Molecular subtyping studies to characterize *Listeria monocytogenes* diversity and ecology in the retail environment

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Overview

- *Listeria monocytogenes* subtyping
- *Listeria monocytogenes* diversity
- *Listeria monocytogenes* distribution and ecology in urban, rural (farm), and food associated environments
- Summary and implication for control of *Listeria monocytogenes*
**L. monocytogenes** strain differentiation (subtyping/fingerprinting)

- Tools which allow sensitive differentiation of bacterial subtypes
  - Facilitates detection of outbreaks
  - Facilitates detection of contamination sources
- Strain differentiation methods commonly applied to *L. monocytogenes* include serotyping, ribotyping, Pulsed Field Gel Electrophoresis (PFGE)
Ribotyping
Examples of different *L. monocytogenes* ribotypes
Pulsed Field Gel Electrophoresis
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DNA sequence data support that *L. monocytogenes* represents three lineages

Concatenated *prs, sigB, gap*  

Lineage I, lineage II, lineage III  

Cluster analysis of genome wide gene presence/absence patterns further supports *L. monocytogenes* lineages

- Analysis were performed using both a randomer serotype 1/2a and a randomer 4b chip
- 16 regions of difference (RD) were absent in lineage I strains,

## Molecular characterization of human, animal, and food isolates

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Human isolates (n=507)</th>
<th>Food isolates (n=502)</th>
<th>Animal isolates (n=126)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lineage I</td>
<td>54.4%**</td>
<td>37.3%**</td>
<td>40%</td>
</tr>
<tr>
<td>Lineage II</td>
<td>42.6%**</td>
<td>62.4%**</td>
<td>52%</td>
</tr>
<tr>
<td>Lineage III</td>
<td>2.4%*</td>
<td>0.4%*</td>
<td>8%</td>
</tr>
</tbody>
</table>

** P<0.0001; * P<0.01

Each lineage contains between 30 and 50 EcoRI ribotypes
<table>
<thead>
<tr>
<th>Ribotype</th>
<th>Number of isolates</th>
<th>P-value 1)</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Food</td>
<td>Human</td>
<td></td>
</tr>
<tr>
<td>DUP-1030A</td>
<td>8</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>DUP-1030B</td>
<td>0</td>
<td>10</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>not found in food</td>
</tr>
<tr>
<td>DUP-1038B</td>
<td>15</td>
<td>63</td>
<td>****</td>
</tr>
<tr>
<td>DUP-1039A</td>
<td>12</td>
<td>31</td>
<td>**</td>
</tr>
<tr>
<td>DUP-1039B</td>
<td>18</td>
<td>43</td>
<td>**</td>
</tr>
<tr>
<td>DUP-1039C</td>
<td>35</td>
<td>25</td>
<td>NS</td>
</tr>
<tr>
<td>DUP-1042A</td>
<td>11</td>
<td>16</td>
<td>NS</td>
</tr>
<tr>
<td>DUP-1042B</td>
<td>18</td>
<td>72</td>
<td>****</td>
</tr>
<tr>
<td>DUP-1042C</td>
<td>14</td>
<td>0</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>multiple food types, not in humans</td>
</tr>
<tr>
<td>DUP-1043A</td>
<td>30</td>
<td>16</td>
<td>*</td>
</tr>
<tr>
<td>DUP-1044A</td>
<td>11</td>
<td>28</td>
<td>**</td>
</tr>
<tr>
<td>DUP-1044B</td>
<td>1</td>
<td>19</td>
<td>***</td>
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<tr>
<td></td>
<td></td>
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<td>rarely found in food</td>
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<td>10</td>
<td>0</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>blue cheese only</td>
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<td>DUP-1045B</td>
<td>14</td>
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<td>NS</td>
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<td>DUP-1052A</td>
<td>58</td>
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<tr>
<td>DUP-1053A</td>
<td>24</td>
<td>41</td>
<td>*</td>
</tr>
<tr>
<td>DUP-1062A</td>
<td>151</td>
<td>9</td>
<td>****</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rarely found in humans</td>
</tr>
<tr>
<td>DUP-1062D</td>
<td>28</td>
<td>1</td>
<td>****</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rarely found in humans</td>
</tr>
<tr>
<td>rare</td>
<td>22</td>
<td>42</td>
<td>*</td>
</tr>
<tr>
<td>uncommon</td>
<td>22</td>
<td>33</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>502</td>
<td>507</td>
<td>****</td>
</tr>
</tbody>
</table>

1) P-values refer to comparison of origin between ribotype specified in that row vs. all other ribotypes where NS = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$
Human virulence attenuation of ribotype DUP-1062A

- Isolates with ribotype DUP-1062A carry a premature stop codon in \textit{inlA}, which leads to reduced invasion of human epithelial cells

**Wildtype**

<table>
<thead>
<tr>
<th>inlA (800 aa)</th>
<th>MA</th>
</tr>
</thead>
</table>

**DUP-1062A**

<table>
<thead>
<tr>
<th>inlA (700 aa)</th>
</tr>
</thead>
</table>

Human intestinal epithelial cell
*inlA* premature stop codons in other strains

**Panel A**
- Food isolate from France (NV4; Rousseaux et al., 2002)
- Human fecal carriage strains from France (Olier et al., 2002)
- Human fecal carriage strain L028 from France (Jonquieres et al., 1998)
- Food isolate from France (NV7; Rousseaux et al., 2002)
- Food isolate from France (NV5; Rousseaux et al., 2002)
- Food isolate from France (NV4; Rousseaux et al., 2002)
- Food isolate from France (NV5; Rousseaux et al., 2002)
- Food isolate from France (NV6; Rousseaux et al., 2002)

**Panel B**
- Mutation type 1; DUP-1052A & DUP-16635A
- Mutation type 2; DUP-1025A & DUP-1031A
- Mutation type 3; DUP-1046B & DUP-1062A

**Panel C**
- EGD-e (Glasser et al., 2001)
In vitro evaluation of invasion into human intestinal epithelial cells of *L. monocytogenes* with and without premature stop codons in *inlA*. 

![Graph showing invasion of *L. monocytogenes* strains](graph.png)
L. monocytogenes with premature inlA stop codon: summary

• Found more commonly in food isolates than human isolates
  • France: inlA premature stop codon strains represent 35% of food isolates and 4% of human clinical isolates (Jacquet et al. 2004. JID 189:2094-2100)
  • Also found in China and Portugal
  • Attenuated virulence in guinea pigs

• Not all L. monocytogenes are equally likely to cause human disease and many L. monocytogenes in foods have reduced ability to cause human disease
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

K. K. Nightingale,¹ K. Windham,¹ K. E. Martin,¹ M. Yeung,² and M. Wiedmann¹*

*inlA* Premature Stop Codons Are Common among *Listeria monocytogenes* Isolates from Foods and Yield Virulence-Attenuated Strains That Confer Protection against Fully Virulent Strains⁷,†

K. K. Nightingale,¹,²*, R. A. Ivy,¹ A. J. Ho,¹ E. D. Fortes,¹ B. L. Njaa,³,⁴ R. M. Peters,³ and M. Wiedmann¹


A. Van Stelten and K. K. Nightingale*
L. monocytogenes subtype data for 121 retail operations

- 156 isolates represented lineage I (55.8%) and II (44.2%)
- Molecular serotyping classified isolates into profiles B (serotypes 1/2b, 3b, and 7; 44 isolates), D (serotypes 4b, 4d, and 4e; 43 isolates), and A (serotypes 1/2a and 3a; 59 isolates)
- Isolates include ribotypes linked to human outbreaks, including DUP-1042B (13.5%), DUP-1038B (3.2%), and DUP-1044A (3.2%)
- Some ribotypes identified at retail include only isolates with inlA mutations (one ribotype, 8.3%) and some include isolates with and without inlA mutations (5 ribotypes, 38.4%)
  - Isolates will be specifically characterized for inlA mutations
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• Summary and implications for control of *Listeria monocytogenes*
**L. monocytogenes prevalence**

- Pristine environments: 1.3% (n=900) [23.4% for *Listeria* spp.]
- Urban environments: 7.3% (n=900) [22.3% for *Listeria* spp.]
- Ruminant farms
  - Bovine farms with listeriosis cases: 24.3% (n=616)
  - Bovine farms without listeriosis cases: 20.1% (n=643)
  - Small ruminant farms with listeriosis: 32.9% (n=322)
  - Small ruminant farms without listeriosis: 5.9% (n=475)
- Raw foods
- Ready-To-Eat foods: 0.17 – 4.7 % (Gombas et al., 2004)
**L. monocytogenes** in urban environments

- Albany, NY
  - 214 samples tested
  - 27 positive for LM
  - 10 isolates were ribotype DUP-1038B (over three samplings and >1 year)
Cattle

Small ruminants
L. monocytogenes ecology and contamination patterns in processing plants and retail operations
### Plant A1

#### Raw Product

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</tbody>
</table>

#### Raw Environment

**E2: Drain**
- 1043A
- 1052A
- 1045B
- 1045B
- 1039C
- 1039C
- 1043A
- 1043A
- L.spp
- 1052A
- 1039C
- 1048A
- 1039C
- 1043A
- 1043A
- 1043A
- 1043A
- 1043A
- 1062A
- 1027A
- 1052A

**E8: Apron**
- 1062A
- 1062A
- -
- -
- -
- -
- -
- -
- -
- -
- L.spp
- 1044A
- -

**Fillet knife**
- 1043A

#### Finished Environment

**E1: Drain**
- 1039C
- 1043A
- 1042B
- 1039C
- L.spp
- L.spp
- L.spp
- L.spp
- 1043A
- 1043A
- -
- -
- -
- -
- -
- -
- 1039C
- 1039C

**E3: Drain**
- 1043A
- 1043A
- 1043A
- 1039C
- L.spp
- L.spp
- L.spp
- L.spp
- 1043A
- 1043A
- 1042C
- 1042C
- 1042C
- L.spp
- L.spp
- 1043A
- 1042C
- 1042C
- 1042C
- 1043A
- 1052A
- 1038B
- 1052A

**E4: Cooler Floor**
- 1062A
- L.spp
- -
- 1043A
- L.spp
- L.spp
- 1052A
- 1043A
- L.spp
- -
- -
- -
- -
- -
- -
- -
- -

**Floor**
- L.spp

**Floor mat**
- L.spp
- 1052A

**E6: Cart wheels**
- L.spp
- 1043A
- 1052A
- 1027A
- 1043A
- L.spp
- 1043A
- 1052A
- 1052A
- -
- -
- -
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- -

**E5: Under Slicer**
- -
- -
- -
- L.spp
- L.spp
- -
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**E9: Sliding Door**
- -
- -
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#### Food Contact Surfaces

**E7: Gloves**
- -
- -
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**E10: Slicer**
- -
- -
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- -
- -
- -

**E11: Skinner**
- -
- -
- -
- L.spp
- -
- -
- -
- -
- 1027A
- 1039C
- -

**E12: DeBone**
- -
- -
- -
- -
- -
- 1042B
- -
- -
- -
- -
- -
- 1044A

**E13: Sal. Table**
- -
- L.spp
- -
- L.spp
- L.spp
- L.spp
- 1044A

**Tubs-dirty**
- 1062A

**Tubs-clean**
- 1043A

**New Mixer**
- -
- -
- -
- -
- -

**New Table**
- -
- -
- -
- -

#### Finished Product

<table>
<thead>
<tr>
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</tbody>
</table>
2000 US outbreak - Environmental persistence of *L. monocytogenes*?

- 1988: one human listeriosis case linked to hot dogs produced by plant X
- 2000: 29 human listeriosis cases linked to sliced turkey meats from plant X
Persistent *L. monocytogenes* in food processing plants


- Persistent environmental contamination in meat plants, >4 years in at least one plant (Nesbakken et al., 1996, Int. J. Food Micro. 31:161-171)

- Persistent environmental contamination in poultry processing plants (Ojeniyi et al., 1996, J. Appl. Bacteriol. 80: 395-401)

- Persistent environmental contamination in seafood plants (Rorvik et al., 2000, Appl. Environ. Micro. 66: 4779-4784)
**L. monocytogenes in retail environments**

- Subtyped 98 food and 40 environmental *L. monocytogenes* isolated from 50 supermarkets in New York State between 1997 and 2002.
- 16 supermarkets showed evidence for persistence of one or more specific *L. monocytogenes* strains as indicated by isolation of the same *EcoRI* ribotype from food and/or environmental samples collected in a given establishment on different days.
- 17 ribotypes were found among human clinical isolates as well as among food and environmental isolates.

Distribution of *Listeria monocytogenes* Molecular Subtypes among Human and Food Isolates from New York State Shows Persistence of Human Disease–Associated *Listeria monocytogenes* Strains in Retail Environments

BRIAN D. SAUDERS,1 KURT MANGIONE,2 CURTIS VINCENT,2 JON SCHERMERHORN,2 CLAUDETTE M. FARCHIONE,2 NELLIE B. DUMAS,3 DIANNA BOPP,3 LAURA KORNSTEIN,4 ESTHER D. FORTES,1 KATY WINDHAM,1 AND MARTIN WIEDMANN1*

1Department of Food Science, Cornell University, Ithaca, New York; 2Food Laboratory, New York State Department of Agriculture and Markets, Albany, New York; 3Wadsworth Center, New York State Department of Health, Albany, New York; and 4New York City Department of Health and Mental Hygiene, New York, New York, USA

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ABSTRACT
### L. monocytogenes in retail environments - example

<table>
<thead>
<tr>
<th>Est. Code</th>
<th>Source</th>
<th>Sample Description</th>
<th>Date Collected (No. isolates)</th>
<th>Ribotype</th>
<th>Persistent Ribotype (Lineage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>Food</td>
<td>Oven roasted turkey</td>
<td>08/17/00</td>
<td>DUP-1062A</td>
<td>DUP-1062A (II)</td>
</tr>
<tr>
<td></td>
<td>Food</td>
<td>Smoked turkey breast</td>
<td>08/25/00</td>
<td>DUP-1062A</td>
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</tr>
<tr>
<td></td>
<td>Environment</td>
<td>Swab; display cooler</td>
<td>08/29/00</td>
<td>DUP-1062A</td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>Environment</td>
<td>Swab</td>
<td>10/27/00</td>
<td>DUP-1053A</td>
<td>Not applicable</td>
</tr>
<tr>
<td></td>
<td>Environment</td>
<td>Swab</td>
<td>10/27/00</td>
<td>DUP-1053A</td>
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</tr>
<tr>
<td>X</td>
<td>Food</td>
<td>Ham</td>
<td>11/07/00</td>
<td>DUP-1042C</td>
<td>DUP-1042C(I)</td>
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<td>Swab</td>
<td>11/20/00</td>
<td>DUP-1042C</td>
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<td>11/20/00</td>
<td>DUP-1042C</td>
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<td>Environment</td>
<td>Swab</td>
<td>12/04/00</td>
<td>DUP-1042C</td>
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<td>Environment</td>
<td>Swab</td>
<td>12/04/00</td>
<td>DUP-1042C</td>
<td></td>
</tr>
</tbody>
</table>
Subtype data from environmental sampling of 121 retail establishments

- 27 establishments that had two or more *L. monocytogenes* with the same ribotype
  - in 19 est. two samples had LM with the same subtype, in 6 three samples had LM with the same subtype, in 1 four samples had LM with the same subtype, and in two five samples had LM with the same subtype
- In 11 establishments that had isolates with identical ribotypes in multiple environmental samples, at least one isolate was from a food contact surface
- In 7 establishments isolates with the same ribotype were found in multiple drain or floor samples
<table>
<thead>
<tr>
<th>Date</th>
<th>Sample Description</th>
<th>Ribotype (lineage)</th>
<th>PFGE type (Ascl/ApaI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/21/06</td>
<td>(C) Deli sink</td>
<td>DUP-1052A (I)</td>
<td>I (052/090)</td>
</tr>
<tr>
<td></td>
<td>(D1) Deli area floor drain</td>
<td>DUP-1062A (II)</td>
<td>II (085/098)</td>
</tr>
<tr>
<td></td>
<td>(E1) Raw meat area floor drain</td>
<td>DUP-1052A (I)</td>
<td>I (052/090)</td>
</tr>
<tr>
<td></td>
<td>(G) Dry aisle</td>
<td>DUP-1052A (I)</td>
<td>I (052/090)</td>
</tr>
<tr>
<td></td>
<td>(I) Grocery cart wheels</td>
<td>DUP-1052A (I)</td>
<td>I (052/090)</td>
</tr>
<tr>
<td></td>
<td>(J1) Produce area floor drain</td>
<td>DUP-1052A (I)</td>
<td>I (052/090)</td>
</tr>
<tr>
<td>3/13/07</td>
<td>(C) Deli sink</td>
<td>DUP-1052A (I)</td>
<td>Ia (084/091)</td>
</tr>
<tr>
<td></td>
<td>(D1) Deli area floor drain</td>
<td>DUP-1038C (I)</td>
<td>III (082/058)</td>
</tr>
<tr>
<td></td>
<td>(E1) Raw meat area floor drain</td>
<td>DUP-1062A (II)</td>
<td>IIa (025/097)</td>
</tr>
<tr>
<td></td>
<td>(F1) Seafood area floor drain</td>
<td>DUP-1062A (II)</td>
<td>IIa (025/097)</td>
</tr>
<tr>
<td>11/28/05</td>
<td>(B) Deli case</td>
<td>DUP-1062A (II)</td>
<td>IIa (025/097)</td>
</tr>
<tr>
<td></td>
<td>(C) Deli sink</td>
<td>DUP-1042B (I)</td>
<td>IV (065/040)</td>
</tr>
<tr>
<td></td>
<td>(I) Grocery cart wheels</td>
<td>DUP-1042B (I)</td>
<td>IVa (065/040)</td>
</tr>
<tr>
<td></td>
<td>(J1) Produce area floor drain</td>
<td>DUP-1042B (I)</td>
<td>IVa (065/040)</td>
</tr>
<tr>
<td>3/6/07</td>
<td>(J1) Produce area floor drain</td>
<td>DUP-1062A (II)</td>
<td>IIb (026/097)</td>
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<tr>
<td>Date</td>
<td>Sample Description</td>
<td>Ribotype (lineage)</td>
<td>PFGE type (Ascl/ApaI)</td>
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<tr>
<td>7/10/06</td>
<td>(Food) Intact, deli ham</td>
<td>DUP-1042B (I)</td>
<td>V (064/032)</td>
</tr>
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<td>(C) Deli sink</td>
<td>DUP-1042B (I)</td>
<td>V (064/032)</td>
</tr>
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<td>(D1) Deli area floor drain</td>
<td>DUP-1042B (I)</td>
<td>V (064/032)</td>
</tr>
<tr>
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<td>(E1) Raw meat area floor drain</td>
<td>DUP-1042B (I)</td>
<td>V (064/032)</td>
</tr>
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<td>(G) Dry aisle</td>
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<td>V (064/032)</td>
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<td>(H) Dairy case</td>
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<td>VI (061/083)</td>
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<td>V (064/032)</td>
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<td>(F1) Seafood area floor drain</td>
<td>DUP-1051D (I)</td>
<td>VII (068/037)</td>
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<td>DUP-1051D (I)</td>
<td>VII (068/037)</td>
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<td>(J4) Bakery floor drain</td>
<td>DUP-1051D (I)</td>
<td>VIIa (069/036)</td>
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<td>DUP-1052A (I)</td>
<td>VIII (040/070)</td>
</tr>
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<td>(F1) Seafood area floor drain</td>
<td>DUP-1051D (I)</td>
<td>VII (068/037)</td>
</tr>
<tr>
<td>Date</td>
<td>Sample Description</td>
<td>Ribotype (lineage)</td>
<td>PFGE type (Ascl/ApaI)</td>
</tr>
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<td>DUP-1052A (I)</td>
<td>VIII (040/070)</td>
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<td>DUP-1052A (I)</td>
<td>VIIIa (044/070)</td>
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<td>IX (044/085)</td>
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<td>IXa (040/088)</td>
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<td>(G) Dry aisle</td>
<td>DUP-1052A (I)</td>
<td>IX (044/085)</td>
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Patterns of human listeriosis

Geographic Distribution of Human Listeriosis Cases

Example: Cluster G

- Single localized geographic cluster

Temporal Clusters: A, B, C, D, E, F, G, H, I

Spatial Clusters: B*, D, G*, H, I

*=Epidemiologically-linked cluster (outbreak)
Overview

• *Listeria monocytogenes* subtyping
• *Listeria monocytogenes* diversity
• *Listeria monocytogenes* distribution and ecology in urban, rural (farm), and food associated environments
• Trends in human listeriosis
• **Summary and implications for control of* Listeria monocytogenes**
Summary

• *L. monocytogenes* contamination in retail environments is not uncommon
  • Not surprising as *L. monocytogenes* is not uncommon in surrounding environments
  • Size and inspection scores seem to have little predictive value for *L. monocytogenes* presence
• *L. monocytogenes* can persist in retail environments
• At least some *L. monocytogenes* found in retail environment appear to have potential to cause human listeriosis
• Patterns of cases and small outbreaks suggest that “local events” may contribute to human listeriosis cases
  • Risk assessment suggests that contamination at retail is one contributor to human listeriosis cases
Establishment size and *L. monocytogenes* prevalence

No significant association between the prevalence of *L. monocytogenes* (positive samples/number of samples) and the number of employees (*P* = 0.229) and area (in square feet) of the food facility (*P* = 0.834).
Conclusions

• Control of *L. monocytogenes* at retail is challenging
• Contamination of RTE products from environmental sources at retail is a major concern
• Identification of “high risk” retail operations may be best achieved by environmental testing/monitoring
  • Environmental testing not only identifies the problem but also provides initial insights into potential control strategies
  • Environmental testing should focus on *L. monocytogenes*
• Persistence of *L. monocytogenes* in environmental niches is an important issue
  • Identification and elimination of niches is important
  • Niches can and maybe outside of retail environment
• Need for innovative strategies to reduce *L. monocytogenes* introduction into retail
• Need to comprehensively quantify contributions, to human listeriosis, of *L. monocytogenes* contamination at processing, retail, restaurant, and home and institutional kitchens
Acknowledgments

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