Incidence of *Listeria* spp. in domestic refrigerators in Portugal

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Abstract

The main objectives of this study were to determine the incidence of *Listeria* spp. in Portuguese domestic refrigerators and to evaluate some of the hygienic practices in the domestic environment that might contribute to the persistence of the organisms. It was found that *L. monocytogenes* was present in 3 domestic refrigerators of the 86 investigated. *L. grayi* and *L. innocua* were also isolated from 4 and 1 refrigerators, respectively.

Overall, the information obtained from our survey demonstrates the need for consumer education in Portugal regarding safe food handling practices. For the refrigerators investigated, ≈71% were operating at a temperature higher than 6.1 °C, 87% were cleaned only monthly or less frequently, and only 8% were cleaned with appropriate proprietary cleaning products available in supermarkets.

Introduction

In order to answer consumers' demands, e.g. for minimally processed foods and products with longer shelf-life, the use of refrigeration has increased considerably during the past several years. A potential hazard in refrigerated foods, particularly in products which are eaten without further cooking, is *L. monocytogenes*. This pathogen is widely distributed in many environments and often found in foods (Farber & Peterkin, 1991; Jørgensen & Huss, 1998), and is well known for its survival and growth at refrigeration temperatures. *L. monocytogenes* has also been shown to adhere to various surface materials normally in contact with foods such as stainless steel, rubber, glass and polypropylene (Blackman & Frank, 1996; Mafu, Roy, Goulet, & Magny, 1990) and colonisation of refrigerators by *L. monocytogenes* has been previously demonstrated (Cox et al., 1989; Sergelidis et al., 1997).

The work presented here had as its main objectives to determine the incidence of *Listeria* spp. in Portuguese domestic refrigerators and to evaluate some of the hygienic practices in the domestic environment that might contribute to the persistence of the organisms. To our knowledge, this is the first survey into domestic practices that may affect the safety of food products in Portugal, although similar studies have been conducted in other countries (Cox et al., 1989; Jackson et al., 1993; Sergelidis et al., 1997).

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Materials and methods

During the period October 2001–May 2002, 86 refrigerators located at private homes in the North of Portugal (around Porto) were sampled for the presence of *Listeria* spp. Before sampling, the temperature of each refrigerator was measured and recorded using a portable digital thermometer (HD 9214, Delta OHM, Caselle di Selvazzano, Italy) and the refrigerator's owners were asked to answer a simple questionnaire, including the following:

How do you clean your refrigerator?
How often do you clean your refrigerator?
When was the last time you cleaned your refrigerator?
Do you pack all your foods before storage in the refrigerator?

What are the foods you normally store in the refrigerator without any packaging?

From each refrigerator two surface samples were collected (≈100 cm^2 from locations where vegetables were stored and ≈100 cm^2 from locations where cheese or meats were stored) by swabbing at various points of the selected location with sterile cotton swabs, previously immersed in sterile Ringer's solution. The swabs were transferred to 10 ml of half-Fraser broth (Biokar Diagnostics, Beauvais, France) and incubated at 30 °C for 48 h. Aliquots (1 ml) of these primary enrichments were transferred to 10 ml of secondary enrichment Fraser broth (Biokar Diagnostics) and incubated at 30 °C for 48 h. A loopfull of each primary enrichment culture and of the secondary enrichments after 24 h and 48 h of incubation, was streaked separately onto PALCAM and Oxford agar plates (Merck, Darmstadt, Germany). After incubation at 37 °C for 48 h, five typical colonies per plate (when possible) were transferred onto Plate Count Agar and incubated at 37 °C for 48 h. Pure cultures were tested for the Gram reaction, catalase, oxidase, fermentation of the sugars mannitol (0.5%w/v), rhamnose (1%w/v) and xylose (0.5%w/v), CAMP with *Staphylococcus aureus* NCTC 1621 and *Rhodococcus equi* NCTC 25923, and API *Listeria* (BioMérieux 10300).

Results and discussion

In the present study, it was found that *L. monocytogenes* was present in 3 domestic refrigerators out of the 86 investigated. *L. grayi* and *L. innocua* were also isolated from 4 and 1 refrigerators, respectively (Table 1). Since the selective media PALCAM and Oxford do not allow a clear distinction between colonies of different *Listeria* spp. it is possible that the incidence of *L. monocytogenes* presented in this work might be underestimated. Johansson (1998) demonstrated that the selection of five colonies for confirmation from the standard selective plating media (Anonymous, 1996) may not be sufficient if other *Listeria* species are present. Additionally, the higher growth rate of *L. innocua* in selective liquid media (Curiale & Lewus, 1994; MacDonald & Sutherland, 1994) compared with *L. monocytogenes*, can result in false negative results on the PALCAM and Oxford media, and Yokoyama, Maruyama, and Katsube (1998) concluded that most *L. innocua* strains produce a bacteriocin-like substance against *L. monocytogenes* that may inhibit growth of the latter organism during enrichment culture. It is also important to comment that the presence of any *Listeria* spp. may be indicative of poor hygiene and cross-contamination scenarios which could favour the persistence of *L. monocytogenes*.

Colonisation of refrigerators by *L. monocytogenes* has already been demonstrated. Sergelidis et al. (1997) in Greece and Cox et al. (1989) in Holland recovered the organism from 2 of 136 and 1 of 35 household refrigerators tested, respectively. However, Jackson et al. (1993) in the USA did not recover *L. monocytogenes* from any of the 195 domestic refrigerators sampled.

According to the results presented here and to those previously published (Cox et al., 1989; Sergelidis et al., 1997), the potential for ready-to-eat products to be cross-contaminated via contaminated surfaces in the refrigerator, must be recognised. Studies in northern Europe and Australia indicate that most of the populations of these countries are not aware of this organism and hygienic practices in domestic environments often pose a risk to the safety of food (Jay, Comar, & Govenlock, 1999; Scott, 1996). There is no data to suggest that the level of awareness of the Portuguese consumer is higher than in these reported studies.

Although no correlation was found between the presence of *L. monocytogenes* in refrigerators and their temperature (Table 1), the results obtained in this study demonstrate that a significant number of the refrigerators investigated were operating at a temperature that

Table 1
Listeria spp. in domestic refrigerators

Organism	Place of isolation	Frequency of cleaning	Last cleaning prior to sampling	Product used for cleaning	Products stored without packaging	Temperature (°C)
L. monocytogenes	Cheese shelf	Monthly	2 weeks	Water	Cheese, fermented sausages	6.0
L. monocytogenes	Meat shelf	Monthly	2 weeks	Water	Cheese, fermented sausages	4.8
L. grayi	Vegetables shelf	Each 6 months	>3 months	Water/vinegar	Meat	8.2
L. grayi	Vegetables shelf	Weekly	1 week	Detergent	Vegetables	10.0
L. grayi	Vegetables shelf	Each 2 months	1 month	Water	Fermented sausages	6.1
L. inoccua	Vegetables shelf	Monthly	3 weeks	Detergent	Fermented sausages	8.5
L. grayi	Vegetables shelf	Each 3 months	1 month	Water/vinegar	Vegetables	5.4
L. monocytogenes	Meat shelf	Monthly	1 month	Water	Vegetables	10.0

Table 2
Operating temperatures of 86 domestic refrigerators located in private homes in the North of Portugal

Temperature (°C)	Refrigerators (%)
2.0–4.0	13
4.1–6.0	16
6.1–8.0	31
8.1–10.0	27
10.1–12.0	10
>12.1	2

Table 3
Products used in the cleaning of 86 domestic refrigerators located in private homes in the North of Portugal

Product	% of respondents
Water	20
Detergent	40
Detergent for refrigerators	8
Bleach	5
Vinegar	20
Water and vinegar	20
Other	6

can compromise the safety of the foods stored inside them (Table 2). As an example, predictions effected using the Food MicroModel (Leatherhead Food International Ltd., Surrey, UK) software indicated that after 5 days of storage at 0–5 °C, the final concentration of *L. monocytogenes* would be 10^3/g in a fresh cheese having an initial concentration of 10 cells per gram. This final concentration, however, would rise to 10^7 cfu/g for a storage temperature of 10 °C (Mena et al., 2004). Temperature abuse is quite frequent in the cold chain, both in commercial and domestic situations. Sergelidis et al. (1997) reported that 25% of the 136 domestic refrigerators and 13.6% of the 228 supermarket refrigerators investigated in Greece, were operating at temperatures higher than 10 °C. A study of the temperatures of domestic refrigerators in 85 households in Melbourne and Sydney during 1997–98 revealed that the temperature of the fresh food compartments was higher than 10 °C in 55% of the measurements (Meat Research Corporation, 1998). Studies in Portugal have previously demonstrated that only 19.4% of 72 fresh cheeses sampled at the time of purchase were at temperatures below 5 °C (Anonymous, 2001).

Various studies have indicated that a significant proportion of food-borne illness arises from poor food-handling and hygiene practices in the domestic environment (Scott, 1996; Speirs, Anderton, & Anderson, 1995). According to the results presented here, although the presence of *Listeria* spp. in the refrigerators does not seem to correlate with the frequency of cleaning, the products used for cleaning appear to be important factors. As observed in Table 1, all the refrigerators that tested positive for the presence of *L. monocytogenes* were normally cleaned only with water, and 3 of the 5 refrigerators that tested positive for the other *Listeria* spp. were normally cleaned with water or water/vinegar solutions. It should be mentioned that none of the respondents knew in which proportion they were using water and vinegar. In general, refrigerator manufacturers recommend that the plastic interiors of domestic refrigerators should be cleaned with solutions of bicarbonate, partly to restrict the growth of moulds, but the alkalinity will also minimise the growth of bacteria. Nevertheless, there are several suitable cleaning products available on the domestic market designed for use on food preparation surfaces, that usually contain a mixture of ionic and non-ionic surface active agents that claim activity against a range of food-associated pathogens. It was interesting to find that none of the Portuguese domestic refrigerators were cleaned with alkali, and only 8.14% were cleaned with the surface cleaning products available in supermarkets (Table 3). The low number of positive samples, however, necessitates a larger study in order that valid conclusions can be drawn on the general occurrence of *Listeria* spp., and *L. monocytogenes* in particular in domestic situations. The frequency of cleaning is also too low. As observed in Table 4, 87.21% of the refrigerators investigated are cleaned only monthly or less frequently.

Foods, especially raw materials, that are stored in refrigerators, frequently contain pathogenic organisms including *L. monocytogenes* (Mena et al., 2004). The food industry makes considerable efforts to reduce the presence of *Listeria* spp. in foods as far as possible, but it must be recognised that this pathogen will probably enter the domestic kitchen from a variety of sources. If allowed to contaminate and persist on internal surfaces of refrigerators, *L. monocytogenes* might pose an additional risk to that presented by food contaminated earlier in the production chain. From the results presented in Table 4 it can be concluded that the population interviewed probably is not aware of this, since many products are stored without packaging. Although the number of samples positive for *Listeria* spp. is too low to allow valid conclusions, it was found in this study that in all the refrigerators that tested positive, some products were stored without packaging (Table 5).

Table 4
Frequency of cleaning of 86 domestic refrigerators located in private homes in the North of Portugal

Frequency	% of respondents
Weekly	6
Each 2 weeks	8
Monthly	38
Each two months	9
Each three months	29
Each six months	8
Annually	2

Table 5
Products stored without packaging

Product	% of respondents
None	42
Fermented meats	28
Vegetables	35
Raw meat	11
Cheese	17
Other	8

Overall, the information obtained from our survey demonstrates an urgent need for consumer education in Portugal regarding safe food handling practices.

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