Origin of *Listeria monocytogenes* on meat products.

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Abstract

*Listeria monocytogenes* is a relevant food safety hazard in ready to eat products. Inactivation during processing, prevention of recontamination and control of multiplication are the main instruments to secure the safety of meat products. Intensive microbiological monitoring of products and the production environment are valuable tools to assess the level of control in a meat processing plant. During the course of a year all isolates found during hygiene monitoring at a meat processing plant were stored at -70 degrees Celsius. A total of 94 *L. monocytogenes* isolates have been analyzed by pulsed-field gel electrophoresis (PFGE) and were divided into 30 different types.

Some types were observed both in samples taken from the environment and machinery as well as on products, indicating cross contamination of the final product. Two types could be identified at different moments during the year, indicating persistent contamination.

Processing equipment is playing an important role in *L. monocytogenes* contamination of meat products because it was contaminated before the start of processing.

Good hygienic practices, effective cleaning and disinfection and hygienic design of machinery are important to control *L. monocytogenes* on meat products. Genotyping of harvested *L. monocytogenes* isolates is a valuable tool to review the status of hygiene control in processing of meat products.

Introduction

*Listeria monocytogenes* is a relevant food safety hazard in ready-to-eat (RTE) products with potentially severe consequences (1,2,3). Preventing contamination of RTE products is therefore important. *L. monocytogenes* is an ubiquitous organism, also found in pigs and pork (4). Inactivation during pork processing, preventing recontamination and controlling the multiplication are therefore important points of an HACCP based quality system to assure the control of this hazard.

Food processing facilities can be contaminated with *L. monocytogenes* for long periods of time (5,6). Pulsed field gel electrophoresis (PFGE) has shown to be an effective tool to identify genetic relations of *L. monocytogenes* isolates recovered from food products and processing facilities (7,8). Genetic analysis shows some *L. monocytogenes* strains to be persistently present in a plant where other strains are not (5). Raw materials entering a meat product processing facility are a known source of environmental and product contamination (9,10). Complex processing machines, which are difficult to clean, are known to be the source of persistent contamination (8,11).

The objective of this study was to find the source of contamination of RTE products in a meat processing plant by means of PFGE typing and to determine its effectiveness for hygienic control at a processing plant accordingly.

Material and Methods

Description of the processing plant

The processing plant produces RTE products like fermented products (e.g. salami type), cooked products (e.g. bologna type) and oven-heated pork products (e.g. meatballs). Raw materials are cut, minced and mixed in a designated area for raw material processing. These processed raw materials are either fermented or heat treated and taken to an area with higher hygiene status (“high care area”). Here the finished products are sliced and/or packed to be dispatched. Employees from either area are not allowed to come in contact with each other or to enter the other area to minimise risk of cross contamination.

Sampling and isolates of *L. monocytogenes*

According to the processing plant’s sampling plan as prescribed by their HACCP-based quality assurance system, RTE products are sampled on a weekly basis. Incoming raw materials as well as the production area (e.g. floor, drains) and processing equipment are sampled on a regular basis and sample collection was intensified after positive findings in the RTE
products. For RTE and raw materials the product itself (25 gram) was sampled. For environmental and equipment sampling swabs and sponges were used.

In total 1522 samples were taken; 635 from the environment and 887 from RTE and raw products. Isolation was done by plating on selective agars after a two-step selective enrichment (12). One single colony was selected and stored at -70 degrees Celsius resulting in the recovery of 94 isolates of *L. monocytogenes* in the course of 17 months.

**Pulsed field gel electrophoresis**
Genetic relatedness of *L. monocytogenes* isolates was studied using PFGE analysis. Briefly, colonies were collected from blood agar plates and resuspended in TE buffer to an OD610 between 1.0 and 1.2. After addition of Lysozyme and incubation at 54 ºC proteinase K was added and agarose gel blocks were prepared by mixing the cell suspension with an equal volume of 1% low-melting point agarose. The plugs were incubated for 2 hours at 54ºC in cell lysis buffer (Tris, EDTA, 1% Sarcosyl, proteinase K solution). After incubation cell lysis mixture was removed and plugs were washed four times in TE buffer. Next, a slice of each plug was pre incubated with 200 µl of the restriction buffer. Subsequently, the DNA was digested by incubating the agarose plug in 200 µl restriction buffer containing 20 units Ascl or 20 units of Apal for 4 hours at 37ºC or 30ºC respectively. Before electrophoresis, the plugs were rinsed and loaded on a 1% (w/v) PFGE certified agarose.

Electrophoresis was performed using the CHEF-DR III system (Bio-Rad Laboratories). Agarose gels were run in 0.5 X TBE at 14ºC and 6 V/cm with an included angle of 120º. Pulse times varied from 4 to 40 sec over 19 hours. Agarose gels were stained with GelRed for 30 minutes and photographed.

**Analysis and clustering of PFGE**.
DNA fingerprints were analyzed using the BioNumerics software package. Band matching was performed using the 5% relative to max filtering criteria. Uncertain bands were excluded from the analysis. The similarity coefficients were calculated using the band-based DICE algorithm with a 1% band position tolerance window and 1% optimization. The unweighted pair-group method with arithmetic means (UPGMA) was used for clustering. Fingerprints were assigned to the same PFGE Pulsotype when similarity was 95% or higher and were based on the composite data of both Ascl and Apal restriction endonuclease patterns.

**Results/Discussion**
The 94 isolates were divided in 30 PFGE pulsotypes of which 6 formed clonal clusters (pulsotype 9, 11, 15, 19, 21 and 22) based on 95% similarity and 24 were non related fingerprints (fig. 1 and Table1)

![Fig 1. Clustering based on PFGE fingerprints from 94 L. monocytogenes isolates obtained by Ascl and Apal restriction endonuclease. Pulsotypes based on 95% similarity (---), Origin of the isolate and Product (♦) and or Environment (Δ) relatedness are indicated.](image)

![Table 1.](image)
Distribution of PFGE pulsotypes in 94 isolates of different origin collected in a meat processing plant.

<table>
<thead>
<tr>
<th>Origin</th>
<th>n</th>
<th>PFGE types</th>
</tr>
</thead>
<tbody>
<tr>
<td>product</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>RTE</td>
<td>25</td>
<td>8, 9(2), 11(13), 15, 19(4), 23, 26, 29, 30</td>
</tr>
<tr>
<td>raw material</td>
<td>4</td>
<td>12, 13, 16, 17,</td>
</tr>
<tr>
<td>environment</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>area</td>
<td>27</td>
<td>1, 2, 5, 6, 7, 9(2), 11, 18, 19(9), 20, 21(3), 22, 24, 25, 27, 28</td>
</tr>
<tr>
<td>machine</td>
<td>38</td>
<td>3, 4, 10, 11(28), 14, 15, 19(4), 22</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td></td>
</tr>
</tbody>
</table>

Numbers in **Bold** indicate the frequency the PFGE types are found if found more than once.

Genetically identical PFGE pulsotypes in RTE products and in the processing area as well as the processing machines were found. This indicates cross contamination of RTE from the environment.

Only PFGE pulsotype 11 and 19 are considered persistent when using the definition of persistent strains as described by Keto-Timonen (6), (Fig. 2).

This distribution of the PFGE pulsotypes according to the origin (Table 1) combined with the occurrence in time (Fig. 2) suggests that PFGE pulsotype 11 was most likely originating from a persistent contamination of the processing machines. The machine was identified as a former unit used for altering the texture of cooked meat. After this machine was subjected to improved cleaning and disinfection procedures, including sterilizing the unit in an industrial steam cooker before each use, no *L. monocytogenes* could be recovered after intensive sampling.

PFGE pulsotype 19 appears to be persistently present both in the production environment and processing machines. Contamination of RTE could occur from either source further demonstrating the importance of good hygiene practices during food production.

The single PFGE pulsotypes from RTE and production area underline that also new introductions and contamination was occurring.

**Conclusion**

Using PFGE for genotyping of *L. monocytogenes* isolates is a valuable tool to review the status of hygiene control in a meat processing facility. It can reveal important contamination pathways and point out main sources of *L. monocytogenes* contamination which are specific for the processing facility. This provides valuable insights for changing hygienic operating procedures to improve food safety control.

**References**


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**Fig 2.** Distribution over time of different PFGE pulsotypes recovered from the environment and RTE products. When a PFGE pulsotype was recovered more than once on the same date and the same sample type, it is represented as a single point in the graph. An arrow is used to indicate a persistent PFGE pulsotype.


