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Transfer of Persistent *Listeria monocytogenes* Contamination between Food-Processing Plants Associated with a Dicing Machine

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ABSTRACT

The possibility of the transfer of persistent *Listeria monocytogenes* contamination from one plant to another with a dicing machine was evaluated, and possible reasons for persistent contamination were analyzed. A dicing machine that diced cooked meat products was transferred from plant A to plant B and then to plant C. After the transfer of the dicing machine, *L. monocytogenes* PFGE type I, originally found in plant A, was soon also found in plants B and C. This *L. monocytogenes* PFGE type I caused persistent contamination of the dicing lines in plants B and C. The persistent *L. monocytogenes* strain and three nonpersistent *L. monocytogenes* strains found in the dicing line of plant C were tested for adherence to stainless steel surfaces and minimal inhibitory concentrations of a quaternary ammonium compound and sodium hypochlorite, disinfectants widely used in the dicing lines. The persistent strain showed significantly higher adherence to stainless steel surfaces than did the nonpersistent strains. The minimal inhibitory concentrations of sodium hypochlorite were similar for all strains, and the minimal inhibitory concentrations of the quaternary ammonium compound for three of the *L. monocytogenes* PFGE types, including the persistent PFGE type, were high. All persistent *L. monocytogenes* PFGE type I isolates were found in an area with high hygienic standards, with the dicing machine being the first point of contamination. These observations show that the dicing machine sustained the contamination and suggest that the dicing machine transferred the persistent *L. monocytogenes* PFGE type from one plant to another.

Listeria monocytogenes has been shown in several studies to be capable of contaminating food-processing machines, resulting in the contamination of a variety of products (5, 15, 18). It has also been shown that *L. monocytogenes* contamination may become persistent for several months or even years in food-processing plants (5, 18, 23), with complex machines sustaining the contamination (5, 18). Processing machines that manipulate cooked products should be free of *L. monocytogenes* to prevent postprocessing contamination and contamination of new production sites when processing machines are relocated. However, the eradication of *L. monocytogenes* from contaminated processing lines and machines has proved to be difficult, requiring special measures, including targeted and improved cleaning and disinfection procedures (5, 18).

Cleaning and disinfection procedures for food-processing machines involve mainly mechanical, chemical, and thermal energy (11). When these procedures are adequately applied, *L. monocytogenes* is eliminated. However, treatments need to reach the site of contamination to a sufficient extent to be effective. This may not always be possible with routine cleaning and disinfection procedures because of the complex construction of the processing machines. Small spaces and narrow openings are difficult or impossible to reach, resulting in poor mechanical cleaning. However, me-

chanical cleaning is the most effective way to detach adhered cells and biofilms (11).

Adherence to surfaces of processing machines is important for the survival of bacteria, because adherence increases the resistance of bacteria to mechanical and chemical stresses (8, 9, 20). *L. monocytogenes* adheres to stainless steel, Buna-n rubber, polypropylene, and several other food contact surfaces (7, 16, 20) commonly found in equipment used in the food-processing industry. Persistent *L. monocytogenes* strains have been shown to adhere more efficiently to stainless steel surfaces after short contact times than do nonpersistent strains (14), which may influence the effectiveness of cleaning and disinfection treatments for the processing machines. The survival of the bacteria is also affected by other properties, e.g., adaptation to low acidic conditions (12) and to high temperatures (13, 19). Evidence further suggests that persistent *L. monocytogenes* strains are resistant to benzalkonium chloride, which could lead to increased survival of these bacteria in food-processing facilities (1).

Slicing, dicing, packaging, and brining machines have been shown to harbor *L. monocytogenes* (5, 18, 22). This is a matter of concern not only in everyday disinfection but also when processing machines are moved to new production sites. In addition to the contamination of a dicing line by *L. monocytogenes*, we report for the first time to our knowledge the transfer of persistent *L. monocytogenes* contamination from one plant to another with a dicing machine.

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TABLE 1. Cleaning and disinfection^a of the dicing lines in plants A, B, and C

Plant	Cleaning agent	Disinfectant	Dismantling	Other procedures
A	Sodium hypochlorite, anionic surfactant (P, daily) Phosphoric acid, anionic surfactant (S, once/week)	Isopropanol and QAC (P, daily) Peracetic acid (S, once every 2–3 wk) QAC (S, once per mo)	Once per wk	No
B	Sodium hypochlorite, anionic surfactant (P, daily)	Isopropanol and QAC (P, daily)	Once per wk	No
C	Sodium hypochlorite, anionic surfactant (P, daily) Phosphoric acid, anionic surfactant (S, once every 2–3 wk) Sodium hydroxide, nonionic surfactant (S, once every 2–3 wk)	Isopropanol and QAC (P, daily) Peracetic acid (S, once every 2–3 wk) QAC (S, once every 2–3 wk) Amphoteric surfactant (S, once per mo)	Daily	Alkali-acid-alkali treatment (once every 2 wk)

^a Dicing lines were cleaned and disinfected daily. Each plant used one cleaning agent and one disinfectant as principal agents (P). The principal agents were substituted at plants A and C with substitutional compounds (S) at predetermined intervals (frequency indicated after the agent). Cleaning agents and disinfectants were used according to the manufacturers' recommendations. QAC, quaternary ammonium compound.

MATERIALS AND METHODS

L. monocytogenes isolates and collection of sampling data.

L. monocytogenes isolates were recovered as a part of quality control programs in meat-processing plants. Samples were collected from the environment, from equipment, and from products. Detection was carried out according to Nordic Committee on Food Analysis (2) or International Organization for Standardization (3) standards. Briefly, both methods involved two-step enrichment in selective medium and plating on selective agar. Identification was based on hemolytic activity, Gram staining, catalase reaction, and motility at 25°C, and further identification was achieved with the API Listeria kit (BioMérieux SA, Marcy l'Etoile, France). The isolates were stored at -70°C. Sample data were collected retrospectively from the available sampling records of each plant.

DNA isolation and pulsed-field gel electrophoresis (PFGE) typing. Pure *L. monocytogenes* cultures were grown on blood agar for 24 h at 37°C, and then a single colony was transferred into brain heart infusion broth (Difco Laboratories, Detroit, Mich.). Cells were harvested from 2 ml of brain heart infusion broth after overnight incubation at 37°C. DNA isolation was performed as described by Björkroth et al. (6) with the modifications described by Autio et al. (5). Briefly, plugs were lysed for only 3 h, and the 1-h wash with ESP (0.5 M EDTA [pH 8.0], 10% sodium lauroyl sarcosine, 100 µg of proteinase K per ml) at 50°C was performed only once. Two rare-cutting restriction enzymes, *AscI* and *Apal* (New England Biolabs, Beverly, Mass.), 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 at 14°C in a Gene Navigator system with a hexagonal electrode (Pharmacia, Uppsala, Sweden). Pulse times ramped from 1 to 35 s for *AscI* and *Apal* for 18 h. The gels were stained with ethidium bromide and visualized and digitally photographed with the Alpha Imager 2000 documentation system (Alpha Innotech, San Leandro, Calif.). Fragment size was determined with a Low Range PFG marker (New England Biolabs).

Interpretation of PFGE patterns. Every restriction enzyme pattern was assigned a number for both restriction enzymes. 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 those of other PFGE patterns.

Serotyping. Serotyping was carried out with commercial *Listeria* antisera (Denka Seiken, Tokyo, Japan) as directed by the manufacturer.

Dicing machine. The dicing machine was purchased and transferred from plant A to plant B in October 1998 and was then transferred to plant C in March 1999. It was used to dice cooked pork meat products, but not raw or minimally processed products, at each plant. The dicing machine was located in an area with high hygienic standards. Raw products were not present in the area and personnel traffic was restricted.

Disinfection of dicing machine. The dicing machine was cleaned and disinfected in each plant before it was put to use. The machine was cleaned and disinfected every day after processing was finished and was regularly dismantled to allow access to hard-to-reach places (Table 1). The motor house was not cleaned or disinfected. Lubricants were not replaced, except for refilling. The improved cleaning and disinfection methods used in plant C included thorough dismantling, heating of smaller parts in water, and alkali-acid-alkali washes every 2 weeks.

Adherence test. The test for the adherence of *L. monocytogenes* strains to stainless steel was performed according to Lundén et al. (14) with some modifications. Stainless steel surfaces (type 316, Outokumpu Steel, Tornio, Finland) were cut into coupons (5 by 15 by 1 mm), washed, and then autoclaved in test tubes. *L. monocytogenes* strains were grown in tryptic soy broth (TSB) at 25°C to the log phase and resuspended in TSB to attain a test suspension of 6×10^5 CFU/ml. The test suspension was transferred into the test tubes with test coupons. The contact time was 2 h at 25°C. After the contact time, the coupons were rinsed with sterile water and stained with 0.05% acridine orange (Certistain, Merck, Darmstadt, Germany), and adhered cells were enumerated with an epifluorescence microscope (Optiphot-2, Nikon, Japan) at a magnification of ×400. At least 30 random fields of each coupon were analyzed. The test was repeated six times.

Resistance to disinfectants. Minimal inhibitory concentrations (MICs) of the disinfectants sodium hypochlorite (active

TABLE 2. *Listeria monocytogenes* PFGE type I contamination sites in the dicing lines in plants A, B, and C

Sampling site	No. of positive samples/total no. of samples
Plant A	
NS ^a	1/8
Plant B	
Between oven and dicer	0/8
Exterior surface of dicer	1/41
Conveyor belt after dicer	2/15
Cooked diced pork product	9/85
NS	0/19
Plant C	
Between oven and dicer	0/305
Exterior surface of dicer	2/422
Cutting blades	2/16
Control panel	1/16
Tool kit	2/18
Cooked diced pork product	13/1,600
NS	0/26

^a NS, sampling site not specified.

chlorine 10%; Finnish Chemicals, Äetsä, Finland) and alkyl-benzyl-dimethylammonium chloride (Goldschmidt, Pandino, Italy), a quaternary ammonium compound (QAC), for *L. monocytogenes* strains were determined by the microdilution broth method according to the National Committee on Clinical Standards (4).

RESULTS

L. monocytogenes PFGE type I was recovered from the dicing line in plant A in November 1997. After the removal of the dicing machine from plant A, *L. monocytogenes* PFGE type I was found once in the raw area but never in the post-heat-treatment area over a 1-year sampling period. The dicing machine was sold to plant B at the beginning of October 1998, and *L. monocytogenes* PFGE type I was found in a plant B sample taken on 28 October 1998. The organism was found on the exterior surface of the dicing machine and on the conveyor belt that followed the dicing machine on the processing line (Table 2). It was also frequently detected in diced products. The organism was not found in any part of the processing line preceding the dicer or anywhere else in the plant. The dicing machine was sold to plant C at the beginning of March 1999, and *L. monocytogenes* PFGE type I was recovered from this plant for the first time on 30 March 1999. Following this finding, several positive samples were encountered in 1999 and 2000 (Table 2). *L. monocytogenes* PFGE type I contamination persisted in the dicing line and was detected in diced products and in different parts of the dicing machine, including the cutting blades, the exterior surface, the control panel, and the tool kit used for disassembling the machine. However, *L. monocytogenes* PFGE type I was not found in the processing areas preceding the dicer, including the post-heat-treatment area.

The dicing line in plant C was subjected to improved cleaning and disinfection procedures when it became ob-

TABLE 3. *Listeria monocytogenes* PFGE types found in the dicing line in plant C

PFGE type	Time of contamination (mo/yr)	Contamination site	No. of positive samples/total no. of samples
I	3/99–6/00	Exterior surface of dicer Cutting blades Control panel Tool kit Cooked diced pork product	2/422 2/16 1/16 2/18 13/1,600
II	6/99, 10/99	Exterior surface of dicer Cooked diced pork product	1/422 2/1,600
III	4/99	Exterior surface of dicer	1/422
IV	4/99, 9/99	Exterior surface of dicer	2/422

vious that conventional treatments led to unsatisfactory results. These conventional treatments included the use of cleaning and disinfecting agents and dismantling of the dicing machine once a week, whereas the improved treatments included daily and thorough dismantling of the machine, alkali-acid-alkali washes every second week, and the heating of small parts. The *L. monocytogenes* PFGE type I contamination was eradicated following the implementation of the improved treatments and was not found over a 6-month follow-up period.

The dicing line in plant C was also found to be contaminated with three other *L. monocytogenes* PFGE types (types II, III, and IV), but to a lesser extent and over a shorter period than the contamination with the persistent PFGE type I (Table 3).

L. monocytogenes PFGE types showed differing levels of adherence to stainless steel after a 2-h contact time (Table 4). The adherence level of the persistent *L. monocytogenes* PFGE type I was significantly higher ($P < 0.05$, Students *t* test) than that of the nonpersistent PFGE types

TABLE 4. Persistence, adherence to stainless steel surfaces, and minimal inhibitory concentrations (MICs) of disinfectants for *Listeria monocytogenes* strains recovered from the dicing line

PFGE type	Serotype	Persistent (P) or nonpersistent (N)	Adherence (cells/cm ²) ^a	MIC (μg/ml) of:	
				Sodium hypochlorite ^b	QAC ^c
I	1/2c	P	1,102 ± 158 A	2,500	5
II	1/2c	N	780 ± 106 B	2,500	5
III	1/2a	N	882 ± 116 B	2,500	1.25
IV	1/2a	N	730 ± 166 B	2,500	5

^a Mean ± standard deviation of six replicates. Values with the same letter are not significantly different according to the paired *t* test ($P < 0.05$).

^b The results represent the MICs of sodium hypochlorite. The active chlorine concentration was 10%.

^c QAC, quaternary ammonium compound (alkyl-benzyl-dimethylammonium chloride).

II, III, and IV. The MICs of sodium hypochlorite were similar for all *L. monocytogenes* strains, but the MICs of the QAC differed between strains (Table 4). *L. monocytogenes* PFGE types I, II, and IV were resistant to the QAC, and *L. monocytogenes* PFGE type III was sensitive to it.

DISCUSSION

L. monocytogenes PFGE type I was found in the dicing line of plant A in 1997, but after the removal of the dicing machine, the organism was no longer found in the postprocessing area. However, soon after the transfer of the dicing machine to plant B and then to plant C, *L. monocytogenes* PFGE type I was recovered from the dicing machine and the dicing line in both plants. All *L. monocytogenes* PFGE type I isolates were found in an area with high hygienic standards, with most isolates being found on the dicing machine and the conveyor belt transporting diced products to the packaging facilities. *L. monocytogenes* PFGE type I was not found in processing areas preceding the dicer or in any other parts of the processing plants. These observations indicate that the *L. monocytogenes* PFGE type I contamination was due to the dicing line, and especially the dicing machine. These observations further suggest that the contamination was transferred from one plant to the next by the dicing machine.

The persistent *L. monocytogenes* PFGE type in the dicing line of plant C was type I, but the dicing line was also found to be contaminated with three other *L. monocytogenes* PFGE types. These PFGE types did not cause persistent contamination of the dicing line as PFGE type I did. The reasons for persistent contamination are unclear. Continuous contamination of processing lines by incoming raw material does not seem probable. Rørvik et al. (21), Autio et al. (5), and Miettinen et al. (18) did not find persistent *L. monocytogenes* PFGE types in raw materials. Autio et al. (5) observed that *L. monocytogenes* contamination was associated with processing machines, and Miettinen et al. (18) showed that the packaging machine sustained the contamination. It seems that the persistence of a strain in a compartmentalized food-processing line is more probably caused by the properties of the *L. monocytogenes* strain (1, 14, 17) and the prevailing conditions in the food-processing facilities than by contaminated raw materials.

The *L. monocytogenes* PFGE types recovered from the dicing lines showed differing levels of adherence to stainless steel surfaces after short contact times. The persistent *L. monocytogenes* PFGE type I showed an adherence level that was significantly higher ($P < 0.05$) than those of PFGE types II, III, and IV after a 2-h contact time. Large numbers of adhering cells promote the survival of bacteria in food-processing plants, as adherence to food contact surfaces increases resistance to stresses (9, 20). Strong adhering abilities alone or in combination with other factors, such as low susceptibility to disinfectants, may contribute to persistent plant contamination.

The MICs of sodium hypochlorite were similar for all *L. monocytogenes* strains, but the MICs of the QAC differed between strains. The MIC of the QAC for *L. monocytogenes* PFGE types I, II, and IV was 5 µg/ml, which is

considered high by Aase et al. (1). Aase et al. observed that the MIC of benzalkonium chloride, a QAC, for *L. monocytogenes* strains ranged from <2 to 7 µg/ml, and strains for which MICs ranged from 4 to 7 µg/ml were considered resistant. A high MIC of the QAC and efficient adherence to stainless steel are properties that promote the survival of *L. monocytogenes* PFGE type I in food-processing plants. A high MIC may result from adaptation to QACs, since these compounds were widely used in the dicing lines. Also, the MICs for the resistant PFGE types may have been initially high when these types entered the plants. PFGE type III was susceptible to the QAC, which may be a result of a lack of sublethal exposure to QACs or an inability to adapt to QACs.

Despite the cleaning and disinfection of the dicing machine after partial dismantling, all spots could not be properly reached by mechanical cleaning. Such hard-to-reach places may potentially harbor *L. monocytogenes*. Organic matter should be removed by mechanical cleaning, since it reduces the efficiency of disinfectants such as QACs and especially chlorine (10). In addition, organic matter may prevent disinfectants from reaching bacteria at the recommended concentrations (11), leading to adaptive responses (1). Adaptive responses may also occur in response to heat and acid stresses (12, 13), factors that are frequently involved in cleaning and disinfection treatments.

Dicers and other food-processing machines that manipulate cooked meat products provide sites at which *L. monocytogenes* may become established. Once *L. monocytogenes* is established, it may be very difficult to eradicate, since adherence and adaptive responses may have occurred. The processing line may recurrently become contaminated, as *L. monocytogenes* organisms may be driven out of the spots that harbor them when the machine is running. However, it is possible to eradicate persistent contamination from a compartmentalized processing line provided that the complex machines are regularly dismantled and that the mechanical cleaning procedure and the disinfectant reach the site of contamination. After the dicing machine in plant C was subjected to improved cleaning and disinfection procedures, including daily dismantling, alkali-acid-alkali washes, and heating of small parts, PFGE type I contamination was eradicated.

In conclusion, processing lines containing complex machines with poor hygienic properties, such as dicers, slicers, and packaging machines, are susceptible to persistent *L. monocytogenes* contamination. Relocation of processing machines from one plant to another may transfer persistent *L. monocytogenes* contamination. Appropriate measures to prevent the contamination of new production sites must be taken when processing machines are relocated.

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