



CONTAMINATION OF COLD-SMOKED FISH WITH *LISTERIA MONOCYTOGENES*

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Summary

In the Netherlands, 41% of ready-to-eat cold-smoked fish is contaminated with the human pathogenic bacterium *Listeria monocytogenes*. Therefore sources of contamination in fish smoking plants were investigated. A survey in three plants shows that both *L. monocytogenes* as *L. spp* are easy to isolate from floors, machines, tables, and hands and aprons of employees. Total elimination of contamination will not be possible, so the focus will be on measures to control contamination like the use of risk assessment in the evaluation of product safety, improvement of cleaning and disinfection procedures, and monitoring of processing steps. To enable tracing of contamination routes, two methods of typing of *L. monocytogenes* are set up. A combination of limited serotyping and resistance to tetracycline, cadmium, and arsenic provides a set of 9 biotypes. Analysis of samples from different European countries shows large differences in the presence of the various biotypes. A more discriminatory typing method, AFLP, will be used further in attempt to identify contamination routes.

Introduction

L. monocytogenes, a human pathogen, is mainly found in contaminated food (1). Reported cases are associated with industrially processed food (2). Other suspected sources are cross-contamination in the distribution of foods (3) and the kitchen (4). A survey on Dutch fish and fish products, carried out in the past year by our institute, shows a high incidence of contamination with *L. monocytogenes* (Table 1). Fresh fish fillets are quite often contaminated with *L. monocytogenes*. However, when properly heated during cooking, this opposes no risk to the consumer. With the ready-to-eat smoked fish there is a higher risk of infection, in 41% of the cold-smoked fish samples *Listeria monocytogenes* was present.

This raises the need for control of contamination of *L. monocytogenes* of fish and fish products, especially those products which do not undergo any final listericidal treatment, like cold-smoked salmon. In order to find measures to control

contamination during manufacturing of cold-smoked salmon, a survey on the presence of *Listeria* species was carried out in fish smoking plants. Critical points of contamination and control were investigated and evaluated. The results of this study, including typing, will be presented in this paper.

Table 1. Incidence of *L. monocytogenes* in Dutch fish and fish products (1998-1999), obtained from supermarkets, fish markets. Isolation method according to ISO 11290-1: 1996 (5).

Product	Number of samples	Number and % of samples positive for	
Fresh fillets		<i>L. spp</i>	<i>L. monocytogenes</i>
Atlantic salmon	1	1(100)	1(100)
Plaice	3	2(67)	2(67)
Pollack	1	0(0)	0(0)
Rainbow trout	2	2(100)	2(100)
Redfish	1	1(100)	1(100)
Whiting	2	2(100)	2(100)
Total	10	8(80)	8(80)

Product	Number of samples	Number (%) of samples positive for	
Cold-smoked fillets		<i>L. spp</i>	<i>L. monocytogenes</i>
Atlantic salmon	54	31(57)	23(43)
Cod	4	3(75)	1(25)
Halibut	8	5(63)	4(50)
Herring	6	2(33)	1(17)
Marlin	1	1(100)	1(100)
Rainbow trout	2	2(100)	1(50)
Trout	3	0(0)	0(0)
Tuna	4	4(100)	3(75)
Total	82	48(59)	34(41)

Product	Number of samples	Number (%) of samples positive for	
Other		<i>L. spp</i>	<i>L. monocytogenes</i>
Salmon cubes, smoked	1	0(0)	0(0)
Salmon cuts, smoked	1	1(100)	0(100)
Sprat, smoked	5	2(40)	1(20)
Shrimps, fresh	1	0(0)	0(0)
Total	8	2(25)	1(13)

Materials and Methods

Sampling method and microbiological analysis

Swab samples (5 sterile swabs containing 3 ml peptone saline solution) taken from surfaces (10 cm²) and product samples (25 g) were incubated in 225 ml Half Fraser broth

for 1 day at 30°C as a pre-enrichment and further analysed according to ISO 11290-1 (5). This means enrichment in Fraser broth for 2 days at 37°C, isolation on PALCAM and Oxford media, confirmation of typical colonies with Gram staining, catalase, oxidase, glucose and xylose fermentation, and β -haemolysis on sheep-blood agar. Identification of *L. spp* isolates was carried out by using API-ListeriaTM (6), enabling the distinction between species (Table 2). Differentiation on species level is useful, not only to identify the pathogenic *L. monocytogenes*, but also to use the other identified species for identifying contamination.

Table 2. *Listeria* species able to identify with API-ListeriaTM (6)

Species
<i>Listeria monocytogenes</i>
<i>Listeria innocua</i>
<i>Listeria ivanovii</i>
<i>Listeria grayi</i>
<i>Listeria seeligeri</i>
<i>Listeria welshimeri</i>

Typing

Further identification of *L. monocytogenes* isolates was carried out according to (7), using a typical set of reactions enabling to obtain 9 different biotypes based on a combination of serotyping and resistance / sensitivity tests to cadmium, arsenic, and tetracycline.

Table 3. Differentiation of *L. monocytogenes* in nine biotypes, based on a combination of serotyping and resistance / sensitivity to cadmium, arsenic, and tetracycline (7)

Biotype	Serogroup	Sensitivity to ⁽¹⁾		
		Tetracycline	Cadmium	Arsenic
1	1/2	s	s	s
2	1/2	s	r	s
3	1/2	s	s	r
4	1/2	s	r	r
5	4	r	r	s
6	4	s	r	s
7	4	s	s	r
8	4	s	s	s
9	4	s	r	r

1) s: sensitive, r: resistant

Since typing as mentioned above will not provide enough information to differentiate between isolated strains (7, 8), experiments have started using AFLP, a method based on DNA restriction analysis with a high discriminatory power (9).

Sampling strategy

Three different Dutch fish smoking plants have been visited. Samples were taken in the consecutive process areas, in the opposite direction starting with packaging area. Sampled sites are categorised as shown in Table 4, in order to compare results between the three fish smoking plants. In one plant the filleting and packaging took place in one room, but were separated from each other.

Table 4. Sampling sites, ordered by category in four process areas

Area	Category	Sampling sites
fresh fish area	floor	personnel entrance, product entry
	product	skins, flesh
	waste product	heads, skins, bones
	waste water	water from wash basin, brine
	work surface	tables
	employee	hands, aprons
	machines	deboner, brine injector, knives
smoking area	containers	trays
	floor	entrance to smoking cabinet
	product	salmon fillets
processing of smoked product	smoking cabinet	floor, racks
	floor	personnel entrance, product entry
	product	salmon fillets
	waste product	fish skins, smoke offcuts, pinbones
	work surface	tables
	employee	hands, aprons
	machines	cutting blades, conveyor belts
cutting and packaging area	containers	trays
	floor	personnel entrance, product entry
	product	salmon fillets, cuts
	waste product	offcuts
	work surface	tables, scales
	employee	hands, aprons
	machines	cutting blades, conveyor belts
	containers	trays

Results and discussion

Fish smoking plant survey

Results of the first series of surveys of three fish smoking plants are presented in Table 5. In all three fish smoking plants, species of *Listeria* were found and in plant I and III *L. monocytogenes* was found. In plant I *L. monocytogenes* was found only in the fresh fish area, whereas in plant III *L. monocytogenes* was found in all areas, except the smoking area. No *L. monocytogenes* was found in the final products of all three plants.

According to the sites of isolation in plant III, *L. monocytogenes* does not seem to inhabit any particular site. For example, *L. monocytogenes* was found in waste water

(deboner), but no *L. monocytogenes* has been identified in any product afterwards. Therefore no specific measures to eliminate *L. monocytogenes* can be put forward yet. The three plants are now improving their code of practice, cleaning and disinfection. At the end of this year, all plants will be sampled again.

Table 5. Contamination of fish processing plants with *Listeria* species and *Listeria monocytogenes* in particular

Area	Sample Category	Fish smoking plant		
		I	II	III
Fresh fish area	Floor	<i>L. monocytogenes</i> <i>L. seeligeri</i>	<i>L. innocua</i>	<i>L. monocytogenes</i>
	Product	-	-	-
	Waste product	n.t.	-	<i>L. welshimeri</i> , <i>L. innocua</i>
	Waste water	n.t.	-	<i>L. monocytogenes</i>
	Work surface	<i>L. monocytogenes</i>	-	-
	Employee	<i>L. monocytogenes</i>	-	-
	Machines	-	-	-
	Containers	<i>L. monocytogenes</i>	-	-
Smoking area	Floor	<i>L. monocytogenes</i> <i>L. seeligeri</i>	-	<i>L. grayi</i> , <i>L. innocua</i> -
	Product	-	<i>L. innocua</i>	-
	Smoking cabinet	-	-	-
Processing of smoked product ¹⁾	Floor	-	<i>L. seeligeri</i>	<i>L. innocua</i> , <i>L. welshimeri</i>
	Product	-	-	<i>L. monocytogenes</i> , <i>L. welshimeri</i>
	Waste product	-	<i>L. innocua</i>	<i>L. welshimeri</i> , <i>L. seeligeri</i>
	Work surface	-	<i>L. innocua</i>	-
	Employee	-	<i>L. innocua</i>	-
	Machines	-	<i>L. innocua</i>	-
	Containers	-	-	-
	Containers	-	-	-
Cutting and packaging area ¹⁾	Floor	<i>L. seeligeri</i>	<i>L. seeligeri</i>	<i>L. welshimeri</i> , <i>L. innocua</i> -
	Product	-	-	<i>L. welshimeri</i> , <i>L. seeligeri</i>
	Waste product	-	<i>L. innocua</i>	<i>L. seeligeri</i>
	Work surface	-	-	<i>L. monocytogenes</i>
	Employee	-	-	<i>L. welshimeri</i> , <i>M. L. monocytogenes</i>
	Machines	-	-	-
	Containers	-	-	-
	Containers	-	-	-
End product		-	-	-

1): In factory I Filleting and Packing was carried out in one room.

-: no *Listeria* spp isolated, n.t.: not tested

Typing

Twelve isolates of *L. monocytogenes* of cold-smoked salmon from the market survey show that 7 biotypes are present in the Netherlands, in comparison with Iceland (1 biotype out of 21 samples of cold-smoked fish), France (8 out of 150), and Denmark (2 out of 9). The large variation in biotypes and number of different biotypes between countries has yet not been explained.

Table 6. Differentiation of 12 isolates of *L. monocytogenes* from Dutch fish samples (see Table 3).

Biotype	Incidence
1	2
2	4
3	-
4	1
5	-
6	3
7	1
8	1
9	1
Total	12

AFLP fingerprinting

Six of the 12 isolates mentioned above are used for AFLP fingerprinting. Each isolate used is from a different biotype according to table 3. Fig. 1 shows that each different biotype can be discriminated from another by looking at the position and color of the bands (variation in concentration of molecules with the same number of base-pairs). This means that AFLP is at least as discriminating as the method of Duarte (7). Experiments are to be continued, analysing isolates from fish smoking plant surveys.

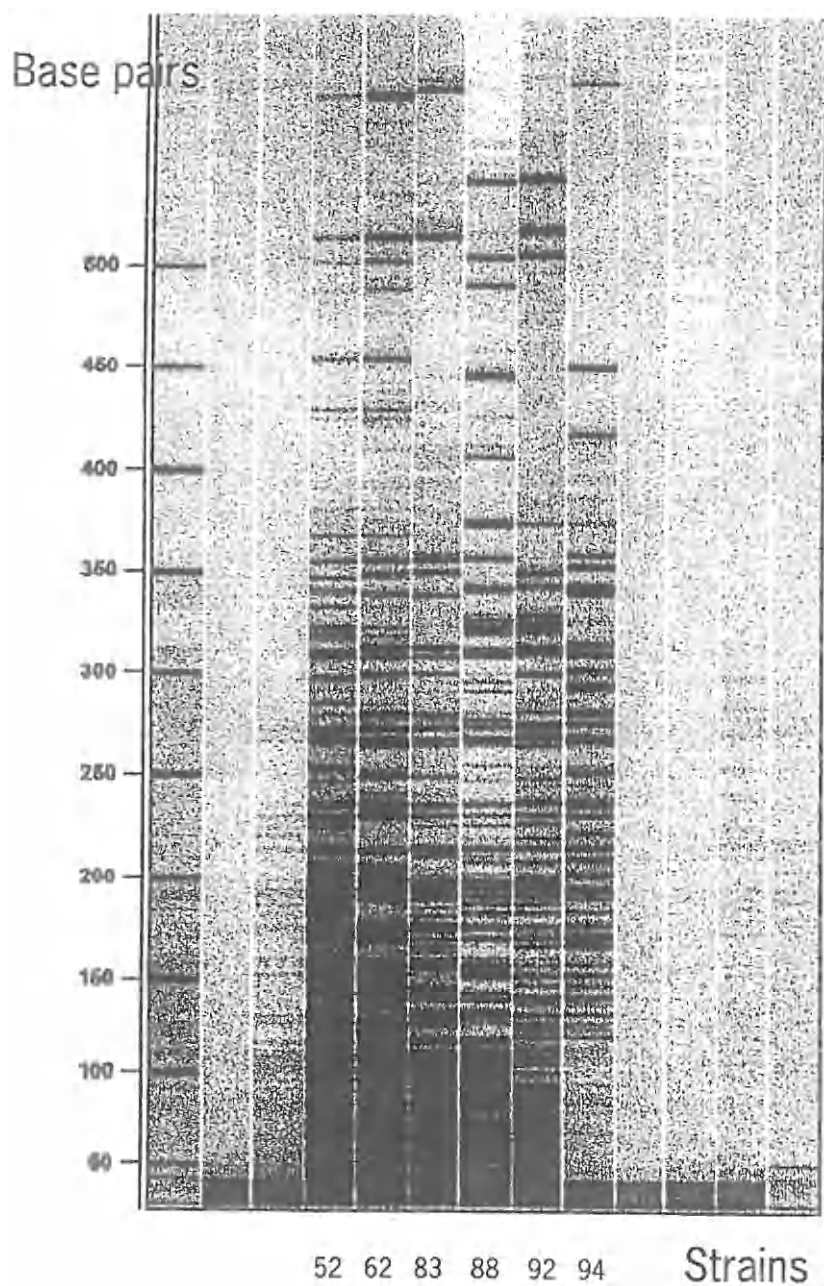


Fig. 1. Digital electrophoresis image of AFLP fragments of *Listeria monocytogenes* run on an ALF-Express automatic DNA sequencer. Strain numbers do not refer directly to any strain mentioned in this article.

Conclusions

The high incidence of Dutch fish products contaminated with *Listeria monocytogenes* puts a pressure on smoking plants to comply with the Dutch legislation: *Listeria monocytogenes* should be absent in 25 g of product. Emphasis should be put on means to control contamination: e.g. the use of risk assessment for the evaluation of process safety, monitoring of processing steps instead of relying on end-product testing, as also recently has been advised a.o. by the FAO (10).

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