Review

Listeria monocytogenes Persistence in Food-Associated Environments: Epidemiology, Strain Characteristics, and Implications for Public Health

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ABSTRACT

Over the last 10 to 15 years, increasing evidence suggests that persistence of Listeria monocytogenes in food processing plants for years or even decades is an important factor in the transmission of this foodborne pathogen and the root cause of a number of human listeriosis outbreaks. L. monocytogenes persistence in other food-associated environments (e.g., farms and retail establishments) may also contribute to food contamination and transmission of the pathogen to humans. Although L. monocytogenes persistence is typically identified through isolation of a specific molecular subtype from samples collected in a given environment over time, formal (statistical) criteria for identification of persistence are undefined. Environmental factors (e.g., facilities and equipment that are difficult to clean) have been identified as key contributors to persistence; however, the mechanisms are less well understood. Although some researchers have reported that persistent strains possess specific characteristics that may facilitate persistence (e.g., biofilm formation and better adaptation to stress conditions), other researchers have not found significant differences between persistent and nonpersistent strains in the phenotypic characteristics that might facilitate persistence. This review includes a discussion of our current knowledge concerning some key issues associated with the persistence of L. monocytogenes, with special focus on (i) persistence in food processing plants and other food-associated environments, (ii) persistence in the general environment, (iii) phenotypic and genetic characteristics of persistent strains, (iv) niches, and (v) public health and economic implications of persistence. Although the available data clearly indicate that L. monocytogenes persistence at various stages of the food chain contributes to contamination of finished products, continued efforts to quantitatively integrate data on L. monocytogenes persistence (e.g., meta-analysis or quantitative microbial risk assessment) will be needed to advance our understanding of persistence of this pathogen and its economic and public health impacts.

Listeria monocytogenes is a gram-positive, facultative intracellular foodborne pathogen that can cause severe invasive illness (listeriosis) in humans and other animal species, including mammals (e.g., ruminants) and birds (155). Although invasive human listeriosis can occur in healthy individuals, the vast majority of cases occur in young, elderly, or immunocompromised individuals and manifest as septicemia, meningitis, or other infections of the central nervous system. In pregnant women, infections may lead to spontaneous abortion, still birth, or fetal death (177). Scallan et al. (154) estimated that this foodborne pathogen causes approximately 1,460 hospitalizations each year in the United States, resulting in 260 deaths. In developed countries worldwide, the incidence of listeriosis is 0.36 to 5 cases annually per million people (42, 43, 154); however, the number of reported cases can be very low in countries with limited surveillance for this disease. Although some authors have suggested that the majority of human listeriosis cases are sporadic, findings from some studies that have included cluster analysis of human cases based on molecular subtyping data suggest that more human listeriosis cases than previously assumed may represent outbreaks (28, 147), supporting use of improved molecular subtyping methods for improved outbreak detection. In the first decade of the 21st century, coinciding with enhanced implementation of foodborne pathogen subtyping procedures, a large number of human listeriosis outbreaks have been reported in various countries, including the United States (23, 58, 165), Canada (76, 136), Chile (77, 78), Germany and Austria (52), the United Kingdom (37), Sweden (20), Czech Republic (178), Japan (104), and Australia (128).

Although L. monocytogenes is a facultative intracellular pathogen usually of homeothermic animals, it also can grow and survive outside a host at a wide pH range (4.7 to 9.2) (131, 132), at high salt concentrations (10%, wt/vol) (109), and most importantly at refrigeration temperatures (−0.5 to 9.3°C) (180). This growth range allows this pathogen to subsist in the food processing plant environment, survive...
various food processing hurdles, and proliferate in food products. L. monocytogenes has been isolated from a variety of raw and processed food products, including milk and dairy products, meat products, egg products, seafood, vegetables, and other ready-to-eat (RTE) foods (44). In addition to food processing plant environments, this pathogen has been isolated from other environments, including natural, urban, and farm environments (66, 117, 149, 152), and types of samples, including soil, decaying vegetation, stream water, sewage, and human and animal feces (46, 125, 181, 182). Its frequent presence in different environments and its unusual ability to adapt to and survive under stressful conditions make control of L. monocytogenes in the food processing environment a considerable challenge. L. monocytogenes also has been isolated from environmental samples at retail establishments (148, 151) and in consumer homes (12, 31). However, our understanding of L. monocytogenes transmission and ecology in food-associated environments other than those used for commercial food processing is limited. Results from two independent but similar risk assessments in the United States (41, 135) suggest that in >50% of human listeriosis cases associated with consumption of RTE deli meats the contamination may have occurred at the retail level. Cross-contamination of retail products was recently found during a major listeriosis outbreak in Canada linked to contaminated cheese made from pasteurized milk (55). Although L. monocytogenes is inactivated by thermal treatments used for production of RTE foods, postprocessing cross-contamination from equipment and the environment represents a major concern (93, 95). Various approaches are used to control postprocessing contamination in food processing plants, including stringent implementation of good manufacturing practices, sanitation standard operating procedures, sterile packaging technologies (36), in-package postlethality treatments, and reformulation of products with antimicrobial agents. Use of molecular subtyping approaches to better understand L. monocytogenes ecology and transmission in various food-associated environments will continue to improve control of L. monocytogenes and prevention of cross-contamination. For example, molecular typing methods such as pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism, and ribotyping, which have high discriminatory power, have been essential in studies of L. monocytogenes sources and contamination routes in food processing plants (4, 56, 66, 130). Studies including various molecular subtyping methods have revealed that specific L. monocytogenes subtypes can persist in food processing plants over long periods of time, whereas other subtypes are recovered only sporadically (96, 101, 113, 121). Subtypes that persisted were occasionally isolated from contaminated foods and were sometimes associated with listeriosis outbreaks. Although many field studies have revealed L. monocytogenes persistence in various food-associated environments and laboratory studies have revealed L. monocytogenes characteristics that facilitate persistence, our understanding of L. monocytogenes persistence and its impact on food safety and public health is still fragmented. This review includes a discussion of our current knowledge on some key issues regarding L. monocytogenes persistence: (i) persistence in food processing plants and other food-associated environments (farm, retail, and consumers home environments), (ii) persistence in the general environment (natural and urban environments), (iii) phenotypic and genetic characteristics of persistent strains, (iv) environmental niches, and (v) public health and economic implications of L. monocytogenes persistence.

**PERSISTENCE OF L. MONOCYTOGENES**

The term “persistence” can acquire a variety of meanings within the context of a bacterial foodborne pathogen. Persistence can describe the long-term survival of a pathogen in a human host (a persistent infection). Although persistent human or animal infections have been described for various pathogens (e.g., persistent infection of the nostrils with Staphylococcus aureus), including foodborne bacterial pathogens (e.g., persistent infection of food handlers with Salmonella) (150, 167), no clear evidence of persistent infection of humans with L. monocytogenes has been published. Persistence also is used to describe long-term survival (typically without growth) of a foodborne pathogen in a food or food matrix (often in laboratory incubation experiments), e.g., meat products (57, 75), cheese (94, 115), smoked salmon (60, 142), and vegetables (11). Persistence can similarly be used to describe long-term survival of a pathogen in a simple, defined matrix (e.g., soil, water, or stainless steel surfaces) or a complex natural or human-made environment (e.g., a processing plant). In this review, we use the term persistence to describe the presence over time of L. monocytogenes in complex natural or human-made environments. This persistence likely requires both growth and survival in specific compartments of the complex environment. For example, L. monocytogenes could persist in a processing plant in a specific abiotic environment (e.g., a hollow roller in a piece of equipment or an uncleanable part of a slicer) or as a long-term host population inside a plant (e.g., in rodents in a plant lacking appropriate pest control).

Differences in criteria used to define persistence complicate determination of persistence in a complex environment (e.g., a food processing plant or a farm). Typically, persistence is defined by repeated isolation on different dates of L. monocytogenes strains that are subsequently identified as identical subtypes (as determined by phenotypic or genotypic methods). However, some subtyping methods have limited discriminatory power, either for L. monocytogenes as a species or for specific subtypes (e.g., serotype 4b strains). Thus, a method with limited discriminatory power may not be useful for determining persistence of the same subtype in multiple sample collections. Some L. monocytogenes subtypes (e.g., specific PFGE types) are relatively common and widely distributed, whereas others appear rare. Fugett et al. (53) used the standard Centers for Disease Control and Prevention (CDC) two-enzyme PFGE protocol for L. monocytogenes and found one PFGE type that had caused...
outbreaks in Switzerland in 1983 through 1985 and in the United States in 1985; this type also was isolated from multiple farms and urban environments in New York state in 2001 and 2002. Isolation of a very common subtype from multiple sample collections provides weaker evidence of persistence compared with isolation of a rare subtype from multiple sample collections.

The relative frequency of a given subtype can be determined with a statistical testing approach developed by Malley et al. (105) that compares the observed frequency of the subtype to the historical frequency of that subtype. Of the five instances in which a subtype was observed more than once in a food processing plant, only three were identified as showing persistence; the other two cases of repeated isolation (two observations of the given subtype) were not significantly different from isolation expected by chance. Appropriate frequency information for subtypes may be very costly to acquire. The relative frequency of particular L. monocytogenes subtypes among human clinical isolates may be available, but the same information may not be available for other source populations (e.g., turkeys and turkey farms, small ruminants, and raw sheep milk cheeses), limiting frequency comparison within understudied environments.

Differentiation between repeated reintroduction of a specific L. monocytogenes subtype into a facility and true persistence in the facility is challenging based on subtyping information alone and almost always also involves information on facility set up and design. To illustrate extremes, isolation of the same subtype over multiple sampling times in a facility that uses only pathogen-free ingredients and is separated from its surrounding environments (e.g., through premises design and construction and/or implementation of good manufacturing practices) is likely to indicate true persistence. In contrast, isolation over multiple sampling times in a facility that has few measures to prevent introduction of L. monocytogenes from ingredients or surrounding environments (e.g., a farm or a retail facility) may indicate either reintroduction or persistence.

In the following examples, criteria used to define persistence differ among studies and often include only isolation of the same subtype in a given facility over at least two or three sampling times. Although these repeat isolations may indicate persistence of the pathogen, we cannot exclude the possibility that in some instances, particularly when subtyping methods with low discriminatory power were used for isolate characterization, repeat isolation of a subtype may not necessarily indicate persistence. We recommend careful evaluation of data on strain persistence and hope to stimulate ongoing research to establish methods to quantify the likelihood that a set of subtyping data truly indicates persistence.

Persistence of L. monocytogenes in food processing plants. Harvey and Gilmour (61) were among the earliest authors to suggest colonization of food production and processing environments by persistent L. monocytogenes subtypes (Table 1). Multilocus enzyme electrophoresis (MEE) and restriction fragment length polymorphism (RFLP) of L. monocytogenes isolates obtained from raw milk and nondairy food products from various producers and manufacturers revealed the recurrent isolation of L. monocytogenes of the same subtypes. Although lacking environmental samples, the authors suggested that specific subtypes of L. monocytogenes can persist in food processing and farm environments for long periods and recontaminate raw milk.

Rørvik et al. (141) first reported persistence of L. monocytogenes subtypes within a fish plant: a salmon smokehouse in Norway (Table 2). MEE of the L. monocytogenes isolates revealed that those belonging to one electroforetic type (ET-6) were present during the entire 8-month investigation in the environment, in the fish during processing, and in vacuum-packed smoked salmon. In the same year, Lawrence and Gilmour (95) (Table 3) reported characterization of L. monocytogenes isolates from a poultry meat processing plant by random amplification of polymorphic DNA (RAPD). Two of 18 RAPD types found (A and B) persisted throughout a 6- and 5-month period, respectively, with one type more prevalent in raw poultry products and equipment and the other more prevalent in the cooked poultry processing environment. Samples were contaminated with the same RAPD types up to 1 year later. Subsequently, persistence of L. monocytogenes strains for months to several years has been reported in a variety of food processing plants, including those for meat, fish, dairy, and RTE products (Tables 1 through 4).

In some studies, environmental sources have been identified as the main source of postprocessing L. monocytogenes contamination of food products within processing plants (124). Persistent L. monocytogenes strains are more often isolated from food processing environments (e.g., drains and equipment), including sites close to food contact surfaces (e.g., dicing machines), rather than from raw materials (99, 113, 156, 179). The recovery of persistent strains from the environment and equipment after cleaning and disinfection emphasizes the risk of growth and establishment of L. monocytogenes, particularly in sites difficult to access, leading to ongoing food product contamination (4, 124, 186). Microbiological testing of the processing environment and equipment is therefore necessary to detect particular niches of L. monocytogenes and validate the efficiency of sanitation procedures. Persistent strains also can be transferred between facilities through a contaminated environment. For example, a meat dicer harboring L. monocytogenes in an internal niche transferred identical PFGE types to two subsequent processing plants that purchased the contaminated equipment (99).

Other authors have emphasized the importance of cross-contamination by raw materials. Berrang et al. (10) reported that three of the four resident L. monocytogenes strains in a chicken processing plant were recovered from the raw product at some point during the 1-year study, suggesting persistent reintroduction of this strain from raw product into the plant environment.

Persistence of L. monocytogenes in other food-associated environments. Although molecular subtyping
Table 1. Selected studies of the persistence of *L. monocytogenes* in dairy processing plants

<table>
<thead>
<tr>
<th>Type of processing plant (country)</th>
<th>No. of plants</th>
<th>No. of isolates (no. of persistent isolates)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Subtyping methods for establishing persistence&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Duration of observed persistence</th>
<th>Source of isolate(s)</th>
<th>Comments</th>
<th>Publication yr</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk; dairy farm (Northern Ireland)</td>
<td>4; 3</td>
<td>62 (32); 16 (14)</td>
<td>MEE, PFGE, serotyping</td>
<td>1–6 mo</td>
<td>Raw milk</td>
<td>All studied plants had persistent subtypes; persistent strains recovered from two dairy farms were of the same subtype; persistent strains belonged to serogroup 1/2</td>
<td>1994</td>
<td>61</td>
</tr>
<tr>
<td>Cheese (Scandinavia)</td>
<td>1</td>
<td>10 (10)</td>
<td>PFGE, serotyping</td>
<td>7 yr</td>
<td>Wash water, cheese, and environment</td>
<td>In spite of thorough attempts to eliminate the source of contamination, the persistent clone became part of the domestic flora in the dairy; the persistent strain belonged to serovar 3b</td>
<td>1996</td>
<td>171</td>
</tr>
<tr>
<td>Ice cream (Finland)</td>
<td>1</td>
<td>41 (26)</td>
<td>PFGE, serotyping</td>
<td>7 yr</td>
<td>Production environment, production equipment (especially packaging machine), and ice cream</td>
<td><em>L. monocytogenes</em> was not found in raw materials; the persistent strain belonged to serotype 1/2a</td>
<td>1999</td>
<td>113</td>
</tr>
<tr>
<td>Latin-style fresh cheese (USA)</td>
<td>3 (1)</td>
<td>80 (20, 3)</td>
<td>Ribotyping, virulence gene allele characterization <em>(actA and hly)</em></td>
<td>6 mo</td>
<td>Environmental sites (drains, floors), food contact surfaces (plastic connecting tube, processing table), and finished products</td>
<td>One of the studied plants had two persistent ribotypes, one of which (DUP-1044A) has been previously linked to a 1998 multistate human listeriosis outbreak in the United States</td>
<td>2004</td>
<td>85</td>
</tr>
<tr>
<td>Cheese (Ireland)</td>
<td>16</td>
<td>13 facilities had <em>L. monocytogenes</em> contamination; persistent isolates were identified at two facilities (NA&lt;sup&gt;c&lt;/sup&gt;)</td>
<td>PFGE, serotyping</td>
<td>6–20 mo</td>
<td>Cheese and processing environment</td>
<td>In one facility, persistent strains had a pulsotype indistinguishable from that of a strain isolated in 1999, indicating the probable persistence of this strain for 10 yr</td>
<td>2011</td>
<td>51</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of persistent isolates for each subtype is given in parentheses; multiple numbers in parentheses indicate multiple persistent subtypes in a given plant.

<sup>b</sup> MEE, multilocus enzyme electrophoresis; PFGE, pulsed-field gel electrophoresis.

<sup>c</sup> NA, data not available.
TABLE 2. Selected studies of the persistence of L. monocytogenes in fish processing plants

<table>
<thead>
<tr>
<th>Type of processing plant (country)</th>
<th>No. of plants</th>
<th>No. of isolates (no. of persistent isolates)</th>
<th>Subtyping methods for establishing persistence</th>
<th>Duration of observed persistence</th>
<th>Source of isolate(s)</th>
<th>Comments</th>
<th>Publication yr</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon slaughterhouse and smokehouse (Norway)</td>
<td>1</td>
<td>82 (63)</td>
<td>MEE, serotyping</td>
<td>8 mo</td>
<td>Processing environment, fish during process, and vacuum-packed smoked salmon</td>
<td>Persistent electrophoretic type (ET-6) has been isolated from sporadic human listeriosis cases in Norway</td>
<td>1995</td>
<td>141</td>
</tr>
<tr>
<td>Fish (Finland)</td>
<td>1</td>
<td>33 (19)</td>
<td>PFGE, serotyping</td>
<td>14 mo</td>
<td>Salting, skinning, and slicing machines; packaging unit; and finished products</td>
<td>Same PFGE type has been involved in sporadic human listeriosis cases in Finland; serotype 1/2a</td>
<td>1999</td>
<td>84</td>
</tr>
<tr>
<td>Salmon slaughterhouses and smokehouse (Norway)</td>
<td>3</td>
<td>50 (50), 23 (19), 23 (17)</td>
<td>MEE, REA</td>
<td>9–21 mo</td>
<td>RTE seafood, raw seafood, processing environment, seawater, gull, and transport terminal</td>
<td>Specific persistent subtypes were found in each plant; subtypes isolated from the smokehouse have also been isolated from human patients</td>
<td>2000</td>
<td>140</td>
</tr>
<tr>
<td>Cold smoked salmon (France)</td>
<td>1</td>
<td>95 (65)</td>
<td>PFGE, serotyping</td>
<td>2 mo</td>
<td>Environmental samples, environment after cleaning and disinfection, salmon during processing, and final product</td>
<td>One clone of serotype 1/2a persisted in the processing plant and was the only pulsotype detected in the final product but never in raw fish</td>
<td>2001</td>
<td>34</td>
</tr>
<tr>
<td>Smoked fish (United States)</td>
<td>3</td>
<td>22 (8), 16 (4), 47 (23)</td>
<td>Automated ribotyping</td>
<td>4–6 mo</td>
<td>Environmental samples, in-process samples, raw fish, and finished product</td>
<td>Each processing facility had a unique contamination pattern, and specific ribotypes persisted in the environment over time</td>
<td>2001</td>
<td>121</td>
</tr>
<tr>
<td>Cold-smoked salmon (Denmark)</td>
<td>2</td>
<td>263 (147), 58 (48)</td>
<td>RAPD, PFGE, AFLP</td>
<td>4 yr; 5 mo</td>
<td>Contact surfaces, production environment, and final product</td>
<td>Persistent RAPD type in plant I found in slicing environment and products; persistent RAPD type in plant II found in all sections of the plant</td>
<td>2001</td>
<td>179</td>
</tr>
<tr>
<td>Smoked fish (United States)</td>
<td>1</td>
<td>NA (32.1% of total isolates)</td>
<td>Automated ribotyping</td>
<td>4 mo</td>
<td>Raw fish and environmental samples</td>
<td>This subtype had been isolated from the same plant in a previous study (120) and appears to have persisted in this plant for &gt;2 yr</td>
<td>2003</td>
<td>69</td>
</tr>
<tr>
<td>Fish (Poland)</td>
<td>1</td>
<td>71 (5, 5, 24)</td>
<td>RAPD, PFGE</td>
<td>8–10 mo</td>
<td>Finished products, salmon fillets, and sliced vacuum-packed cold-smoked salmon</td>
<td>Three RAPD types persisted in the plant environment; strains entering the plant via raw fish seem to be eliminated, and fish are recontaminated with persistent resident strains</td>
<td>2003</td>
<td>111</td>
</tr>
</tbody>
</table>
### TABLE 2. Continued

<table>
<thead>
<tr>
<th>Type of processing plant (country)</th>
<th>No. of plants</th>
<th>No. of isolates (no. of persistent isolates)</th>
<th>Subtyping methods for establishing persistence</th>
<th>Duration of observed persistence</th>
<th>Source of isolate(s)</th>
<th>Comments</th>
<th>Publication yr</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTE smoked fish (United States)</td>
<td>4</td>
<td>88 (5, 16, 5, 25, 15), 24 (12, 2), 50 (32), 10 (0)</td>
<td>EcoRI ribotyping</td>
<td>2-yr study period</td>
<td>Raw materials, food contact surfaces, employee contact surfaces, nonfood contact surfaces, finished product</td>
<td>Persistent ribotypes were widely distributed in plants 1 and 3; persistent ribotype DUP-1053A in plant 2 seemed to be concentrated in the raw material processing area; subtype data revealed that the meat-bone separator was persistently contaminated with subtype DUP-1044A, which seemed to be established in a niche and was extremely difficult to eliminate using routine sanitation procedures; plant 3 persistent ribotype DUP-1039A continued to be isolated after implementation of intervention strategies</td>
<td>2004</td>
<td>93</td>
</tr>
<tr>
<td>Cooked peeled shrimp (Iceland)</td>
<td>2</td>
<td>106 (63), 66 (43)</td>
<td>PFGE, serotyping</td>
<td>≥1 to &gt;3 yr²</td>
<td>Raw material, shrimp shell, transporters and equipment after cleaning and during processing, floor during processing, and outdoor environment</td>
<td>Each plant had one persistent PFGE type; the persistent pulsotype from one plant was also isolated in the second plant as a nonpersistent type</td>
<td>2006</td>
<td>59</td>
</tr>
<tr>
<td>Fish smokehouse (Denmark)</td>
<td>1</td>
<td>43(25)</td>
<td>RAPD, AFLP</td>
<td>1.5 yr</td>
<td>Raw fish, raw fish area, slicing area, and final product</td>
<td>This RAPD type was also isolated from two other fish smokehouses and two fish slaughterhouses; it persisted in the processing environment in three of these plants for 1–3 mo</td>
<td>2006</td>
<td>186</td>
</tr>
<tr>
<td>Mussels (New Zealand)</td>
<td>3</td>
<td>40 (29), 15 (11), 46 (36)</td>
<td>PFGE, serotyping</td>
<td>6–10 mo</td>
<td>Mussel processing environment, raw mussels, and finished products</td>
<td>Persistent pulsotype 3814 was found in all plants; pulsotype 5132 was found in plants B and C; pulsotype 6502 was found in only plant A; one persistent pulsotype was previously isolated in human listeriosis cases in New Zealand</td>
<td>2011</td>
<td>32</td>
</tr>
</tbody>
</table>

² Number of persistent isolates for each subtype is given in parentheses; multiple numbers in parentheses indicate multiple persistent subtypes in a given plant.

⁶ MEE, multilocus enzyme electrophoresis; PFGE, pulsed-field gel electrophoresis; REA, restriction enzyme analysis; RAPD, random amplification of polymorphic DNA; AFLP, amplified fragment length polymorphism.

⁵ NA, data not available.

⁴ According to data in Table 1 (59).
<table>
<thead>
<tr>
<th>Type of processing plant (country)</th>
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<th>Duration of observed persistence</th>
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<th>Publication yr</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry meat (United Kingdom)</td>
<td>1</td>
<td>289 (184, 35)</td>
<td>RAPD, MEE</td>
<td>5–6 mo</td>
<td>Raw poultry products, raw poultry processing environment, and cooked poultry processing environment</td>
<td>Two persistent RAPD types were subsequently identified in the cooked poultry processing environment and cooked poultry products up to 1 yr after the initial study</td>
<td>1995</td>
<td>95</td>
</tr>
<tr>
<td>Meat (Norway)</td>
<td>1</td>
<td>32 (15)</td>
<td>MEE, RFLP</td>
<td>4 yr</td>
<td>Production environment, waste from the slicer, and cold cuts</td>
<td>Two clone variants colonized different areas of the plant; persistent ET-6 was isolated from sporadic human listeriosis cases in Norway (145)</td>
<td>1996</td>
<td>116</td>
</tr>
<tr>
<td>Pork slaughtering and cutting (France)</td>
<td>2</td>
<td>287 (186, 16)</td>
<td>RAPD, PFGE, PCR-REA, serotyping</td>
<td>1 yr</td>
<td>Carcasses, pork cuts, slaughterhouse after sanitizing and during activity, cutting room after sanitizing and during activity, and chilling room after sanitizing</td>
<td>Each plant had one specific persistent genotype that was also detected in other plants; three and five isolates representing the persistent genotypes for each plant were selected for serotyping and belonged to serotype 1/2a</td>
<td>1999</td>
<td>56</td>
</tr>
<tr>
<td>Turkey (Denmark)</td>
<td>1</td>
<td>53 (32, 15)</td>
<td>PFGE</td>
<td>2 mo</td>
<td>Raw meat, finished products, critical control points of the cleaned and disinfected abattoir, and subsequently along the processing line</td>
<td>Two PFGE types were isolated during two visits; the same PFGE patterns were obtained for isolates from human listeriosis cases</td>
<td>2000</td>
<td>124</td>
</tr>
<tr>
<td>Meat (Switzerland)</td>
<td>1</td>
<td>89 (43, 26)</td>
<td>PFGE</td>
<td>2 yr</td>
<td>Machines and equipment of the slicing and packing area of the raw ham production line, packing area of nonsliced raw ham, and meat products</td>
<td>Persistence of closely related strains; processing environment was a major source of cross-contamination of products</td>
<td>2000</td>
<td>156</td>
</tr>
<tr>
<td>Poultry meat; pork meat (France)</td>
<td>1; 1</td>
<td>502 (18.8%)</td>
<td>PFGE, serotyping</td>
<td>9 mo; 3 mo</td>
<td>Environment, equipment, processed meat products, and various areas after cleaning operations</td>
<td>Same genotype was isolated in both plants; persistent strain belonged to serotype 1/2a</td>
<td>2001</td>
<td>25</td>
</tr>
<tr>
<td>Meat</td>
<td>1</td>
<td>NA</td>
<td>PFGE, serotyping</td>
<td>1 yr</td>
<td>Dicing machine, dicing line, and diced products</td>
<td>Persistent PFGE serotype 1/2c was found in the dicing machine and dicing line of plant A in 1997; after transfer of dicing machine to plant B (Oct. 1998) and then plant C (Mar. 1999), the same PFGE type was recovered from the dicing machine and the dicing line in both plants; in plant C several samples contaminated with the persistent strain were found over 1 yr</td>
<td>2002</td>
<td>99</td>
</tr>
<tr>
<td>Type of processing plant (country)</td>
<td>No. of plants</td>
<td>No. of isolates (no. of persistent isolates)</td>
<td>Subtyping methods for establishing persistence</td>
<td>Duration of observed persistence</td>
<td>Source of isolate(s)</td>
<td>Comments</td>
<td>Publication yr</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------------------</td>
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</tr>
<tr>
<td>Poultry, further processing (United States)</td>
<td>1</td>
<td>161 (NA)</td>
<td>actA sequence-based typing</td>
<td>1 yr</td>
<td>Environmental surfaces and sites and raw product</td>
<td>Only the four most common persistent types and no transient types were detected on the cooked product side; three of these subtypes were also detected in raw product</td>
<td>2002</td>
<td>10</td>
</tr>
<tr>
<td>Beef and pork; poultry (Finland)</td>
<td>3; 1</td>
<td>18 (11), 92 (67); 307 (278), 179 (133)</td>
<td>PFGE, serotyping</td>
<td>≥3 mo</td>
<td>Environment, equipment, and final product</td>
<td>All plants were contaminated with one or more persistent and several nonpersistent strains; 9 of 19 persistent PFGE types were nonpersistent in another plant; persistent strains belonged to serotypes 1/2a, 1/2c, 3a, 1/2b, and 4b</td>
<td>2003</td>
<td>101</td>
</tr>
<tr>
<td>Cattle slaughterhouse (Italy)</td>
<td>1</td>
<td>7 (7)</td>
<td>PFGE</td>
<td>16 mo</td>
<td>Excised meat samples, carcass swab, and knife swabs</td>
<td>Persistent subtype was unique; equipment and environment were the probable sites of cross-contamination for several months</td>
<td>2003</td>
<td>130</td>
</tr>
<tr>
<td>Turkey (United States)</td>
<td>2</td>
<td>5 (4), 13 (10)</td>
<td>PFGE</td>
<td>4–10 mo&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Raw product and environment</td>
<td>Both plants had two persistent PFGE types that were encountered on more than one sampling occasion; persistent strain belonged to serotype 4b</td>
<td>2005</td>
<td>40</td>
</tr>
<tr>
<td>Pig abattoir and processing plant (Spain)</td>
<td>1</td>
<td>161 (85, 29, 35)</td>
<td>PFGE, serotyping</td>
<td>1 yr</td>
<td>Environment, whole pieces of meat, and ground pork products</td>
<td>Three predominant PFGE types persisted for 1 yr and accounted for 92% of the isolates; two persistent genotypes belonged to serotype 1/2a, and one belonged to serotype 1/2b</td>
<td>2008</td>
<td>96</td>
</tr>
<tr>
<td>Chicken, further processing (United States)</td>
<td>1</td>
<td>NA</td>
<td>actA sequence-based typing</td>
<td>1–15 mo</td>
<td>Drains and cooked product</td>
<td>Several subtypes categorized as persistent drain contaminants in a previous study (9) were found in only raw products</td>
<td>2010</td>
<td>8</td>
</tr>
<tr>
<td>Fermented meat sausages (Portugal)</td>
<td>7</td>
<td>240 (127)</td>
<td>PFGE, serotyping</td>
<td>10–32 mo</td>
<td>Finished products</td>
<td>Three processors had one persistent strain (which was not persistent in other processors); two processors had two persistent strains; two other processors had persistent strains with same pulsotype</td>
<td>2011</td>
<td>49</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of persistent isolates for each subtype is given in parentheses; multiple numbers in parentheses indicate multiple persistent subtypes in a given plant.

<sup>b</sup> RAPD, random amplification of polymorphic DNA; MEE, multilocus enzyme electrophoresis; RFLP, restriction fragment length polymorphism; PFGE, pulsed-field gel electrophoresis; PCR-REA, PCR plus restriction enzyme analysis.

<sup>c</sup> NA, data not available.

<sup>d</sup> According to data in Table 1 (40).
studies of *L. monocytogenes* ecology and persistence in food processing plants has allowed for improved control there, limited information is available on the ecology and persistence of *L. monocytogenes* in other food-associated environments, such as farms, retail facilities, and consumer and commercial kitchens.

**Farm environment.** *L. monocytogenes* appears be common on livestock farms. Cattle in particular transmit this pathogen, including subtypes linked to human listeriosis (117). Contamination of silage by *L. monocytogenes* has been identified as a risk factor for farm animal listeriosis due to feed consumption, resulting either in herd outbreaks or dissemination of the pathogen in the farm environment through animal fecal material (47, 185). *L. monocytogenes* also can survive in different types of animal feeds and in liquid manure for several months (153). Use of fecal material from infected or shedding animals as agricultural fertilizer can contaminate raw foods. The 1981 listeriosis outbreak in Nova Scotia involved 42 human cases linked to consumption of coleslaw made from cabbage fertilized with untreated sheep manure collected from a farm with a history of ovine listeriosis (155).

*L. monocytogenes* persistence in produce and on other types of farms is less well understood. *L. monocytogenes* has been isolated at low levels from a variety of environmental samples associated with the primary production of food, including vegetation, growing grass, and vegetables (47). Watkins and Sleath (181) found that levels of *L. monocytogenes* in sewage sludge sprayed on agricultural land remained unchanged for at least 8 weeks. Strawn et al. (163) isolated identical sigB allelic types of *L. monocytogenes* from four surface water sites over at least 1 year on produce farms, including one case in which the four individual isolates were indistinguishable by PFGE.

Further research is needed to address the prevalence and persistence of specific *L. monocytogenes* subtypes within both livestock and produce farm environments. These data will allow a better understanding of the role that farm environments and specific persistent strains play in human listeriosis, either through a direct route (e.g., contamination of raw vegetables or raw milk) or an indirect route (e.g., contamination of processing plants via physical proximity between production animals and food processing areas or cross-contamination via contaminated raw materials).

**Retail environment.** The majority of human listeriosis cases have been linked to the consumption of contaminated RTE foods (173), specifically delicatessen meats, which have been estimated to be associated with the highest per serving and per population risk of listeriosis of all foods in the United States (174). Slicing at retail facilities is a key factor in the postprocessing contamination of RTE foods; retail-sliced products are 1.7 times more likely to be associated with fatal listeriosis than are prepackaged deli meats (41). An example of the impact of *L. monocytogenes* cross-contamination of RTE foods at the retail level is the large 1992 listeriosis outbreak in France involving 279 cases. Although contaminated pork tongue of a specific
brand had been identified as the major vehicle of human infection, several delicatessen items contaminated with the same PFGE subtype were found at retail sites where the contaminated pork tongue had been sold and were associated with human illness in patients that did not consume pork tongue (80). Therefore, cross-contamination is a likely mechanism by which environmentally persistent \textit{L. monocytogenes} strains can be introduced into RTE delicatessen foods.

\textit{L. monocytogenes} can frequently be found in the retail environment, predominantly on nonfood contact surfaces such as floors, floor drains, sinks, and walk-in cooler shelves (68). Hands and gloves of food handlers also have been recognized as an important potential contamination source (67). Sauders et al. (148) found evidence for persistence of \textit{L. monocytogenes} subtypes in 16 retail establishments; the same EcoRI ribotype was isolated from prepared or handled foods or environmental samples from an establishment on different days. One persistent ribotype, found in two of the retail environments investigated, was identical to a ribotype responsible for two listeriosis outbreaks in the United States (21, 22), suggesting that subtypes that persist in retail facilities may cause human disease. Sauders et al. (151) also conducted a cross-sectional study of \textit{L. monocytogenes} contamination patterns in retail environments, focusing on RTE deli meats and salads, the deli area environment, and other areas. Of the 121 retail establishments studied, 73 (60\%) produced at least one sample that was positive for \textit{L. monocytogenes}. In five of seven establishments where follow-up sampling was conducted 8 to 19 months after the initial sampling, isolates with the same ribotype and pulstype were obtained on several sampling dates, further supporting \textit{L. monocytogenes} persistence at the retail level.

The results indicate that \textit{L. monocytogenes} strains are often present and widely distributed in retail facilities and that these strains can persist in these environments for more than 1 year and are common among human listeriosis cases (148). Further studies are needed to specifically understand the role of retail facilities in the contamination of food products, particularly RTE food products that may become contaminated at the point of sale (e.g., deli cheeses and meats during slicing).

\textbf{Private and public kitchens.} Little attention has been given to \textit{L. monocytogenes} ecology in private (consumer) or public kitchens (e.g., restaurants, caterers, schools, hospitals, and care centers). A few researchers have reported frequent isolation of \textit{L. monocytogenes} from domestic kitchens (5, 12, 31, 79, 91) without evaluating pathogen persistence. In one study, an outbreak of gastroenteritis was reported among 39 adults (nonpregnant and previously healthy) that consumed contaminated foods prepared in a private household in Italy (144). Epidemiological investigation identified rice salad as the most likely vehicle of the pathogen, and \textit{L. monocytogenes} isolates of the same phage and MEE types were isolated from patients, leftover foods, and the kitchen freezer.

Listeriosis outbreaks related to consumption of contaminated foods prepared in health care center and hospital kitchens have also been described. Between August 2006 and June 2007, six hospitalized elderly patients in Brazil (median age of 80 years, with immunocompromising conditions) were diagnosed with listeriosis, and five of these patients died (108). Cases occurred in different wards of the hospital and stopped after an intervention in the kitchen. A tuna salad prepared in a New York City hospital kitchen was implicated as the source of \textit{L. monocytogenes} infection in some cases reported between August and September 2008 (29).

Public health effects of persistent contamination at facilities that process RTE food can be magnified in immunocompromised consumers. Lyytikäinen et al. (103) linked a listeriosis outbreak that occurred from June 1998 to April 1999 among patients of a tertiary care hospital in Finland to butter samples collected from the hospital kitchen; six of these patients died. The outbreak strain had been previously isolated from an internal quality control sample of butter produced in 1997 at the dairy plant that supplied the hospital. In another outbreak, pregnant women were thought to have eaten prepacked sandwiches from a retail outlet at a hospital in the United Kingdom in autumn 2003. Sampling at the supplier revealed \textit{L. monocytogenes} isolates with a molecular subtype indistinguishable from that of the clinical isolates (37).

These data highlight the importance of developing guidelines for prevention of \textit{L. monocytogenes} persistence within food service institutions, particularly when large quantities of food are prepared and served to immunocompromised populations in such facilities as hospitals and homes for the elderly.

\textbf{Persistence of \textit{L. monocytogenes} in the general environment.} Few studies have been conducted on the prevalence of \textit{L. monocytogenes} and other \textit{Listeria} species in soil and vegetation of natural environments other than those associated with farms (47, 182), and little is known about the ecology and persistence of \textit{L. monocytogenes} in natural or urban environments. \textit{L. monocytogenes} isolates can survive from 12 to more than 730 days in soil and from less than 7 to 928 days in water (153). Sauders et al. (146) characterized 80 \textit{L. monocytogenes} isolates collected over a 2-year period from natural and urban environments and found prevalences of 0.77 to 12.6\%. Several ribotypes associated with human disease were found among urban and pristine environments, suggesting that these environments are potential primary sources for the introduction of human pathogenic \textit{L. monocytogenes} subtypes into the human food chain and food processing plants. The extent to which these sources contribute to the presence of \textit{L. monocytogenes} in the human food system and their consequent role in the transmission of disease remain to be ascertained.

\textbf{PHENOTYPIC AND GENETIC CHARACTERISTICS OF PERSISTENT \textit{L. MONOCYTOGENES} STRAINS}

Although persistence of \textit{L. monocytogenes} in various food-associated environments has been well documented, it
is not well understood. Several hypotheses have been proposed to explain persistence, including physical adaptation or enhanced tolerance to processing factors (72, 186) such disinfectants (1, 100) and improved ability to adhere to food contact surfaces (16, 102). The following sections include research on the phenotypic characteristics of isolates reportedly representing persistent and sporadic strains with regard to biofilm formation, disinfectant resistance, stress resistance, and virulence.

One difficulty researchers face in studies comparing persistent and nonpersistent isolates is the classification of isolates into these categories. Typically, isolates classified as persistent are those with identical subtypes that have been isolated over time; this classification method is often qualitative and nonstatistical. Identification of nonpersistent isolates is more challenging. Even though a given subtype has not been isolated over time, it may yet become persistent. This caveat applies to all studies discussed below.

**Biofilms.** Biofilms are aggregates of microbial cells that adhere to each other and/or to surfaces and are enclosed in an extracellular polymeric matrix (30). Biofilms contribute to microbial persistence in unfavorable environments because they provide phenotypic versatility and ecological advantages, including (i) protection from the environment conferred by the surrounding extrapolymeric substance matrix, (ii) enhanced nutrient availability and metabolic cooperation through nutrient exchange and removal of potentially toxic metabolites, and (iii) acquisition of new genetic traits (e.g., antibiotic resistance genes) by horizontal gene transfer (35). Microorganisms that can attach to food contact surfaces and form biofilms can contaminate food and lead to spoilage or transmission of foodborne pathogens (92).

Although *L. monocytogenes* is capable of rapid attachment to various food processing surfaces such as buna-N rubber, cast iron, stainless steel, nylon, Teflon, polyester floor sealant, and glass (14, 24, 27, 160), few studies have produced strong evidence (e.g., scanning electron micrographs) that this pathogen actually forms biofilms. Therefore, debate continues about whether *L. monocytogenes* forms a classic biofilm or whether the more common methods used to quantify biofilm formation (e.g., enumeration or crystal violet staining of adhered cells) merely reflects strong cell adsorption to a surface rather than a true biofilm (which requires adherence of planktonic cells followed by production of extracellular polysaccharides). For example, Kalmokoff and coworkers (87) use scanning electron microscopy to determine that only 1 of 36 *L. monocytogenes* strains (representing food and clinical strains with different serovars) formed a true rudimentary biofilm on a stainless steel surface after 72 h of incubation period, although significant differences in number of adhered cells (per square millimeter) were found among the various strains after 2 h of contact.

Some authors have found that persistent strains adhere better to stainless steel surfaces than do sporadic strains. Norwood and Gilmour (122) tested the ability of 35 persistent and 24 sporadic *L. monocytogenes* strains from food processing establishments and farm environments to adhere to stainless steel coupons. Mean counts of adherent cells over a 24-h period at 25°C were significantly higher for persistent strains. Lundén et al. (102) compared *L. monocytogenes* adherence to stainless steel surfaces in 3 persistent strains (2 that have persisted in poultry plants for at least 2 years and 1 that persisted in an ice cream plant for 7 years) and 14 sporadic strains collected at the same processing plants. Although the persistent strains had higher counts of adhering cells after short contact times (1 and 2 h), half of the sporadic strains achieved equal or greater adherence than the persistent strains after 72 h. Borucki et al. (16) tested 11 persistent and 15 sporadic *L. monocytogenes* strains isolated from bulk milk tanks at a dairy plant for biofilm formation on a polyvinyl chloride (PVC) surface. Persistent strains were significantly better biofilm formers than were strains obtained sporadically from bulk milk tanks after incubation for 40 h at 30°C, as determined with a microtiter plate assay using crystal violet and ruthenium red staining (a carbohydrate-binding dye). Scanning electron microscopic examination of a persistent strain revealed a dense, three-dimensional composite of cells with well-distributed channels and pores on both stainless steel and PVC, whereas the sporadic strain produced only sparse aggregates of cells on stainless steel and predominantly single attached cells on PVC.

Other authors have found no differences in attachment or biofilm formation between persistent and sporadic strains. Using crystal violet staining and epifluorescence microscopy assays, Djordjevic et al. (38) found no differences in biofilm formation between five persistent and five sporadic *L. monocytogenes* strains recovered from a fish processing plant after 40 h of incubation on PVC microtiter plates. Using 48-h microtiter plate and 14-day petri dish assays with quantification by crystal violet staining, Harvey et al. (63) also found no differences in attachment among several persistent and sporadic *L. monocytogenes* isolates collected from different food sources. Jensen et al. (81) found no systematic differences in adhesion to PVC based on a microtiter plate assay of 8 clinical and food *L. monocytogenes* isolates and 10 persistent isolates, including 4 isolates belonging to one RAPD type that had been recovered from different fish processing plants for up to 10 years.

Although Norwood and Gilmour (122) and Lundén et al. (102) have reported that serotype 1/2c strains have significantly better adsorption, no clear relationship has been found between serotype and ability to form biofilms. Kalmokoff et al. (87) found no relation between *L. monocytogenes* serotype (1/2a, 1/2b, 1/2c, 4b, and 4c) or source (food, environmental, and clinical) among 36 strains. However, Djordjevic et al. (38) found a correlation between phylogeny and biofilm formation, reporting that the majority of lineage I strains (4b and 1/2b serotypes) were significantly better able to form biofilms than were lineage II (1/2a and 1/2c serotypes) and lineage III (4a, 4b, and 4c serotypes) strains. Opposite results were obtained by Borucki et al. (16), who observed increased biofilm formation among lineage II strains, providing another
example of a lack of a consistent relationship between L. monocytogenes subtypes or clonal groups and the ability to form biofilms or adhere to surfaces.

Overall the association between the ability of L. monocytogenes strains to adhere to surfaces and/or form biofilms and the persistence of various strains in the food processing plant environment is not clear. Differences in the results of the several studies may be related to differences in methods applied, including sample size. Several researchers have suggested that the ability of L. monocytogenes to adhere and form biofilms is strain dependent and influenced by experimental conditions such as temperature, pH, salt, nutrients, and surface material (114). Biofilm formation also is affected by the growth medium (50).

Most in vitro studies on the ability of persistent and sporadic L. monocytogenes strains to adhere to surfaces and form biofilms have not sufficiently reproduced conditions of an in vivo system. The background microflora can affect the ability of L. monocytogenes to form a biofilm, and interactions with other species (e.g., Pseudomonas fragi, Flavobacterium spp., and Bacillus spp.) might contribute to formation of biofilms that are thicker and more stable than monospecies biofilms (19, 145).

Disinfectant resistance. Disinfectants (i.e., chemicals with lethal activity against microorganisms) are used extensively in the food industry to sanitize environmental surfaces, equipment, and utensils. The most common chemical agents used in the food industry are halogens, peroxide, alcohols, anhydrides, aldehydes, quaternary ammonium compounds (QACs), (poly)biguanidines, chlorhexidine, polyamines, imidazoline, amphoteric polyamines, phenol, cresol, and their derivatives and metals (161). Disinfectants have different mechanisms of action targeting various sites of the bacterial cell, including cellular constituents (e.g., nucleic acids, proteins, or enzymes), cell membranes (proteins and transport pumps), or thiol groups (enzymes and coenzymes) (110).

Widespread use of disinfectants may create selective pressure for the development of resistance mechanisms by mutation or acquisition of genetic material (e.g., plasmids or transposons) (110). The proton motive force-driven multidrug efflux pump is one well-studied adaptation mechanism conferring resistance to a diverse range of chemicals (164). A similar efflux pump mechanism has been induced in L. monocytogenes by exposure to benzalkonium chloride (BC; a QAC) (1, 159, 166). Mereghetti et al. (112) reported an overexpression of the multidrug efflux pump MdrL among some of the 7% of strains studied in vitro that were adapted to increased concentrations of BC (<4 to >7 mg/liter, well below the maximum recommended concentration of 200 mg/liter or ppm (157)).

Persistent L. monocytogenes strains have been isolated from food processing environments after cleaning and disinfection (101, 111, 159). Although this isolation may simply indicate presence of these strains in locations that cannot be reached by sanitizers (niches), resistance to cleaning and disinfection agents also could explain the persistence of specific L. monocytogenes strains in these environments. In this context, resistance is implicitly defined as a capacity for adaptation and survival of microorganisms in response to recommended concentrations of disinfectants. Empirically, long-term exposure to sublethal concentrations of three disinfectants did not lead to survival at higher concentrations of disinfectants used in industrial settings (89). Aase et al. (1) reported that one strain that persisted in a Norwegian fish processing plant was more resistant to BC. For this strain and 19 others isolated from samples from different products and processing plants, the MIC (determined by microtiter assay) of BC was 4 to 7 μg/ml, and for the remaining 174 strains studied the MICs was 2 μg/ml or lower. However, the final MICs did not exceed the U.S. Food and Drug Administration (FDA) maximum recommended concentrations for QACs used in food processing plants (200 mg/liter or ppm) (157).

Other authors found no relation between persistence and increased MICs of commonly used commercial disinfectants when comparing susceptibility of persistent and sporadic isolates (39, 72, 88). For example, Heir et al. (64) determined MICs of BC for 112 L. monocytogenes strains, including clinical and food isolates and sporadic and persistent strains, by microtiter assay. Seventeen strains had relatively higher MICs (4 to 8 μg/ml), but no correlation was found between persistence and relatively higher MICs. Earnshaw and Lawrence (39) and Holah et al. (72) evaluated the effect of QACs and alkaline metal hydroxide–based disinfectants (at recommended concentrations) on levels of viable bacteria. The first study was conducted with 20 L. monocytogenes strains (including two persistent strains of different RAPD types), and the second was conducted with 3 persistent strains and 1 laboratory control strain. In both studies no major differences in the log reduction was found between persistent and sporadic strains. Lundén et al. (100) reported that initial MICs of various disinfectants were higher for two persistent strains than for two sporadic strains. However, both types of strains adapted after short-term exposure (2 h) to sublethal disinfectant concentrations, and the MICs became similar. Final MICs did not exceed the concentrations used in working solutions in food processing plants.

Although some findings support the hypothesis that L. monocytogenes cells organized in a biofilm are less susceptible to disinfectants than are cells in the planktonic state, presumably because biofilms shield against cleaning and disinfection procedures (45, 123, 129), Kastbjerg and Gram (88) found that 14 strains (including 5 persistent strains representing three RAPD types) did not differ in their sensitivity to two commercial disinfectants (QACs and peroxide-based disinfectants) when planktonic and when attached to stainless steel coupons.

Stress resistance. L. monocytogenes strains are sometimes directly exposed to frequent and dramatic environmental changes. In the food industry environment, this pathogen faces severe conditions (e.g., scarcity of nutrients, acidic pH, high osmolarity, classical heat shock conditions, and competing bacteria) that inflict different levels of stress, defined here as ‘‘exposure to any
environmental situation that results in damage of cellular components in the absence of a cellular response” (162). Stresses may be lethal, causing irreversible damage to microbial cells, or sublethal, permitting survival. Under sublethal stress conditions, the microbial cell exhibits stress adaptation, where mild forms of particular stresses may result in increased resistance against subsequent exposures to lethal levels or in some cases cross-protection against other unrelated stresses (65). For example, adaptation of L. monocytogenes to osmotic stress significantly increased the resistance of this pathogen to peroxide stress (7); acid-adapted cells were more tolerant of lethal acid conditions, and hydrogen peroxide, ethanol, and low pH significantly increased the resistance of this pathogen to heat (97, 133).

L. monocytogenes stress responses to sublethal conditions result in global changes in gene and protein expression profiles of the cells. In L. monocytogenes and other gram-positive bacteria, σB (encoded by sigB) has been identified as the general stress responsive alternative sigma factor (15). σB is activated after exposure to various environmental stresses (6) and contributes to bacterial osmotolerance, detergent stress response, and survival under acid and oxidative stress and carbon starvation (26, 48, 86, 143, 184).

Proteomic and transcriptional analysis of stress response profiles of L. monocytogenes led to identification of several stress-activated proteins, including cold shock proteins (e.g., Fri, Ctc, GroEL, DnaK, Csp1-Csp4, CspA, and CspD), heat shock proteins (e.g., Hsp6, DnaK, and GroES), salt stress–induced proteins (e.g., Ctc, DnaK, GbuA, and AppA), acid stress response proteins (e.g., ASP, GbuA, GroEL, and ClpP), alkaline stress response proteins (e.g., DdaA, GroEL, and Dnak), and high hydrostatic pressure response proteins (e.g., Fri, GroES, PepF, and PepT) (reviewed by Soni et al. (158)). The SOS regulon of L. monocytogenes encodes proteins that function in translesion DNA synthesis and DNA repair (175). The SOS response is activated after stress exposure, playing an important role in L. monocytogenes resistance to heat, H2O2, and acid exposure (175). L. monocytogenes stress response triggers multiple protein expression changes, and key proteins have cross-protective effects against various environmental stresses.

Based on the likely importance of different stress response systems for survival of L. monocytogenes under various stress conditions, including those likely encountered in food processing environments, researchers have evaluated the resistance of persistent and nonpersistent strains to various stresses. Lundén et al. (98) examined the acid and heat tolerance of 40 persistent and sporadic L. monocytogenes isolates, representing 12 persistent and 23 sporadic RFLP types. The persistent isolates had a significantly higher acid tolerance (pH 2.4 for 2 h) than did sporadic isolates, but no significant differences in heat tolerance (55°C for 40 min) were observed. Porsby et al. (134) evaluated a persistent L. monocytogenes strain (representing a RAPD type isolated from several fish smokehouses over many years) to determine whether it survived hurdles encountered during the processing of cold smoked salmon (salting, drying, and cold-smoking steps) better than did a clinical reference strain (EGD). Results obtained in both laboratory model systems and pilot plant scale experiments that replicated an industrial cold-smoking process revealed that the persistent strain did not survive processing steps better than did the clinical strain. Harvey and Gilmour (62) studied recurrent and sporadic L. monocytogenes isolates recovered from raw milk (23 isolates) and nondairy foods (22 isolates) and found no difference in monocin production, but plasmid carriage and cadmium resistance were more frequent in recurrent than in sporadic isolates.

Although some phenotypes associated with persistent strains have been identified in individual studies, most of the existing literature does not support any robust stress response generally associated with persistent L. monocytogenes subtypes. Ringus et al. (139) evaluated the transcript levels of genes in the regulons of two stress response regulators, σB and CtsR, for six persistent and six nonpersistent L. monocytogenes strains isolated from fish processing plants and one persistent strain isolated from a meat plant. After exposure to salt stress conditions, no correlation was found between persistence and gene transcript levels.

**Virulence of persistent strains.** For risk analysis, the virulence potential of strains that are likely contaminants of food products, such as strains persisting in the food processing environment, must be assessed. One research group studied the virulence of isolates belonging to a persistent RAPD type isolated from several fish processing environments (82). Strains were characterized in terms of (i) ability to invade Caco-2 human epithelial cells; (ii) adhesion, invasion, and intracellular growth in Caco-2 cells, infection of the fruit fly Drosophila melanogaster, nematode Caenorhabditis elegans, and guinea pigs; and (iii) virulence potential in a pregnant guinea pig model (83). The overall results of the first two studies suggest that persistent isolates belonging to the persistent RAPD type are less invasive and have a lower virulence potential in the tested models when compared with human clinical strains, whereas the third study revealed that one persistent isolate was virulent in the guinea pig model and capable of crossing the fetoplacental barrier. Holch et al. (73) classified strains belonging to a specific molecular subtype (RAPD 9) that persists in Danish fish processing plants as being less virulent because they invaded human placental trophoblasts less efficiently than did other L. monocytogenes strains, including clinical strains, and carried a premature stop codon in inlA.

On average, persistent strains are no more virulent, and may be less virulent, than sporadic strains. This conclusion is consistent with the observation that a significantly greater proportion of L. monocytogenes isolates from RTE foods than from human clinical cases carried the premature stop codon in the key virulence gene inlA (176); this mutation attenuates virulence (118). However, the relevant factor for
public health is not the average virulence of persistent strains but the particular virulence of a strain that contaminates a food product. The risk of listeriosis is greatly increased when a more virulent than average strain becomes established in the environment of a food processing plant, particularly when the strain persists over extended periods of time and continuously contaminates food products. The numerous examples of outbreaks linked to persistent *L. monocytogenes* strains stress the public health impact of persistent strains.

**ROLE OF NICHES IN \( L. \text{MONOCYTOGENES} \) PERSISTENCE**

Because no particular characteristics of *L. monocytogenes* have been robustly linked to persistence, characteristics of the environment itself are likely to be a more critical determinant of persistence, a conclusion also reached by Carpentier and Cerf (18). *L. monocytogenes* can colonize particular niches that provide protection from the environmental stresses, cleaning and sanitation treatments, and physical forces that eliminate bacteria. Niches are “locations harboring the organism after the routine sanitation process for that area has been completed” (74).

**Characteristics of niches in the food processing environment.** Growth niches are sites in an environment that provide both protection from lethal stress and growth permissive conditions, which in sum allow the bacteria to replicate regardless of the application of standard cleaning and sanitation procedures (18). Reviews targeted at industrial audiences have described niches as sites that cannot be adequately cleaned and sanitized because of inherent inaccessibility (e.g., drains), the presence of harborage sites (e.g., hollow rollers or porous floor mats), or the presence of unaddressed wear (e.g., cracks in food contact surfaces). Many other examples have been reported (3, 169, 170). The same design and operation flaws cause accumulation of moisture and organic debris, allowing psychrotolerant *L. monocytogenes* strains to proliferate at the site. Niche locations often are specific to a particular area (e.g., a particular packaging line), and nearby similar sites (e.g., adjacent identical lines) can remain *L. monocytogenes* negative (169). Such specificity suggests that local environmental conditions, such as degradation or wear, may be more responsible for development of a niche than characteristics of the bacteria that inhabit a particular processing line. However, stochastic events (e.g., introduction of *L. monocytogenes* into a niche) likely also play a role.

**Industry response to control niches.** Elimination of bacteria established in a niche (and elimination of the niche itself) requires exceptional effort, using food safety management techniques modeled after engineering process control techniques (74). Routine environmental sampling can detect *L. monocytogenes* in a niche and trigger (i) elimination of *L. monocytogenes* from the niche (e.g., through steam treatment of the equipment or other deep cleaning) and (ii) prevention of recolonization of the niche (e.g., though improved preventative maintenance and sanitary redesign) (2, 169, 170). The “seek-and-destroy” strategy developed in the RTE meat industry (17) formalizes the niche elimination process into three steps: investigation of a positive test result, equipment and process qualification, and process validation. Equipment disassembly, sterilization, and sanitary (re)design is the core intervention in the seek-and-destroy strategy, followed by microbial process control for continued safe operation. The food industry also stresses the need for cooperative, science-based management, such as when the American Meat Institute’s board of directors declared food safety to be noncompetitive (33).

**PUBLIC HEALTH AND ECONOMIC IMPLICATIONS OF *L. MONOCYTOGENES* PERSISTENCE**

Human listeriosis can occur as sporadic cases or outbreaks. Detection of listeriosis outbreaks and their sources is complicated by the long incubation period (1 week to 2 months), the common presence of *L. monocytogenes* in various environments, and the fact that many foods can serve as vehicles in listeriosis outbreaks (168). Although listeriosis outbreaks are rarely reported (typically fewer than five worldwide per year), a larger proportion of sporadic human listeriosis cases probably represent unrecognized outbreaks. Based on cluster analysis of molecular subtype data, Saunders et al. (147) suggested that 13 to 31% of human listeriosis cases reported in New York State may represent common source outbreaks.

**Listeriosis outbreaks linked to persistent strains.** Among the reported human listeriosis outbreaks, some have been traced to persistent contamination from a particular *L. monocytogenes* subtype in the source food processing plant. A multistate listeriosis outbreak in the United States in 2000 that caused illness in 29 people (including four deaths) in 11 U.S. states was linked to the consumption of contaminated delicatessen turkey meat; this outbreak included cases that occurred over an 8-month period (126). The processing plant identified as the source of this outbreak had also been linked to a single listeriosis case in 1988 caused by contaminated turkey frankfurters produced at the same processing facility (183). Subtyping of human and food isolates from the 1988 case and the 2000 outbreak by various methods, including PFGE and ribotyping, revealed that the isolates from 1988 and 2000 were indistinguishable, suggesting that the outbreak strain may have persisted in the same plant for at least 12 years. Full genome sequencing of a food isolate and a human isolate from 1988 and a food isolate and a human isolate from the 2000 outbreak revealed a highly conserved genome among these strains, with differential mutations in only two prophages (127). These data indicate the persistence in this facility of a specific *L. monocytogenes* strain, with limited genomic diversification. This outbreak illustrates the public health importance of *L. monocytogenes* persistence and the economic importance of persistence. In response to the 2000 outbreak, 16 million pounds (7.3 million kilograms) of processed turkey and chicken meat that might have been contaminated with *L monocytogenes* were recalled (126).
A listeriosis outbreak that occurred between 1983 and 1987 in Switzerland represents another example of the public health implications of L. monocytogenes persistence. This outbreak included at least 122 cases (typically occurring during the winter months), of which 31 were fatal (119); more than 80% of cases were caused by serotype 4b strains with one of two characteristic phage types. These two serotype and phage type combinations were uniquely found among L. monocytogenes isolates from a particular Swiss soft cheese consumed only during the winter season. A subsequent case-control study established an association between the outbreak and the consumption of the cheese, and one epidemic strain was isolated from a cheese that had been consumed by a case. After a recall and production cessation, no further cases attributed to the epidemic strain were reported (13). Data available for this outbreak suggest the persistence of the outbreak strains in the cheese processing environment over at least a 5-year period.

Other listeriosis outbreaks also have been linked to L. monocytogenes persistence in processing plants and equipment, including a 2008 outbreak in Canada with 65 cases and 20 deaths (137) and an outbreak in 1998 and 1999 in 22 U.S. states involving at least 101 cases with 15 associated deaths and six miscarriages or stillbirths (21). Persistence of L. monocytogenes has also been implicated as the cause of a large recall of processed meat and poultry products in the United States, even though the products in question were not linked to human listeriosis cases (172). The contamination source was a packaging machine, and the recall resulted in a prolonged plant closure with substantial temporary layoffs (54).

These examples clearly illustrate the public health burdens and economic costs associated with L. monocytogenes persistence in food processing plants, and the vast majority of human listeriosis outbreaks probably have L. monocytogenes persistence as a root cause. Exposure of susceptible individuals to a high level of L. monocytogenes only rarely results in disease. The dose-response model in the risk assessment conducted by the FDA in cooperation with the U.S. Department of Agriculture (USDA) and the CDC (174) predicts a median rate of 1 death per 667 servings for pregnancy-associated and neonatal listeriosis for an exposure at 1 \times 10^{10} CFU per serving. Outbreaks will require a large number of contamination events and exposure incidences before multiple clinical cases of listeriosis with a single subtype occur. These exposures are unlikely to be due to a single lot failure but rather, as supported by most listeriosis outbreak data, represent contamination of food lots over prolonged periods of time. One plausible explanation for multiple contamination events with a single subtype over time is persistent contamination. However, multiple contamination events in a single food processing plant but with different subtypes would rarely be identified as a listeriosis outbreak by typical molecular subtyping surveillance systems.

Economic burden of persistent strains. Although the total economic costs associated with foodborne diseases in general and listeriosis in particular are difficult to quantify, listeriosis has a considerable negative economic impact, partially because of the severity of the disease (154). In one study, the health costs associated with listeriosis in the United States was estimated at $2.6 billion per year (70). Because a considerable proportion of human listeriosis cases may represent outbreaks (147) and many sporadic cases also may have L. monocytogenes persistence as a root cause, the public health–related economic impact of L. monocytogenes persistence in various parts of the food system is significant. L. monocytogenes persistence and the resulting finished product contamination are likely responsible for a large number of recalls, product redesigns, or internal product rejections, which further adds to the economic impact of persistence. Because detection of growth niches and environmental persistence is a key goal of often extensive environmental L. monocytogenes testing programs, the associated costs of testing also are part of the economic impact of persistence. Although comprehensive and specific estimates of the economic impacts of L. monocytogenes persistence are difficult to obtain, a large proportion of the overall economic impact and industry costs associated with L. monocytogenes contamination likely are associated with persistence of this important foodborne pathogen in the food processing environment.

CONCLUSIONS

Although much information is available on L. monocytogenes persistence, critical gaps in our understanding of L. monocytogenes persistence and the contributions of persistence to public health and disease burdens remain. Establishment of persistence and maintenance of persistent populations over time (e.g., in food processing plants) likely is a result of the complex interaction between the pathogen and the environment. Although both pathogen and environmental characteristics likely contribute to L. monocytogenes persistence, current data suggest that environmental characteristics (e.g., presence of growth niches) are the main factors that allow establishment of L. monocytogenes persistence. The factors that determine whether a specific strain establishes persistence may largely be stochastic; almost any strain can become persistent if it is introduced into a suitable niche. However, not all strains are equally likely to establish persistence; some strains may possess certain genetic and phenotypic characteristics that increase the chances of becoming persistent.

Research needs. Additional field studies in different food-associated environments and additional laboratory studies will likely yield further insights into L. monocytogenes persistence, and efforts to quantitatively integrate data (e.g., meta-analysis or quantitative microbial risk assessment) on L. monocytogenes persistence also may help to advance our understanding of persistence. Some key issues that need to be addressed are determination of (i) whether certain L. monocytogenes traits are critical for or contribute to persistence, (ii) whether specific L. monocytogenes subtypes are associated with food and nonfood micro- or macroenvironments (e.g., a geographical region) or animal
populations, and (iii) the risk factors associated with persistence of *L. monocytogenes* in food-associated environments and with transfer of persistent strains to foods at the postprocessing stages. An understanding of the different mechanisms of *L. monocytogenes* stress response may allow better assessment of the food safety risks posed by persistent strains and development of methods to control the growth and survival of this pathogen in various foods and food-associated environments. Quantitative approaches also are needed to define when reisolation of a specific subtype reflects true persistence (ideally with an associated measure of confidence) or is just random reisolation of a common subtype. These and other research efforts in conjunction with continued efforts by the food industry to design and operate facilities and equipment that provide limited opportunity for *L. monocytogenes* growth and survival should contribute to the development of improved strategies for controlling *L. monocytogenes* and to a reduction of human listeriosis cases and outbreaks. Because persistence in food-associated environments of other pathogens (e.g., *Salmonella* Agona in cereal (106) and spoilage organisms (e.g., *Pseudomonas* in the dairy industry (107, 138)) also plays an important role in food safety and quality, an improved understanding of *L. monocytogenes* persistence likely will more broadly improve our ability to assure a safe and high-quality food supply.

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