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Short communication

Temperature distribution and prevalence of *Listeria* spp. in domestic, retail and industrial refrigerators in Greece

D. Sergelidis, A. Abrahim, A. Sarimvei, C. Panoulis, Pr. Karaioannoglou, C. Genigeorgis

Laboratory of Food Hygiene, Department of Food Hygiene and Technology, Veterinary School, Aristotelian University, 54006 Thessaloniki, Greece

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Abstract

The present paper examined the presence of *Listeria* spp. in the environment of domestic, retail and industrial refrigerators. From 136 household refrigerators, 136 surface samples were taken from the walls or shelves, and 125 from cheese compartments. Only two refrigerators harboured *L. monocytogenes*. From 228 food store refrigerators, 335 samples were taken. Of these, 118 were in in contact with cheeses, 69 with sausages, 21 with cheese and sausages, 20 with miscellaneous products and 107 from refrigerator handles. *Listeria* spp. and *L. monocytogenes* were found in 3.1% and 1.7%, of the samples respectively. *Listeria* spp. was not detected in any of the nine dairy plant refrigerators examined. *Listeria monocytogenes* and *L. innocua* were found in 4.5 and 36.4%, respectively, of the 22 refrigerators inside meat processing plants, with only one of 22 refrigerators handles being positive for *L. monocytogenes*. Temperature distribution in the refrigerators was also investigated. Fifty five per cent of the 136 domestic and 32% of the 228 retail store refrigerators had temperatures of greater than or equal to 9°C. The range of refrigeration temperatures of the industrial refrigerators was 0–2°C for meat plants and 2–7°C for dairy plants. No correlation of any kind could be established between the prevalence of *Listeria* spp. and the temperature of the various refrigerators due to the low number of positive samples.

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1. Introduction

A number of foodborne outbreaks of human listeriosis have been reported in recent years (Barker et al., 1989; Ryser and Marth, 1991; McLauchlin et al., 1991; Farber and Peterkin, 1991; McEachern and Styliadis, 1992; Schuchat et al., 1992; Rocourt, 1994; Salvat et al., 1995).

The presence, often in both high frequency and numbers, of *L. monocytogenes* in a variety of foods including fresh and processed meats, fresh and cooked poultry, ready-to-eat minimally processed seafoods, vegetables and in the environment of food processing establishments, is of a serious concern (Cox et al., 1989; Genigeorgis et al., 1989, 1990; Johnson et al., 1990; Ryser and Marth, 1991; Farber and Peterkin, 1991; Gilbert, 1991; Dillon and Patel, 1992; Grau and Vanderlinde, 1992; Embarek, 1994; Farber and Daley, 1994; Wang and Muriana, 1994; Rocourt, 1994; Salvat et al., 1995). This concern is amplified by the ability of the organism to grow at temperatures as low as -0.1 to -0.4°C, at a pH of greater than or equal to 4.4, at salt concentrations up to 12–13% and at an a_w of greater than or equal to 0.90 (Walker et al., 1990; Ryser and Marth, 1991; Razavilar, 1991; Miller, 1992). The microorganism can survive during manufacturing and ripening of soft cheeses (Ryser and Marth, 1991) and fermented sausages (Farber and Peterkin, 1991) and can grow at refrigeration temperatures, in contaminated minimally processed foods (Johnson et al., 1990; Farber and Peterkin, 1991, Ryser and Marth, 1991; Genigeorgis et al., 1991; Grau and Vanderlinde, 1992; Farber and Daley, 1994; Embarek, 1994; Rocourt, 1994; Salvat et al., 1995). Furthermore, it has the ability to attach to equipment and food contact surfaces and form biofilms which withstand cleaning and sanitizing (Krysinski et al., 1992; Zottola and Sasahara, 1994; Genigeorgis, 1995).

The objective of the present paper was to investigate the presence of *Listeria* spp. in the environment of domestic, retail and industrial refrigerators. Also, to determine the effectiveness of refrigerators in maintaining proper temperature, so as to minimise the risk of growth of *L. monocytogenes* and other psychrotrophic pathogens in contaminated ready-to-eat foods (Genigeorgis and Sofos, 1996).

2. Materials and methods

2.1. Sampling

Household, retail store, meat and dairy plant refrigerators were sampled. The sampled surfaces were selected with the possibility of *Listeria* spp contamination taken into account. These latter surfaces are in direct contact with raw and

processed foods and are frequently contaminated with *Listeria* spp. (Genigeorgis et al., 1991; Abrahim et al., 1992).

From each household refrigerator two samples were collected; one from locations where meat or vegetables were ordinarily stored and the other from the cheese case or tray. From each retail store refrigerator, depending on their capacity, usually more than one sample was collected. Swabbed areas corresponded to locations or trays that were in contact with dairy or meat products, which are sold or served after slicing or cutting into pieces (as cheeses and sausages), and thus are often touched by human hands. Samples were collected from retail store and industrial refrigerator handles, since they are touched by personnel both before and after handling foods. Before sampling, the temperature of each refrigerator was recorded by a portable electronic thermometer (Fluke, 51 K/J, Everett, Washington, USA).

2.2. *Listeria* detection methods

Surface samples were collected by swabbing an area of 100 cm^2, with a sterile cotton swab moistened in saline. Swabs were then placed into a tube containing 10 ml of FDA Listeria enrichment broth (LEB; OXOID). The tubes containing the swabs were incubated at 30°C for 24 h (primary enrichment), and then 0.1 ml was transferred into tubes containing 9 ml LEB as well as Frazer broth (BBL). The tubes were incubated for 24 h at 30°C (second enrichment). A loopful of each enrichment culture was streaked onto LPM (BBL) and MOX (MacClain and Lee, 1989) agars which were incubated at 37°C for 48 h.

Suspect colonies (3–5) were transferred from LPM agar onto BHI agar (OXOID) using a dissecting microscope and Henry's illumination to ensure purity. Pure cultures were Gram stained, and tested for motility, catalase production, utilization of esculin, rhamnose, mannitol, xylose and *a*-methyl-*d*-mannopyranoside, production of β-hemolysin on sheep blood agar. The CAMP test was carried out. (Genigeorgis et al., 1990; Cowan, 1985).

3. Results and discussion

The frequency of *Listeria* spp. and *L. monocytogenes* isolation from surfaces and handles of various refrigerators respectively, is shown in Table 1. From 136 household refrigerators, 136 samples from the walls or shelves, and 125 samples from the cheese compartments or cases were taken. Only two (1.5%) contained *L. monocytogenes* (one in the cheese case).

Food stores ($n = 212$) were visited and 228 refrigerators were sampled. Of the 335 samples examined, 118 were surfaces in contact with cheeses, 21 with cheeses and sausages, 69 with sausages, 20 with miscellaneous products such as salads, dressings, etc., and 107 were taken from refrigerator handles. Of the 228 refrigerators (Table 1), 7 (3.1%) were positive for *Listeria* spp., and 4 (1.7%) were positive for *L. monocytogenes* (from surfaces in contact with cheese, sausages and miscelaneous products). Of the 107 handles, one (0.9%) was positive for *L. monocytogenes*.

Table 1
Isolation of *Listeria* spp. and *L. monocytogenes* from various refrigerators and from the handles of retail store and industrial refrigerators

Refrigerators	Number of refrigerators examined	Number of positive refrigerators (%)			Number of handles examined	Number of positive handles (%)
		LM	LI	LS		LM
Domestic	136	2 (1.5)	—	—	NS	
Retail store	228	4 (1.7)	2 (0.9)	1 (0.5)	107	1 (0.9)
Meat plants	22	1 (4.5)	8 (36.4)	—	22	1 (4.5)
Dairy plants	9	—	—	—	9	—
Total	395	7 (1.8)	10 (2.5)	1 (0.2)	138	2 (1.4)

LI, *L. innocua*, LS, *L. seligeri* and LM, *L. monocytogenes*. <TB1>

Out of the 22 refrigerators in meat plants nine (40.9%) were found to be contaminated with *Listeria* spp., with only one (4.5%) containing *L. monocytogenes* (Table 1). None of the nine refrigerators and their handles, belonging to five dairy plants, harboured *Listeria* spp.

Of the domestic and retail store refrigerator 25 and 13.6%, respectively, had temperatures exceeding 10°C. *L. monocytogenes* was detected in home refrigerators with 8 and 9°C and in retail store refrigerators with 4, 8 and 13°C. The range of temperatures for the industrial refrigerators was 0–2°C for meat plants and 2–7°C for dairy plants. Because of the low number of positive samples no correlation of any kind could be established between the prevalence of *Listeria* spp. and the temperature of the various refrigerators.

In the present paper, *L. monocytogenes* and *Listeria* spp. were recovered at a very low frequency from the surfaces of domestic and retail refrigerators. In a similar survey concerning residential refrigerators in the USA, the pathogen was not recovered from any of the 195 refrigerators sampled (Jackson et al., 1993). Cox et al. (1989) recovered the microorganism from only one out of the 35 Dutch household refrigerators being examined. Although these surveys suggest that colonization of refrigerator surfaces by this microorganism may not be common, refrigerators cannot be excluded as potential source of contamination for ready-to-eat foods.

A great number of the refrigerators examined in the present paper were functioning at higher temperatures than 5°C. Of domestic and retail store refrigerators 25 and 13.6% respectively, functioned at temperatures above 10°C. Willocx et al. (1993) also reported that in 50% of the household refrigerators examined, the average temperature exceeded 10°C. These temperatures easily permit the growth not only of *L. monocytogenes* but also of a great number of pathogenic and spoilage organisms.

Genigeorgis et al. (1991) gave evidence that a number of cheeses could support growth of *L. monocytogenes* during refrigeration storage at 4°C, if cross-contamination occurred after the opening of the packages. Temperature fluctuations may also result in undesirable growth (Saguy, 1992).

The high frequency (40.9%) of *Listeria* spp. isolations from the surfaces of meat plant refrigerators is of concern. This may reflect a failure in the overall application of good manufacturing practices. As a result of such conditions, finished products, especially those without protective casings e.g. frankfurters or ham and sliced cooked products before being vacuum packaged, have a high probability of being cross-contaminated. After contamination, potential growth of the pathogen on these products during extended storage under abuse temperature is very likely (Johnson et al., 1990; Farber and Peterkin, 1991). McKeller et al. (1994) reported that 65.6% of surface inoculated vacuum-packaged Canadian retail winers supported the growth of the pathogen at 5°C. In a recent epidemiologic study Pinner et al. (1992) examined the role of foods in cases of sporadic listeriosis; it was found that 11% of more than 2000 food specimens collected from the refrigerators of patients contained *L. monocytogenes*. Of the 79 refrigerators with foods that harbored the pathogen 33% contained at least one food isolate of the same strain

as that in the correspondent patient, a frequency much higher than would be expected by chance ($P < 0.001$; Pinner et al., 1992). It is interesting that given the frequent contamination (40.9%) of the meat plant refrigerators with *Listeria* spp., only 1 (4.5%) of the door handles was positive and contained *L. monocytogenes*.

The present findings revealed that 32% of retail store refrigerators and 55.1% of domestic ones, functioned at temperatures of 9°C or higher, while none of the industrial refrigerators exhibited temperatures higher than 7°C. Therefore domestic and retail store refrigerators can be considered as critical points of the cold chain. Furthermore the situation with respect to domestic refrigerators is even more alarming when one takes into account the absence of indicator thermometers and the possibility that consumers may by overstocking, exceed the refrigeration capacity of the systems.

Good manufacturing and sanitation practices, especially at the industrial level, loading the refrigerators according to their refrigeration capacity and controlling storage temperature, are of significance in the prevention of growth of important psychrotrophic pathogens like *L. monocytogenes* and *Y. enterocolitica*. This is especially important in the production of minimally processed ready to eat foods.

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