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Molecular epidemiology and disinfectant susceptibility of *Listeria monocytogenes* from meat processing plants and human infections

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Abstract

We have investigated the molecular epidemiology of *Listeria monocytogenes* from the meat processing industry producing cold cuts and from cases of human listeriosis by discriminative pulsed-field gel electrophoresis (PFGE). A subset of the isolates was also investigated for susceptibility to a disinfectant based on quaternary ammonium compounds (QAC) frequently used in the meat processing industry. The purpose of this investigation was to obtain knowledge of sources, routes of contamination and genetic types of *L. monocytogenes* present along the production line in the meat processing industry, and to compare meat industry isolates and human isolates. Of the 222 isolates from four meat-processing plants, 200 were from two plants responsible for nearly 50% of the production of cold cuts in the Norwegian market. The strain collection included historical routinely sampled isolates (1989–2002) and isolates systematically sampled through a one year period (November 2001 to November 2002) from fresh meat and production environments in three plants. No isolates were obtained in samples from employees (throat, faeces). Human strains included all available reported isolates from Norwegian patients in selected time periods. The *L. monocytogenes* PFGE data showed a large genetic heterogeneity, with isolates separated into two genetic lineages and further subdivided into 56 different PFGE profiles. Certain profiles were observed on both sides of production (before and after heat treatment) indicating contamination of end products by fresh meat or fresh meat environments. While fresh meat isolates almost exclusively grouped within lineage I, isolates from end products showed a more balanced distribution between lineages I and II. Ten profiles were common among isolates from human and meat industry. Typing of human isolates identified a previously unrecognised outbreak. Generally, a higher QAC resistance incidence was observed among isolates from the meat processing industry than among human isolates although large plant to plant differences were indicated. No correlation between resistance and PFGE profile or resistance and persistence was observed.

Keywords: *Listeria monocytogenes*; PFGE; Molecular epidemiology; Outbreak meat; Resistance; Quaternary ammonium compounds; Disinfectants

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1. Introduction

Human *Listeria monocytogenes* infections are mainly of food-borne origin and occur as both sporadic incidents and epidemic outbreaks (Farber and Peterkin, 1991). Documented food item sources for human disease have included cheese, vegetable, fish and meat products (Rocourt and Bille, 1997). The incidence of human listeriosis in Norway is at a low and fairly stable level. Between 8 and 21 yearly reported cases have been reported the last 10 years. Sporadic cases predominate, although one outbreak with precooked sliced meat as the source has been reported (Lassen and Caugant, 1992). *L. monocytogenes* grows at refrigeration temperatures and has the ability to form a persistent ''in-house flora'' in food processing industry plants with possible contamination of end products. Together with a growing demand for ready-to-eat products with extended shelf life, this poses serious challenges to the food industry and is of great concern. In the meat industry, *L. monocytogenes* is therefore regarded as the most troublesome microorganism to be controlled during processing.

Application of discriminative, molecular typing techniques is important in epidemiological investigations and to detect outbreaks and verify epidemiological associations. Strain characterisation is also essential when contamination routes and tracing of bacteria in food processing plants are investigated. Phenotypic methods often yield a low power of discrimination in strains (e.g., serotyping), suffer from biological variability (e.g., phage typing) and may not be applicable to all strains. Numerous molecular methods including multilocus enzyme electrophoresis (MEE) (Bibb et al., 1990), random amplification of polymorphic DNA (RAPD) (Martinez et al., 2003), ribotyping (Wiedmann et al., 1996), amplified fragment length polymorphism (AFLP) (Aarts et al., 1999)), multilocus sequence typing (MLST) (Salcedo et al., 2003) and pulsed-field gel electrophoresis (PFGE) (Louie et al., 1996) have been used in typing and molecular epidemiological studies of *L. monocytogenes*. PFGE is often considered the standard subtyping method for *L. monocytogenes* (Graves and Swaminathan, 2001). In this study, PFGE was applied due to the documented reproducibility and discriminating power of PFGE together with successful application of this technique in previous epidemiological characterisation of *L. monocytogenes* (Loncarevic et al., 1997; Autio et al., 1999; Miettinen et al., 1999; Senczek et al., 2000).

Previous studies have shown that many *L. monocytogenes* isolates from heat-treated meat products seem to have other origins than fresh meat (Boerlin and Piffaretti, 1991; Nesbakken et al., 1996). In this perspective, three meat processing plants were visited several times within 1 year to collect and analyse samples for *L. monocytogenes* from both deboned fresh meat, production environments, operators and technical staff. We used PFGE to compare isolates from this project to historical isolates from two of these plants, isolates from cold cuts, sporadic human cases and patients originating from the only recognised outbreak from meat in Norway. In addition, we investigated the possible correlation between ability of certain strains to persist in the environment and resistance to a widely used disinfectant based on quaternary ammonium compounds (QAC).

2. Materials and methods

2.1. Collection of samples in three meat processing plants, 2001–2002

During four visits in each of two meat processing plants (plant 1 (visited October/November, February, June and August); plant 3 (visited October, February, May and July)) and two visits (September/October and February) in one plant (plant 4; closed down during the project period), a total of 319 samples from production environment and 21 samples from deboned fresh meat were collected (summarised in Table 1).

While plant 1 carried out slaughtering, deboning and meat processing including cold cuts, plant 3 was only producing cold cuts. Plant 4 was performing meat processing including cold cuts. All three plants were running according to Good Manufacturing Practices. The production of cold cuts was performed in specific rooms and areas that were allocated for cooked products. A Hazard Analysis Critical Control Point (HACCP) system was partly, but not fully implemented. The specific Critical Control Point (CCP) in connection with heat treatment in the production of cold cuts was taken care of.

A total of 70 swab samples from throats and 45 swab samples from faeces were collected from

Table 1
Isolation of *L. monocytogenes* from various samples through different time periods in three meat processing plants

Plant and period of isolation	Samples (total no./*L. monocytogenes* positive)[a]				
	Fresh meat side[b]	End product side		Employees	
		Environment[c]	End product[d]	Throat	Faeces
Plant 1					
1989–1993	nd/11	nd/5	nd/14	ND	ND
1998–2002[e]	nd/38	nd/16	nd/15	ND	ND
2001–2002[f]	8/4	127/13	ND	27/0	7/0
Plant 3					
1989–1993	nd/10	nd/7	nd/17	ND	ND
1998–2002[e]	nd/0	nd/4	nd/24	ND	ND
2001–2002[f]	11/0	143/22	ND	38/0	38/0
Plant 4					
1989–1993	nd/0	nd/0	nd/0	ND	ND
1998–2002[e]	nd/0	nd/0	nd/6	ND	ND
2001–2002[f]	2/1	49/12	ND	5/0	ND

[a] nd: no data available; ND: not done.
[b] Isolates from fresh meat and environments of fresh meat side included.
[c] Isolates from sites in direct contact with products (conveyor belts, tables, slicers, gloves, etc.) and in indirect contact with products (switch boards, cloths, floors, etc.).
[d] Wastes from slices and cold cuts.
[e] Isolates obtained after daily routine testing in the specified plants.
[f] Isolates from samples obtained quarterly through a one year period in 2001 and 2002.

employees (operators and technical personnel) during the first visit in the three plants (autumn 2001; Table 1) using PROBACT transport swabs (Technical Service Consultants, Schofield St., Heywood, UK).

2.2. Isolates from routine analysis of meat processing plants and human isolates

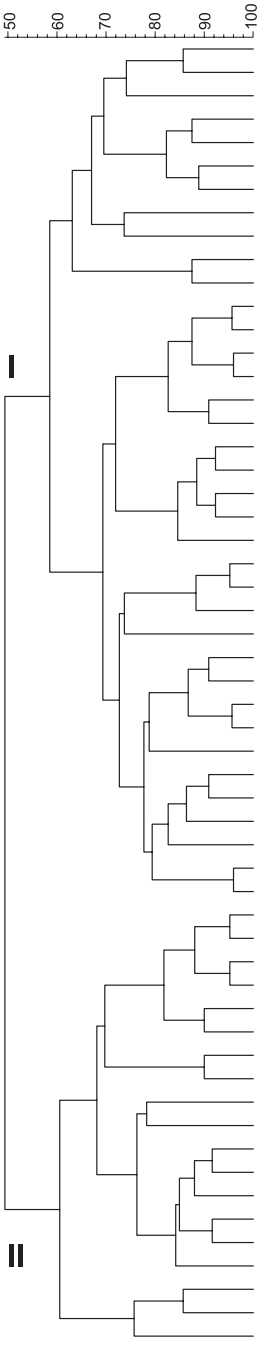
A total of 167 *L. monocytogenes* isolates obtained mainly by daily routine testing of plant 1 ($n=99$; sample years 1989–1993, 1998–2002), plant 3 ($n=62$; sample years 1989, 1991 and 1999–2002) and plant 4 ($n=6$; sample years 2000–2001) were also included (Table 1). Additionally, we included three outbreak-associated isolates from the infected plant involved in the national outbreak in 1992. A list of all reported human cases of *L. monocytogenes* infection in 1992 and 2000–2002 was obtained from the National Notification System for Infectious Diseases (MSIS) in Norway. The corresponding bacteria of all available human cases ($n=42$) were obtained from the Norwegian Reference Laboratory for Enteropathogenic microbes at the Norwegian Institute of Public Health which verifies and collects *L. monocytogenes* isolates in Norway.

2.3. Listeria monocytogenes enrichment procedure for swabs and meat samples

Swabs and meat samples (25 g) collected in the 1-year sampling period 2001–2002 from three meat processing plants (plants 1, 3 and 4) were immersed in 1/2 Fraser broth (BioMérieux, Marcy l'Etoile, France) to enrich *L. monocytogenes*. *L. monocytogenes* was detected by screening using the Vidas LMO ELISA method (BioMérieux). Samples yielding a presumptive positive result with the Vidas LMO method were confirmed using Nordic Committee on Food Analysis No. 136 2nd. ed. (Nordic Committee on Food Analysis, 1999) with additional use of Agar Listeria according to Ottaviani and Agosti (ALOA agar) (AES Laboratoire, Combourg, France) to the selective-differential media used for colony isolation. Isolated colonies were confirmed by Accuprobe (Gen-Probe, San Diego, CA, USA), an assay specific for *L. monocytogenes* and by traditional biochemical assays. The remaining isolates were detected using different microbiological assays in different laboratories, reflecting the progress in food microbiology and isolation of *L. monocytogenes* since 1989. However, the use of one or two selective broths followed by selective plating are the basis for the methods used.

2.4. Plug preparation and pulsed-field gel electrophoresis

Genomic DNA from *L. monocytogenes* isolates was prepared in agarose plugs as described in Graves and Swaminathan (2001). DNA cleavage was achieved by restriction enzyme digestion of agarose-immobilised DNA using 25 U *Asc*I (New England Biolabs, Beverly, MA) for 3 h at 37 °C. Restricted samples were electrophoresed through 1% (wt/vol) agarose (SeaKem Gold) in 0.5 × Tris–borate–EDTA at 6 V/cm on a Chef DR III system (Bio-Rad, Hercules, CA, USA). A linear ramping factor with pulse times from 4.0 to 40.0 s at 14 and 120° were applied for 20 h. *Asc*I-digested plugs of *L. monocytogenes* H2446 (Graves and Swa-



Total no. of isolates	Plant 1	Plant 2	Plant 3	Plant 4	Human	Source of plant isolates[a]	PFGE profile
13	12		1			M, X, E, P	A1
1	1					M	A2
1					1	-	B1
1	1					M	C1
1					1	-	C2
3				3		E	C3
1	1					X	C4
3	2				1	E	D1
1	1					M	E1
1	1					X	F1
2	1				1	M	F2
1	1					X	G1
1	1					X	G2
3	1				2	M	G3
5	3		1		1	M, X	G4
16	16					E, P	G5
2	2					E	G6
35	1		30		4	M, E, P	H1
9	1		7		1	X, E, P	H2
15	5		9		1	M, X, E, P	H3
5	3		2			E, P	H4
2	2					M	H5
1					1	-	I1
1					1	-	I2
1	1					M	I3
1					1	-	J1
4	4					X, E, P	K1
10	10					M, X	K2
2				2		E	K3
12	7			5		M, X, E, P	K4
2	2					X	K5
4	1		3			M, X	L1
12	5		4	1	2	M, X, E, P	L2
6	2			4		E, P	L3
2				2		P	L4
1	1					M	L5
1			1			M	L6
1					1	-	M1
1					1	-	M2
27	3		23		1	M, X, E, P	M3
1					1	-	M4
1					1	-	M5
2					2	-	M6
10	10					E, P	N1
2					2	-	N2
5					5	-	O1
1					1	-	P1
8		2			6	P	Q1
1		1				P	Q2
9	9					P	Q3
4	4					E	Q4
1					1	-	Q5
1	1					P	Q6
1				1		P	R1
1					1	-	R2
1					1	-	S1

[a]M: fresh meat; X: environment fresh meat side; E: environment end product side; P: end product (wastes from slices and cold cuts)

minathan, 2001) were applied as molecular markers. After electrophoresis, PFGE gels were stained with ethidium bromide and photographed with GelDoc 2000 using the Quantity one software (Bio-Rad).

2.5. Computerised analyses of PFGE data

Images generated by Quantity one software on GelDoc 2000 were saved in TIFF format and transferred to the GelComparII software (Applied Maths, Sint-Martens-Latem, Belgium) for computer analyses. Similarity between fingerprints was determined by the Dice coefficient and using a band position tolerance of 1%. Dendrograms were generated by the unweighted pair group method with arithmetic averages (UPGMA). Capital letters (A–S) were used to designate the main clonal groups. Subclones differed by at least one band and were assigned by additional numerical suffixes.

2.6. Disinfection procedures and susceptibility to a quaternary ammonium compound

Cleaning and disinfection procedures were performed at the end of each production day. Disinfectants based on QAC were used on a daily basis in plants 1 and 3. Hypochloritt was used as an alternating disinfectant every second week or each week in plants 1 and 3, respectively. The minimal inhibitory concentration (MIC) of the QAC benzalkonium chloride (BC) was determined in a microtiter assay (Sundheim et al., 1992). From the meat processing industry, isolate representatives of the most prevalent occurring PFGE profiles, as well as random isolates representing other profiles, were included. Isolates from all four plants (1, 2, 3, 4) were investigated in addition to 28 randomly selected human isolates. The BC was provided by Norsk medisinaldepot (Oslo, Norway). A stock solution of 5 mg/g in H_2O was prepared by 10-fold dilutions and stored at 4 °C. Further dilutions were made fresh before each experiment. The lowest concentration of BC in tryptic soy broth preventing growth after 20 h at 30 °C was taken to be the MIC. The screening analyses were done with BC concentrations of 1 μg/ml intervals between 0 and 10 μg/ml. The MIC analyses were performed twice for isolates with MIC higher than 2 μg/ml.

3. Results

3.1. Screening and detection of L. monocytogenes from various sites in the meat industry

A total of 219 meat industry isolates were collected from three meat processing plants (plants 1, 3 and 4). In addition, three isolates were collected from a fourth meat processing plant (plant 2) associated with a human listeriosis outbreak in 1992 (Lassen and Caugant, 1992). The majority of isolates were collected in plant 1 ($n = 116$) and plant 3 ($n = 84$) which are responsible for nearly 50% of the production of cold cuts in the Norwegian market. Plant 4 (19 isolates) was a rather small producer of cold cuts in Southeast Norway. The plant went out of business 6 months into the project.

An summary of *L. monocytogenes* isolates obtained by four screenings performed quarterly through a 1-year period (autumn 2001–2002) from plants 1, 3 and 4 (only two screenings for plant 4) is presented in Table 1. *L. monocytogenes* data obtained by routine tests of the same plants in these and previous time periods (1989–2002) are also included in Table 1.

The quarterly screenings (2001–2002) showed *L. monocytogenes* to be frequently encountered in both production environment ($n = 47$, 15%) and fresh meat ($n = 5$, 24%). None of the 115 samples collected from throat ($n = 70$) or faeces ($n = 45$) of employees at these plants were positive for *L. monocytogenes* (Table 1).

3.2. Molecular typing by pulsed-field gel electrophoresis

Of the 222 isolates from the meat processing plants, 218 were available and subjected to molecular typing by PFGE. In addition, all available reported human isolates from the time periods 1992 and 2000–2002 ($n = 42$) were subjected to PFGE. Dendrogram analyses of the PFGE(*Asc*I) profiles showed that the 260 *L.*

Fig. 1. Dendrogram demonstrating the genetic homology among PFGE (*Asc*I) restriction profiles of *L. monocytogenes* isolated from four meat processing plants in the period 1989–2002 and from human listeriosis cases reported in 1992 and 2000–2002 in Norway. Number of isolates within each profile, isolation source of plant isolates and PFGE profile designations are indicated.

Table 2
Period of isolation, source and number of isolates (in parenthesis) within the various PFGE profiles among *L. monocytogenes* isolated from fresh meat side and end product side in plant 1 ($n = 104$) and plant 3 ($n = 80$)

Period of isolation (year/quarter)	Plant 1			Plant 3		
	Fresh meat side	End product side		Fresh meat side	End product side	
		Environment	End product		Environment	End product
1989/3	H5 (1)				M3 (4)	L2 (1), M3 (9)
1989/4	A1 (1)	A1 (1), Q4 (3)	A1 (2), Q3 (1)	G4 (1)	M3 (3)	M3 (7)
1990/1	L1 (1)					
1990/2			A1 (1)			
1990/3	H3 (2)					
1991/1			A1 (1)	L1 (3), L2 (2)		
1991/2			Q3 (1)			
1991/3	G4 (1), H3 (2)		Q3 (1)	A1 (1), H3 (1), L2 (1)		
1992/3			Q3 (1)			
1992/4			Q3 (1)			
1993/1			Q3 (1)			
1993/2			Q3 (1)			
1993/4		Q4 (1)	Q3 (2)			
1998/2	A1 (1), K4 (3), L2 (3)	K4 (1)				
1999/1	A1 (1), G4 (1), K2 (1)					
1999/3						H1 (4), H2 (1), H3 (1)
1999/4			N1 (2)			H1 (1), H2 (1)
2000/1					H1 (3), H4 (1)	H1 (1), H4 (1)
2000/3						H1 (2)
2001/1	A1 (2), G4 (1), H2 (1), H5 (1), K1 (1), K2 (6), K5 (2), M3 (2)		L3 (1)			H1(2)
2001/2	A1 (2), K1 (1), K2 (3)					
2001/3						H3 (1)
2001/4		G5 (1), K1 (1), N1 (2)	L2 (1)		H1 (4), H2 (1)	H1 (3), H2 (1), H3 (3)
2002/1		D1 (1), G5 (4), N1 (5)	G5 (1), K1 (1), K4 (1), N1 (1)	H3 (1)	H1 (3)	H1 (1)

Table 2 (*continued*)

Period of isolation (year/quarter)	Plant 1			Plant 3		
	Fresh meat side	End product side		Fresh meat side	End product side	
		Environment	End product		Environment	End product
2002/2	M3 (1)	D1 (1), G5 (3), G6 (1)	G5 (5)		H1 (5)	
2002/3	H1 (1)	G5 (1), G6 (1), H3 (1), H4 (3), K4 (2), L2 (1)	G5 (1), L3 (1)		H1 (1), H2 (2), H3 (2)	
2002/4						H2 (1)

Single isolate PFGE profiles in plants 1 and 3 were excluded.

monocytogenes isolates formed 56 different PFGE profiles that were separated into two main lineages showing about 50% genetic homology (Fig. 1). The two lineages could be further divided into 12 different subclusters (78% similarity level) with two to six PFGE profiles within each subcluster. Seven PFGE profiles did not cluster with other profiles.

3.2.1. Isolates from meat processing plants

The 218 *L. monocytogenes* isolates from the meat processing industry grouped into 40 unique PFGE profiles of which 11 were observed in two or more of the investigated plants. A high genetic diversity was observed among plant 1 isolates ($n = 116$) with 33 unique profiles dispersed throughout the dendrogram. Fifteen of the profiles contained a single plant 1 isolate, while the remaining 18 profiles contained between two and 16 isolates. Seven clusters (A, G, H, K, L, N, Q) represented 91% ($n = 105$) of the plant 1 isolates (Fig. 1).

Less heterogeneity was observed among isolates from plant 3 ($n = 81$). Ten unique profiles were identified, of which three contained only a single strain (Fig. 1). The seven remaining profiles contained between two and 30 isolates and were grouped into three clusters (H, L, M). These three accounted for 98% ($n = 79$) of the plant 3 isolates. All except one of the plant 3 profiles were also observed in isolates from plant 1. The 18 PFGE-typed isolates from plant 4 belonged to seven profiles, five of which contained two to five isolates. In processing plant 2, three isolates with two similar profiles (one band difference) were observed.

In plant 1, we observed repeated isolation of identical PFGE profiles during certain time periods (Table 2). Profile A1 was detected in environments of both fresh meat side and product side and in end product in November 1989. Profiles A1, H3, K1, K4, L2 and N1 were also repeatedly detected in various time periods at both the fresh meat side and the end product side. Recurring isolation of PFGE profile G5 from various sources including meat processing surfaces, floors and slicer wastes was evident in the period November 2001 to November 2002. In plant 1, PFGE profile Q3 was detected from sliced meat and slicer wastes (heat-treated products) during the period November 1989 to November 1993.

In plant 3, 23 of 24 isolates sampled in 1989 from end product environments, slicer wastes and cold cuts (after heat treatment) had a single profile (M3; Table 2). In the period August 1999 to July 2002, four other profiles were observed (H1, H2, H3, H4) from the end product side repeatedly collected from various sources (contact surfaces, floors, slicer wastes). Profile H3 was also detected on the fresh meat side in this time period (Table 2). Genetic analyses showed these profiles to be tightly clustered (Fig. 1) and could thus be regarded as variants of the same clone.

The 32 PFGE-typed isolates from fresh meat grouped into 18 PFGE profiles. The profiles observed among isolates from fresh meat and production side (before heat treatment) were seldom identified on the end product side the same years (Table 2). Three exceptions were observed: Profiles A1 and K4 was detected at both the fresh meat side and the end

product side1989 and 1998, respectively. The K1 profile was identified in production environments both before and after heat treatment in 2001.

Among profiles detected more than once in plants 1 and 3, certain profiles were exclusively detected on either the fresh meat side (G4, H5, K2, K5, L1) or the end product side (D1, G5, G6, H4, L3, N1, Q3, Q4 ; Table 2). The dendrogram showed that the majority of fresh meat isolates (31/32) and isolates from the processing environment of plants 1 and 3 (25/27) grouped within clusters of lineage I (Fig. 1). Among the 133 isolates from the finished side (products, environments) of the same plants, 86 and 47 isolates grouped within lineages I and II, respectively (Fig. 1).

3.2.2. Isolates from human cases

The 42 human isolates were separated into 26 PFGE profiles (Fig. 1). Twenty-four isolates of 13 profiles clustered in lineage I while 18 isolates belonged to 13 profiles of lineage II. Ten of the profiles, associated with 20 human cases, were also present among meat processing industry isolates. Only seven profiles contained more than one human isolate. Interestingly, an identical PFGE profile (O1) was observed among five human isolates. Further analyses revealed three of these to be isolated within a 2-week period from patients from a single county. The observed high genetic diversity among human *L. monocytogenes* isolates and the available epidemiological data suggest these cases to be connected. No link to any source was evident. PFGE profile O1 was not detected in isolates from the meat processing industry. Among the 11 available human isolates from 1992, five isolates had the Q1 PFGE profile identical to the profile associated with the 1992 outbreak, traced to contaminated vacuum packed cold cuts produced in plant 2 (Lassen and Caugant, 1992). Two other human isolates from 1992 shared another identical PFGE profile (M6) and were from cases in the same city indicating a possible epidemiological link.

3.3. Disinfectant resistance

A subset of isolates from meat processing industry ($n=84$) and human cases ($n=28$) were selected for determination of the minimum inhibitory concentration (MIC) to BC, a disinfectant based on QAC. Seventeen isolates were resistant to BC having MIC values between 4 and 8 compared to MIC values between 2 and 3 μg/ml for sensitive strains. The resistant isolates belonged to 10 isolates from plant 1, five isolates from plant 4 and 2 human isolates. The results are summarised in Table 3.

Table 3
Susceptibility to the disinfectant benzalkonium chloride for a collection of *L. monocytogenes* isolates from food processing plants and human cases and PFGE profiles of resistant isolates

Source	No. tested	Sensitive	Resistant	PFGE profile of resistant isolates (*n*)
Plant 1	37	27	10	A1(2), K4(3), L2(3), Q4(2)
Plant 2	3	3	0	–
Plant 3	36	36	0	–
Plant 4	8	3	5	C3(3), K4(2)
Human	28	26	2	M4(1), O1(1)

The resistant strains separated into seven PFGE profiles. Isolates within five (A1, C3, K4, L2, Q4) of 20 investigated PFGE profiles from the meat industry contained resistant isolates compared to only two (M4, O1) of 15 investigated human clones. The PFGE profile was not a reliable marker for resistance as certain profiles included both BC-sensitive and resistant isolates. No correlation between persistence and resistance to BC was observed, as certain profiles of persistent strains were sensitive to BC.

4. Discussion

A better understanding of the molecular epidemiology of *L. monocytogenes* and factors involved in survival, spread and persistence of strains is needed to direct powerful preventive measures in the meat processing industry. This could help us in the reduction of possible human listeriosis cases caused by contaminated meat products. In this study, we used PFGE to investigate the molecular epidemiology of *L. monocytogenes* in the meat processing industry. All available strains from reported human listeriosis infections in selected time periods in Norway were also included to compare human and meat industry isolates. Several studies have shown PFGE to be a robust method for discriminative fingerprinting of *L. monocytogenes* (Brosch et al., 1994; Louie et al., 1996; Miettinen et al., 1999; Autio et al., 2002) . The restriction enzymes *Asc*I and *Apa*I are most common-

ly used, with *Asc*I reported to be more discriminatory than *Apa*I. Sauders et al. (2003) reported easier interpretation and thus suitability for electronic database handling for PFGE(*Asc*I) profiles compared to more complex PFGE(*Apa*I) restriction patterns. We therefore applied PFGE(*Asc*I) on the complete strain collection. A subcollection including 23 isolates of four PFGE(*Asc*I) profiles (A1, H1, K1, N1; Fig. 1) was also fingerprinted by PFGE(*Apa*I). The PFGE(*Asc*I) and PFGE(*Apa*I) data were in full concordance except that two of six analysed strains of the PFGE(*Asc*I) A1 profile had a variant *Apa*I profile due to an apparent single fragment mobility shift (data not shown).

L. monocytogenes isolates in the plants may originate from fresh meat, employees or the external environment of the plant. A substantial genetic heterogeneity was observed among both meat industry isolates and human strains. Although differences between plants were observed, a number of profiles were detected on both the fresh meat side (before heat treatment) and the end product side (after heat treatment). Detection of isolates with identical PFGE profiles (A1, H3, K1, K4, L2) from both sides of production (before and after heat treatment) indicates possible contamination of environments and products on the finished side with isolates from meat or processing environments of the fresh meat side to occur. Isolate representative of the A1, H3 and K4 profiles were detected in fresh meat as well as in the production environment (before heat treatment) and at the end product side. In one occasion (plant 1), isolates with an identical profile (K4) were isolated in the same month from fresh meat and both production sides (before and after heat treatment). In other cases, isolates with identical PFGE profiles from different sides of production were collected in different years and time periods. Distinct profiles were recurrently observed from the end product side during certain time periods in both plant 1 (e.g., profiles G5, K1, N1, Q3) and plant 3 (e.g., profiles H1, H2). This indicated the ability of certain clones to persist in the environment and to contaminate products and product environments. Numerical analyses of the PFGE data identified two genetic lineages (I and II; Fig. 1). In plants 1 and 3, a genetically diverse collection was observed among isolates from the end product side where isolates belonged to both lineages (I: 65%; II 35%), while lineage I predominated (95%) among isolates from fresh meat. A previous study based on MEE also indicated that the overall distribution of isolates belonging to separate genetic lineages was different in fresh meat isolates and end-product isolates (Nesbakken et al., 1996). This suggests additional factors than fresh meat to be responsible for contamination at the end product side. However, though GMP was focused, and HACCP was partly implemented, cross contamination from raw products and by personnel due to inadequate routines was possible. No positive samples detected from throat and faeces of employees indicated employee carriage as a minor factor in colonisation and spread of *L. monocytogenes* in the plants. To investigate the sources of the contaminating isolates in more detail, a larger strain collection is needed.

Differences between plants were observed in recurrent PFGE profiles although recurrent profiles of plant 1 occurred sporadically in plant 3 and vice versa. A recent report (Autio et al., 2002) indicated that persistent house-flora strains are not always plant-specific. The study also showed strains with identical PFGE profiles to be recovered in products from several food manufacturers from different years and countries indicating *L. monocytogenes* strains to be geographical and temporally unspecific.

Ten of the 26 PFGE profiles from human isolates were also observed among plant isolates. The human isolate profiles were dispersed throughout the dendrogram. A recent study has indicated isolates of certain PFGE profiles commonly found in food to be less virulent than certain human pathogenic clones (Larsen et al., 2002). Neither this nor previous studies (Nørrung and Andersen, 2000) could indicate the human pathogenic potential of *L. monocytogenes* isolates based on PFGE typing. PFGE analyses of the human strain collection identified a probable and previously unrecognised outbreak. This supports the use of discriminative molecular typing techniques in surveillance of *L. monocytogenes* infections. Identical PFGE profiles may also occur among epidemiologically unlinked isolates from various sources and time periods (Autio et al., 2002). Thus, molecular typing databases are efficient tools for listeriosis surveillance and outbreak investigations (Graves and Swaminathan, 2001), but combinations of fingerprinting and analytical epidemiological data are crucial to avoid erroneous conclusions. This is especially important

when searching for an association between suspected sources (e.g., food items) and human cases.

Although the sources of the contaminating isolates and reasons for the persistence of strains are unclear, improper hygienic barriers at various levels are a plausible cause that may promote spread and contamination of products and end product environments by diverse strains of *L. monocytogenes*. The observed difference in genetic types among the plants may be a result of hygienic strategies and variable ability of the plants to prevent *L. monocytogenes* contamination (cleaning and disinfection strategies, presence and design of hygienic barriers etc.). A range of physical and/or chemical parameters (e.g., presence of dust/organic residues, air humidity, condition and type of working surfaces, temperature, exposure to disinfectants) are involved in modulating the properties of *L. monocytogenes* in the food industry. Such properties influence the ability of strains to survive, grow and colonise plant environments, and may include ability of adherence, biofilm generation and resistance to disinfectants regularly used in the food industry (Lemaitre et al., 1998; Mereghetti et al., 2000; Aase et al., 2000; Chasseignaux et al., 2001). In this study, minimal inhibitory concentrations were determined towards a disinfectant based on QACs because QAC-based disinfectants were used on a daily basis in plants 1 and 3 where the majority of isolates were collected.

The results indicate an overall higher prevalence of resistant clones in the food industry compared to isolates from human cases. Previous findings have also indicated resistance to QAC-based disinfectants to be more prevalent among food-borne isolates than isolates from other sources (Aase et al., 2000). Of note, 13 of the 15 BC resistant plant isolates were collected in the second time period (1998–2002). Another study (Romanova et al., 2002) indicated a general increase in sanitizer resistance in recent isolates. With the current lack of data between applied disinfection strategies and bacterial resistance development, this and previous studies could only indicate that frequent and widespread use of QAC-based disinfectants in the food industry is leading to a general increase in disinfectant resistance among *Listeria*. Interestingly, this study also indicated large plant to plant differences in the prevalence of strains resistant to BC (Table 3). The observed differences cannot be explained by the type of disinfectant used as both plant 1 (10 resistant isolates among 37 isolates tested, Table 3) and plant 3 (no resistance observed among 36 isolates tested, Table 3) use QAC-based disinfectants on a daily basis. However, the general routines and strategies for wash and disinfection may vary between plants and create variable selection pressures that could be a factor in resistance development. Of interest, *L. monocytogenes* obtain stable and increased resistance to QAC-based as well as other disinfectants after laboratory adaptation to sublethal concentrations of QAC-based disinfectants (Lunden et al., 2003). Resistance to QAC appears not to be an independent factor contributing to persistence of certain strains in the meat processing environments (Earnshaw and Lawrence, 1998), but disinfectant resistance may be one among many elements contributing to persistence. Aase et al. (2000) speculated that increased resistance could have a synergistic effect with other resistance mechanisms such as biofilm-related resistance. This is currently being investigated. In this context, the ability of persistent strains to adhere more efficiently to stainless-steel surface after short contact time compared with non-persistent strains is of special interest as this could have a possible effect on initiation of persistent plant contamination (Lunden et al., 2000). The possible synergistic effects between different factors for persistence properties should be investigated. Additional information also needs to be available on procedures for effective wash and disinfection in the food industry, including choice of disinfectants and rotation frequency to avoid adaptation and build-up of resistance.

This study has shown that the molecular epidemiology of *L. monocytogenes* is complex. The high, although variable, genetic diversity observed among *L. monocytogenes* isolates from meat processing industry plants suggests a number of sources and factors to be involved in persistence and product contamination. Indicated plant-to-plant differences, both with regard to genetic diversity and disinfectant resistance properties is interesting and shows that a multi-factorial approach is needed to combat *L. monocytogenes* contamination in the food processing industry.

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