Persistent and Nonpersistent *Listeria monocytogenes* Contamination in Meat and Poultry Processing Plants

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**ABSTRACT**

Contamination analysis of persistent and nonpersistent *Listeria monocytogenes* strains in three meat processing plants and one poultry processing plant were performed in order to identify factors predisposing to or sustaining persistent plant contamination. A total of 596 *L. monocytogenes* isolates were divided into 47 pulsed-field gel electrophoresis (PFGE) types by combining the restriction enzyme patterns of *Ascl* (42 patterns) and *ApaI* (38 patterns). Persistent and nonpersistent strains were found in all plants. Nonpersistent PFGE types were found mostly at one sampling site, with the processing environment being the common location, whereas the persistent strains were found at several sampling sites in most cases. The processing machines were frequently contaminated with persistent *L. monocytogenes* PFGE types, and it was of concern that surfaces having direct contact with the products were contaminated. The role of the processing machines in sustaining contamination and in contaminating the products appeared to be important because the final product of several processing lines was contaminated with the same *L. monocytogenes* PFGE type as found in the processing machine. The proportion of persistent PFGE types in heat-treated products was eight times higher than in the raw products, showing the importance of the persistent PFGE types as contaminants of the final heat-treated products. The contamination status of the processing lines and machines appeared to be influenced by the compartmentalization of the processing line, with poor compartmentalization increasing *L. monocytogenes* contamination. The separation of raw and post–heat treatment areas seemed especially important in the contamination status of post–heat treatment lines.

Meat and poultry processing plants can be contaminated with *Listeria monocytogenes* (5, 7, 8, 16, 20, 25), and contamination analyses have shown that *L. monocytogenes* can survive in food processing plants for extended periods. Some *L. monocytogenes* strains can persist in food processing facilities, whereas other strains are nonpersistent (8, 9, 16, 20). The persistent strains predominate in the plants and cause continuous contamination pressure on the products. The phenomenon of persistent contamination has been observed not only in the meat and poultry industries but also in the fish (4, 11, 22, 23) and dairy industries (19, 26).

Some factors influencing the survival of *L. monocytogenes* strains in food processing plants are recognized. Processing machines play an important role in *L. monocytogenes* contamination (4, 16, 19), especially machines with complex structure and poor hygienic properties (4, 16). The persistence of *L. monocytogenes* might be influenced by strain-specific properties, such as differences in adherence to stainless steel surfaces (18) and susceptibility to disinfectants (1, 17). However, the causes of persistent *L. monocytogenes* plant contamination are still not fully understood, and further research is needed to identify those factors present in food processing plants that contribute to persistent contamination.

Here, we report the results of a study of three meat processing plants and one poultry processing plant. The plants were sampled for *L. monocytogenes* over a period of several years, and the *L. monocytogenes* isolates were characterized by a molecular typing method. The aims of the study were to investigate contamination routes and sites of persistent and nonpersistent strains in food processing plants and to recognize factors in the processing line that predispose the line to persistent contamination. Increased knowledge of the behavior of persistent and nonpersistent *L. monocytogenes* strains is essential for the planning of preventive measures in meat and poultry processing plants.

**MATERIALS AND METHODS**

*L. monocytogenes* isolates. A total of 596 *L. monocytogenes* isolates were recovered at the food processing plants as a part of quality control programs during a period of several years. Samples were collected from the processing environment (walls, floors, and drains), equipment, products, and raw materials. Swabs or sponges were used to sample the processing environment and equipment. Isolation was performed according to the Nordic Committee on Food Analysis (2) or the International organization for Standardization method (3). Both methods include a two-step selective enrichment process followed by plating on selective media. Identification was based on hemolytic activity, Gram staining, catalase reaction, motility at 25°C, and further identification by the API *Listeria* kit (BioMérieux SA, Marcy l’Etoile, France). One isolate
per sample was further characterized. The isolates were stored at 
−70°C.

Food processing plants. Plants A, B, and C produced mainly 
ready-to-eat pork and beef products, and plant D produced poultry 
products. A total of 18 L. monocytogenes isolates (processing 
environment 2, equipment 9, product 7) were collected from plant 
A, 92 isolates (processing environment 24, equipment 49, product 
18, raw material 1) from plant B, 307 isolates (processing 
environment 43, equipment 199, product 63, raw material 2) from 
plant C, and 179 isolates (processing environment 38, equipment 
104, product 37) from plant D. The processing lines investigated 
are presented in Table 1.

DNA isolation and pulsed-field gel electrophoresis (PFGE) 
typing. Pure L. monocytogenes cultures were grown on 
blood agar for 24 h at 37°C, after which a single colony was 
transferred into brain heart infusion broth (Difco, Detroit, Mich.). 
Cells were harvested from 2 ml of brain heart infusion broth after 
night incubation at 37°C. DNA was isolated as described by 
Björkroth et al. (6), with modifications described by Autio et al. 
(4). Briefly, plugs were lysed for 3 h and a 1-h wash with ESP at 
50°C was performed once. Two rare-cutting restriction enzymes, 
Ascl (New England Biolabs, Beverly, Mass.) and Apal (Boehringer 
Mannheim, Mannheim, Germany) were used for restriction 
endonuclease digestion. The samples were electrophoresed 
through 1.0% (wt/vol) agarose gel (Seakem Gold; FMC Bioproducts, 
Rockland, Maine) in 0.5× TBE (45 mM Tris, 4.5 mM boric 
acid [pH 8.3], and 1 mM sodium EDTA) at 200 V and 14°C in 
a Gene Navigator system with a hexagonal electrode (Pharmacia, 
Uppsala, Sweden). Pulse times ramped from 1 to 35 s for Ascl 
and Apal for 18 h. The gels were stained with ethidium bromide 
and visualized and digitally photographed with an Alpha Imager 
2000 documentation system (Alpha Innotech, San Leandro, Ca-
lifornia). Fragment size was determined with a low-range PFG marker 
(New England Biolabs).

PFGE pattern analysis. The PFGE type was obtained by 
combining both restriction enzyme profiles into one unique 
profile. A PFGE pattern was considered unique if one or more bands 
differed from other PFGE patterns.

Serotyping. One randomly selected L. monocytogenes strain 
of each PFGE type was selected for serotyping. Serotyping was 
performed with commercial Listeria antisera (Denka Seiken, 
Tokyo, Japan) as described by the manufacturer.

Persistence of a strain. L. monocytogenes strains that were 
found repeatedly (five times or more) over a period of time (≥3 
months) were considered to be persistent. Strains found 
sporadically (fewer than five times) or within a limited time period (<3 
months) were considered to be nonpersistent.

RESULTS

Distribution of persistent and nonpersistent L. monocytogenes 
PFGE types. A total of 596 L. monocytogenes isolates were divided into 47 PFGE types by combining the macrorestriction patterns (MRP) of Ascl (42 MRP) and 
Apal (38 MRP) and into five serotypes (1/2a, 1/2b, 1/2c, 3a, and 4b) (Table 2). All plants were contaminated with 
one or several persistent strains and several nonpersistent 
strains. The percentage of the persistent PFGE types of the 
total number of PFGE types ranged from 17% in plant A 
to 41% in plant C (Table 3). Thirty-five L. monocytogenes 
PFGE types were plant specific, seven PFGE types were 
common for two plants, and five PFGE types were common 
for three plants (Table 2). Identical PFGE types were found
<table>
<thead>
<tr>
<th>PFGE type</th>
<th>MRP&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Food plant</th>
<th>P or N&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. of contaminated lines</th>
<th>Site of contamination</th>
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<sup>a</sup> MRP: Mobility Repulsion Protein

<sup>b</sup> P or N: Presence or Absence
Table 2. Continued

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<th>PFGE type</th>
<th>MRP**</th>
<th>Serotype</th>
<th>Food plant</th>
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<th>Site of contamination</th>
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* MRP, macrorestriction patterns.

** Persistent (P) or nonpersistent (N) PFGE type.

Both in meat and in poultry processing plants, in all, 19 PFGE types were found to be persistent and 28 PFGE types to be nonpersistent. Nine persistent PFGE types were found to be nonpersistent in another plant (Table 2).

Ten persistent PFGE types and three nonpersistent PFGE types contaminated two or more processing lines in a plant (Table 2). The processing lines contaminated with identical PFGE types were either located in the same compartment, such as processing lines V to VII in plant C, or in different compartments, such as processing lines I and II in plant B.

Differences in the location of persistent and nonpersistent PFGE types were observed (Table 4). All persistent PFGE types, except one, were found at least at two sampling sites, of which one was the equipment. Almost half of the persistent PFGE types were found at all three sampling sites (i.e., in the processing environment, equipment, and products). Two-thirds of the nonpersistent PFGE types were found at only one sampling site. The proportion of persistent and nonpersistent PFGE types differed in raw and cooked products, the proportion of the persistent PFGE types being eight times higher in the cooked product (Table 5). The majority of the persistent PFGE types (13 of 15) were found more than once in the final product, whereas the majority of the nonpersistent PFGE types (11 of 15) were found only once in the final product.

Contamination of process surfaces and products.

Processing machines such as slicing machines, spiral freezers, packing machines, and conveyors were frequently contaminated with L. monocytogenes. Surfaces of the processing machines in direct contact with the product were contaminated, with the exception of peeling machines, which were only contaminated on surfaces having indirect contact with the product. The contamination sites in the processing machines are presented in Table 6.

The same persistent PFGE types were, in several cases, found in a processing line, both from the processing machine and from the products manipulated by the machines. This was observed, e.g., in the slicing and dicing lines in

Table 3. Persistent and nonpersistent Listeria monocytogenes pulsed-field gel electrophoresis (PFGE) types found in food processing plants A, B, C, and D

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<thead>
<tr>
<th>Food plant</th>
<th>PFGE type</th>
<th>P or N*</th>
<th>No. of PFGE types (%)</th>
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<td>A</td>
<td>32</td>
<td>P</td>
<td>1 (17)(^b)</td>
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<tr>
<td></td>
<td>11, 15, 16, 36, 46</td>
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<td>5 (83)(^c)</td>
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<td>B</td>
<td>6, 15, 28, 36</td>
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<td>4 (29)(^b)</td>
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<td>3, 5, 11, 13, 19-21, 30, 38, 45</td>
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<td>10 (71)(^c)</td>
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<tr>
<td>C</td>
<td>11, 17-19, 22, 24, 37, 41, 47</td>
<td>P</td>
<td>9 (41)(^c)</td>
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<tr>
<td></td>
<td>6, 8, 15, 16, 25, 26, 29, 33-35, 40, 42, 44</td>
<td>N</td>
<td>13 (59)(^c)</td>
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<tr>
<td>D</td>
<td>8, 13, 27, 31, 45, 47</td>
<td>P</td>
<td>6 (29)(^c)</td>
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<td>15 (71)(^c)</td>
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</table>

* Persistent (P) or nonpersistent (N) PFGE type.

** Percent persistent PFGE type(s) of total number of PFGE types in the plant.

\(^c\) Percent nonpersistent PFGE type(s) of total number of PFGE types in the plant.

Table 4. Location of persistent and nonpersistent Listeria monocytogenes pulsed-field gel electrophoresis (PFGE) types in plants A, B, C, and D

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<th>Sampling site</th>
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* Persistent (P) or nonpersistent (N) PFGE type.
plants A (processing lines I and II), B (processing lines I and IV), and C (processing lines I and II) where the slicing machines were contaminated, as well as in the end products. This was also observed in processing line IV of plant C and line I of plant D, where post-heat treatment processing—such as the spiral freezers, conveyors leading from the freezer to the packing machine, weighing system in processing line I in plant D, packing machines, and final end products—were contaminated with persistent PFGE types. The PFGE types found in the raw materials or raw products were not established in the post-heat treatment processing machines.

Processing lines IV and V in plant B and II and III in plant D produced raw products and were persistently contaminated, whereas the fermented, uncooked ready-to-eat meat products in plants A (processing line IV) and B (processing line III) were not persistently contaminated.

**Effect of compartmentalization on L. monocytogenes contamination.** Two processing lines (I and II) with differing degrees of compartmentalization in plant B exhibited different contamination levels. The line that was less compartmentalized was found to be more extensively contaminated with *L. monocytogenes* and for longer periods of time. Both processing lines produced a cooked pork and beef product independently of each other; one line cooled, sliced, and packaged the product and the other line chilled the product in a spiral freezer and packaged the product. Schematic layouts of the processing lines, including compartmentalization and contamination status, are presented in Figure 1.

The dicing line (II) and the slicing line (I) in plant C were located next to each other and compartmentalized in a similar manner, but to a lesser degree than processing lines I and II in plant B. Both processing lines were heavily contaminated with several PFGE types. The dicing line was contaminated throughout with PFGE type 37, and the slicing line harbored the processing line-specific PFGE type 11. The slicing line was also contaminated with PFGE type 37, but to a much lesser extent than the dicing line. The processing environment in the area between the ovens and coolers, which was common to both lines, was contaminated with several PFGE types. Schematic layouts of the processing lines, including compartmentalization and contamination status, are presented in Figure 1. A similar effect of compartmentalization on the contamination status that was observed in plant C (processing lines I and II) was also observed in a processing line (I) in plant D.

**DISCUSSION**

The processing machines were persistently contaminated in all plants. It is of concern that the surfaces of the processing machines having direct contact with products were contaminated. Several heat-treated products were contaminated with the same PFGE type found in the processing machines, emphasizing the role of the processing machines in the contamination process of the final products. It also suggests that the processing machines (e.g., slicers, dicers, freezers, and conveyors) were poorly sanitized. Part of the difficulty in sanitizing processing machines is attributable to their complex structure, which prevents sufficient disassembly for cleaning. However, the peeling machines were not found contaminated on surfaces in direct contact with products, which might have been a result of the hot steam applied in the peeling process.

The persistent PFGE types were often widely spread in the processing plants, contaminating several sampling sites and more than one processing line. The persistent PFGE type might have been introduced into different processing lines independently, or the contamination has been transferred from one line to another with the personnel or equipment, splashes from inadequate washing procedures, or air. However, Autio et al. (4) could not show the spread.
of *L. monocytogenes* by air in a heavily contaminated food processing plant. In contrast, relocation of processing machines has transferred *L. monocytogenes* contamination from one site to another (16).

The higher proportion of persistent PFGE types in heat-treated than in raw products emphasizes the importance of the persistent PFGE types as contaminants of final heat-treated products. In fact, the PFGE types found in raw materials or raw products were not able to establish themselves in the post–heat treatment lines. These findings suggest that the persistence of a strain in a food plant is not a result of continuous flow of the strain via raw materials, which is supported by several studies showing that persistent strains are usually not found in the raw materials (4, 21, 23). In contrast, strain-specific properties apparently influence the survival and colonization of the organism in food processing facilities. Enhanced adherence of persistent *L. monocytogenes* strains (18) and differences in disinfectant susceptibility (1, 17) and cadmium resistance (10) have been proposed as having an influence on the survival of persistent and nonpersistent *L. monocytogenes* strains. However, raw materials are often known to be contaminated with *L. monocytogenes* (12, 14, 24) and, therefore, to pose a heavy contamination pressure in the raw area. In fact, Berrang et al. (5) found indistinguishable *L. monocytogenes* strains from the drains on the raw side and the heat-treated products in one out of two poultry processing lines. These findings justify the presumption that the persistent strains are introduced into the food processing plants via the raw materials at some point.

The percent persistent PFGE types of the total number of PFGE types in plant C was higher than in the other
plants. It seems that the barrier between the raw and post-
heat treatment areas in plant C was inadequate, allowing a
large number of PFGE types to enter the food processing
areas and enabling some to become established in the pro-
cessing machines.

The raw product processing lines were persistently
contaminated, but those processing lines that produced fer-
mented uncooked products were not persistently contami-
nated. Competing flora can decrease the amount of \textit{L. monocyto-
gen}{\textit{genes}} on surfaces \citep{13, 15}, suggesting that the pro-
cessing lines producing fermented products might contain
competing flora that would prevent \textit{L. monocytogenes} from
becoming established on the process surfaces.

Some \textit{L. monocytogenes} strains showing a similar
PFGE type were categorized as persistent strains in one
plant and nonpersistent in another plant, emphasizing the
complex nature of persistent and nonpersistent contamina-
tion. It is possible that the typing method was unable to
detect differences in the genome of the strains or that the
phenotypes of the similar PFGE types were different (e.g.,
because of adaptation to environmental stress factors such
as disinfectants). \textit{L. monocytogenes} strains might adapt
(e.g., to quaternary ammonium compounds), with the dif-
ference in resistance between adapted and nonadapted cells
being multifactorial \citep{1, 17}. Also, it is possible that with addi-
tional samples, some of the nonpersistent \textit{L. monocytogenes}
strains were recovered more often, leading to their cate-
gorization as persistent strains. The serotype could not be
shown to influence the persistence of a strain because all
\textit{L. monocytogenes} serotypes were able to cause persistent
contamination.

The contamination status was different in processing
lines with differing degrees of compartmentalization. The
processing line in plant B that was less compartmentalized
was observed to be more frequently contaminated and for
longer periods of time than the processing line with well-
separated operations in the same plant. The slicing and the
dicing lines in plant C were poorly compartmentalized, and
the raw area was not separated from the post–heat treatment
area. The processing lines were contaminated throughout
with persistent \textit{L. monocytogenes} PFGE types, especially the
dicer, slicer, conveyor, and packing machine, which were
difficult to sanitize. These observations indicate that
compartmentalization and separation of different opera-
tions, especially the separation of the raw area from the
post–heat treatment area, is important in the \textit{L. monocyt-
gen}{\textit{ogenes}} contamination status of a processing line. Poor or no
separation of the raw and post–heat treatment areas makes
prevention of personnel or equipment movement between
different processing steps difficult. Proper compartmental-
ization (i.e., fixed walls) prevents undesired traffic between
the raw and post–heat treatment areas.

Persistent contamination of meat and poultry process-
ing plants is a result of many interacting factors. The prop-
erties of the \textit{L. monocytogenes} strain appears to play an
important role, as suggested in earlier studies \citep{1, 19}, but
the design of the processing line, including the processing
machines and the degree of compartmentalization, also
influences the contamination status. The processing machines
and the surfaces that are in direct contact with the products
appear to have a central role in sustaining \textit{L. monocytogenes}
contamination and spreading the contamination to the prod-
ucts. More attention should be focused on the hygiene of
processing machines, not only in the food processing plants
but also at the design stage of the machines. Compartment-
alization of the processing line, especially separation of the
raw from the post–heat treatment area should be adequate
because poor compartmentalization can lead to extensive
contamination of the post–heat treatment processing ma-
chines. Properly compartmentalized processing lines and
the design of more hygienic and easily disassembled pro-
cessing machines should enable better control of \textit{L. monocy-
gen}{\textit{ogenes}} contamination in meat and poultry processing
plants.

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