Surveillance of the contamination by *Listeria* spp of refrigerators

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**SUMMARY**

Prevalence and location of *Listeria* spp were determined by surface sampling on sixty household refrigerators and thirty-four cafeteria cold storage rooms. *Listeria monocytogenes* was not detected. A strain of *Listeria innocua* was isolated in the vegetable compartment of a household refrigerator (1.6%). *Listeria innocua* and *seeligeri* were respectively found in a vegetable compartment of a cafeteria refrigerator (5.9%).

*RÉSUMÉ*

Surveillance de la contamination par *Listeria* spp de réfrigérateurs

La prévalence de *Listeria* spp a été évaluée sur les surfaces internes de 60 réfrigérateurs ménagers et de 34 chambres froides et réfrigérateurs de cuisine de restauration collective. *Listeria monocytogenes* n’a pas été mise en évidence. Une souche de *Listeria innocua* a été isolée dans le bac à légumes d’un réfrigérateur ménager (1,6 %). *Listeria innocua* et *seeligeri* ont été respectivement retrouvées dans une chambre froide et un réfrigérateur en cuisine collective (5,9 %).

Les trois méthodes de recherche de *Listeria* spp (Norme NF V08-055 ; méthodes alternatives ALOA et LISTERIA RAPID TEST) ont donné des résultats cohérents. Les trois facteurs associés au risque *Listeria* relevés lors de l’enquête (température interne des compartiments ; filmage des denrées ; nettoyage et désinfection) apparaissent mieux maîtrisés en restauration collective, principalement celle à caractère social. Une information des consommateurs sur l’hygiène en cuisine et la chaîne du froid paraît nécessaire.

*Mots clés*

listeria, réfrigérateur.
table cold storage room of a cafeteria and in both the vegetable and the meat compartments of a cafeteria refrigerator (5.9%).

The results of the three microbiological methods (NF V08-055; ALOA/L. MONO-DISK; OXOID Listeria rapid Test) involved in this study were consistent.

The three factors associated with the risk of Listeria found during the study (temperature control, food wrapping, cleaning and disinfection procedures) seemed to be better controlled in cafeterias. Then consumers need food safety education to improve temperature control and sanitation of their refrigerator.

**Keywords**

listeria, refrigerator.

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### 1 – INTRODUCTION

Listeria monocytogenes causes recurrent non-contagious outbreaks in France and other industrialized countries (Farber et Peterkin, 1991; Loncarevic et al., 1997; Rocourt, 1994; Salvat et al., 1995). A great variety of foods, mainly of animal origin, is likely to be contaminated by this ubiquitous germ but raw milk cheeses and ready-to-use cooked pork meats are most common vectors. This germ's ability to survive and even develop in a refrigerated environment enables it to last longer within food processing premises (Cox et al., 1989) which can then become a source of cross-contamination of foods during production (Berrang et al., 2002). Humid areas, condensation are favourable environments. The ability of Listeria monocytogenes to create biofilms was proved (Blackman et al., 1998).

Cross-contamination of foods was suspected in listeriosis non-contagious outbreaks at the consumer distribution stage, in retail shops. In the case of a listeriosis infection of humans, the study protocol suggests that the germ should be searched for in foods and on some surfaces of the patient’s refrigerator and of the customer frequented areas in the shops which were the focus of the epidemiological questionnaire.

Very few studies have been carried out to evaluate the contamination by Listeria spp of household refrigerators (Cox et al., 1989; Jackson et al., 1993; Sergelidis et al., 1997). The majority of current studies focuses on the environment and equipment of food industries, from slaughterhouses to meat cutting and processing workshops (Berrang et al., 2002; Lundem et al., 2002).

The objective of this study is to try to trace and locate Listeria spp in household refrigerators and in cafeteria cold storage rooms, in Calvados (Normandy - France).
2 – MATERIALS AND METHODS

2.1 Selection

Sixty household refrigerators belonging to the personnel of Frank Duncombe departmental Laboratory and of the veterinary services were sampled on the basis of voluntary participation, without any statistical analysis including in particular socioeconomic and cultural factors. However, these persons were not representative of the french population. As a matter of fact, most of them were educated at the university.

The selection of social and local cafeterias included thirty-four restaurants whose cold meat and vegetable storing rooms or refrigerator (small restaurants) were sampled.

The survey was composed of a questionnaire on internal compartment temperature, protection of preserved foods and cold room maintenance characteristics.

No survey after a listeriosis infection of humans was included in this study.

2.2 Samples

The samples were taken with a swab soaked in thinner with neutralizer (ATL swabs commercialized by HUMEAU – 44240 La Chapelle-sur-Erdre – France). The neutralizer makes it possible to suppress the inhibiting action of residual disinfectants and antiseptics.

Each refrigerator was swabbed twice, first on the top racks and walls, and then in the vegetable compartment.

The swabbing technique was standardized:

– First swabbing was done using a to-and-fro movement on the racks and walls, while also sampling the occasional organic material deposits, as well as on the inside of the door.

– Second swabbing was done on the inside of the vegetable compartment, bottom and walls, using a vertical and horizontal motion.

The swabbed parts were then rinsed in clear or slightly chlorinated water solution so as not to leave any traces of thinner in the refrigerators.

A total of 120 swabbings were carried out on residential refrigerators and 68 on cafeteria and commercial restaurant refrigerators.

2.3 Temperature control

Before each sampling, the air temperature was recorded in the center of the upper part of the refrigerator and in a bottom compartment by means of a +/- 1 Celsius degree calibrated thermometer.

In cold storage room, the thermometer was placed near the air recovery of the evaporator.
2.4 Questionnaire

A questionnaire was given to each refrigerator user in order to get to know their cleaning practices (frequency, products used) as well as the foods regularly stored in the refrigerators and the way they were stored (with or without protection).

2.5 Methods of analysis

At the laboratory, the swabs were immediately placed into a Half Fraser broth. Then three *Listeria monocytogenes* research methods were applied at the same time.

- **NF V08-055 standard from August 1997** – Within the frame of application of this standard, both PALCAM and Oxford isolating media were systematically used.
- **ALOA/L. MONODISK alternative method**, distributed by AES Laboratory (35270 – COMBOURG, France) and validated by AFNOR (AES 10/3-09/00).
- **LISTERIA RAPID TEST alternative method**, distributed by OXOID (69572 – DARDILLY – France) and validated by AFNOR (UNI-03/02-04/95).

Thus for each of these three methods, the following operative modes were respected after primary enrichment in Half Fraser broth at 30 Celsius degrees for 24 H +/- 2H:

- **NF VO8-055 standard included:**
  - Transfer of 0.1 ml of the culture obtained in Half Fraser broth into a glass test tube containing 10 ml of the enrichment medium (Fraser broth) – incubation of the inoculated medium for 48 H +/- 2 H at 37 Celsius degrees.
  - Isolation done on Oxford and PALCAM agar from the primary enrichment and from the secondary enrichment – incubation of the isolating media for 24 H at 37°Celsius degrees and continued readings at 48 H.
  - Confirmation of the germ and species after purification on TSYEA agar (tryptone soya-yeast extract): reaction of the catalase – search for hemolysis and CAMP trials – API Listeria strip. Confirmation was obtained from 5 *Listeria* spp presumed colonies per isolating medium.
- **ALOA/L. Monodisk alternative method included:**
  - Spreading of 0.1 ml of the culture obtained in Half Fraser broth onto ALOA agar – incubation of the medium for 24 H +/- 2 H at 37 Celsius degrees.
  - Location of the characteristic colonies:
    - *Listeria* spp: blue to blue-green, round, regular with no opaque halo colonies from 1 to 2 mm diameter.
    - *Listeria monocytogenes*: typical *Listeria* spp colonies surrounded with an opaque halo.
  - Extension of incubation at 37 Celsius degrees for 12 to 24 extra hours in case of the absence of typical colonies or in the case of an uncertain halo.
  - Differentiation of *Listeria monocytogenes* from other *Listeria* species with the L. Monodisk reagent when the incubation was extended.
LISTERIA RAPID TEST alternative method included:

- Transfer of 0.1 ml of the culture obtained in Half Fraser broth into a tube containing 10 ml of Buffered Listeria Enrichment Broth (BLEB) – incubation for 21 H to 24 H at 30 Celsius degrees.
- Heat treatment of 2 ml of the secondary enrichment culture: 20 minutes at 80 Celsius degrees.
- Cooling down at ambient temperature and 135 µl placed on a Listeria test plate.
- Observation of the migration and of the antigen-antibody reaction after 20 minutes (the Listeria test plate contains specific monoclonal antibodies targeted at the flagellum B antigen common to the Listeria genre).
- Confirmation of the positive results obtained by doing an isolation from the unheated BLEB on Oxford and PALCAM agar – incubation of isolating media for 24 H at 37 Celsius degrees and continued readings at 48 H.
- Confirmation of germ and species after purification on TSYEA agar following the NF V08–055 standard described above.

3 – RESULTS

3.1 Temperatures

The average and extreme recorded temperatures are reported in table 1. Average preservation temperatures were lower in cafeterias.

The average temperature of the cold storage rooms drew near the highest recommended value in the 0 + 4 Celsius degrees range. For household refrigerators, the average top part temperature was 5 Celsius degrees and was over the 0 to + 4 Celsius degrees recommended temperatures for the storage of sensitive products. There were substantial differences of about 10 Celsius degrees between the minimum and maximum temperatures for household refrigerators and cold storage rooms, which indicate some deficiencies in the equipment care and maintenance as well as in the implementation of effective HACCP-like systems in cafeterias.

The foods were protected by a film or a box in 88.3% of households and in 95.6% of cafeterias. The principal foods stored included now cheese, eggs and earthy vegetables in respectively 25%, 45%, 25% of household refrigerators and 5.9%, 70.6%, and 58.8% of cafeteria’s cold storage rooms. The storage conditions were satisfactory with a quasi-systematic protection of foods. It is not surprising because cafeterias have stopped using meat and traditional pork meat which were not pre-vacuum-packed or packaged in a controlled atmosphere. This protection is important to avoid cross contamination between ready-to-eat foods and other products.
3.2 Frequency of cleaning and disinfection and products used

The frequency of washing and sanitizing (table 2) of cold storage rooms is higher in cafeterias.

Table 2
Frequency of cleaning - disinfection.

<table>
<thead>
<tr>
<th></th>
<th>Household refrigerators (n = 60)</th>
<th>Cafeterias (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once a week or more</td>
<td>1.7%</td>
<td>82.4%</td>
</tr>
<tr>
<td>Once or twice a month</td>
<td>26.6%</td>
<td>17.6%</td>
</tr>
<tr>
<td>Once or twice per trimester</td>
<td>71.7%</td>
<td>0%</td>
</tr>
</tbody>
</table>

The principal disinfectant used in household kitchens is bleach, whereas specialized chemical products are very often used in cafeterias (table 3).
3.3 Results of the Listeria spp research

Three strains of *Listeria* were detected: *L. innocua* in a household refrigerator (top part), *L. seeligeri* (in a “vegetable” cold storage room) and *L. innocua* (in a cafeteria refrigerator: top and bottom parts). *Listeria* spp was recovered in respectively in 1.6% of domestic refrigerators and 5.9% of cafeterias storages.

The three methods were entirely consistent.

### Table 3
Products used during cleaning-disinfection.

<table>
<thead>
<tr>
<th></th>
<th>Household refrigerators (n = 60)</th>
<th>Cafeterias (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleach</td>
<td>73.3%</td>
<td>32.4%</td>
</tr>
<tr>
<td>Washing-up liquid</td>
<td>10.0%</td>
<td>2.9%</td>
</tr>
<tr>
<td>Chemical cleaners and sanitizers for food industry</td>
<td>10.0%</td>
<td>82.4%</td>
</tr>
<tr>
<td>Vinegar</td>
<td>3.3%</td>
<td>0%</td>
</tr>
<tr>
<td>No sanitizer (water only)</td>
<td>3.3%</td>
<td>0%</td>
</tr>
</tbody>
</table>

4 – DISCUSSION

The average temperature of both household refrigerators and cafeterias cold storage rooms were near or above the highest recommended value of 0 to 4 or 5 Celsius degrees for perishable foods (ANONYMOUS, 1995). However, these temperatures are lower than those reported by SERGELIDIS *et al.*, 1997: 55.1% of domestic refrigerators exhibited 9°C or more. Prior survey findings have shown that large proportion of household refrigerators exceeded recommended temperatures (REDMOND *et al.*, 2003).

The differences between households and cafeterias lie in the frequency of cleaning and disinfection procedures. While cleaning and disinfection are very rare in households, they are standard procedures integrated to HACCP-like systems in cafeterias. This deficiency in cleaning and disinfecting household refrigerators is worsened in cases where the person only uses water or washing-up liquid to do what then becomes just a cleaning procedure. The low frequency of cleaning and disinfection of household refrigerators is compensated by their smooth inner casing which is most often dry and unfavourable to bacterial growth (JACKSON *et al.*, 1993). Australian data revealed that almost half of the surveyed consumers failed to use a detergent or cleaner for kitchen surfaces (JAY *et al.*, 1999).
The search for *Listeria* spp was not very fruitful and *Listeria monocytogenes* was not detected. These results are comparable to those of prior surveys. Cox *et al.*, 1989 have isolated only one strain of *Listeria monocytogenes* in a vegetable compartment out of 35 Dutch refrigerators sampled. Jackson *et al.*, 1993, did not isolate *Listeria monocytogenes* in 195 Texan household refrigerators. In a similar study in Greece, Sergelidis *et al.*, 1997 recovered *Listeria monocytogenes* in 2 residential refrigerators from the 136 sampled. This does not mean that the refrigerator surfaces cannot be at the origin of cross-contamination, particularly in the case of listeriosis. Therefore refrigerator surface sampling remains an important element of the study (Ewan *et al.*, 1991; Jackson *et al.*, 1993; Pinner *et al.*, 1992), together with the sampling of the foods eaten by the patients (Ewan *et al.*, 1991; Loncarevic *et al.*, 1997). *Listeria monocytogenes* was found in at least one of the products present in about 64% of listeriosis patients’ refrigerators in the USA (Sergelidis *et al.*, 1997). However, contamination may not be perennial and may disappear in unfavourable environmental conditions. It is impossible to compare the contamination of refrigerators and even of cafeteria cold storage rooms with that of food industry workshops whose size and environment are not comparable and probably enable a longer-lasting contamination.

The more frequent cleaning and disinfection procedures in cafeterias do not prevent a higher contamination than that of household refrigerators. We can however qualify this result. In social cafeterias, only the vegetable cold storage room revealed the presence of *Listeria* spp. The contamination by *L. innocua* of a refrigerator top and bottom compartments was detected in a small local restaurant with a small patronage. The three microbiological methods did not give any conflicting results.

The non-detection of *Listeria monocytogenes* by three different microbiological methods in household refrigerators and cafeteria cold storage rooms is an encouraging result for consumer safety. This absence is all the more surprising since refrigerator disinfection is rarely done in households. It would be worth informing consumers on the subject. A more exhaustive result including a representative selection of the population would be useful.

5 - ACKNOWLEDGEMENTS

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REFERENCES


