *Salmonella* spp. in seafood products using multiplex polymerase chain reaction. Foodborne Pathog Dis. 2012;9(2):108–12. doi:10.1089/fpd.2011.0989.

Zhang L, Yan Z, Ryser ET. Impact of dilution ratios on *Listeria monocytogenes* growth during University of Vermont medium enrichment of deli meats. J Food Prot. 2007;70(11):2656–60.

Zhang Q, Feng Y, Deng L, Feng F, Wang L, Zhou Q, et al. SigB plays a major role in *Listeria monocytogenes* tolerance to bile stress. Int J Food Microbiol. 2011;145(1):238–43. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.12.028.