Detection of *Listeria monocytogenes* in ready-to-eat foods sampled from a catering service in Apulia, Italy

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**Key words:** Listeriosis, Listeria monocytogenes, foodborne diseases, ready to-eat foods

**Parole chiave:** Listeriosi, Listeria monocytogenes, malattie trasmesse da alimenti, alimenti pronti al consumo

**Abstract**

**Objective:** Listeria monocytogenes is currently considered a relevant emerging food-borne pathogen. In particular, the European Centre for Disease Prevention and Control (ECDC) illustrates its widespread presence in different foods. In the present article, L. monocytogenes prevalence was estimated in cooked ready-to-eat foods sampled from a catering service in a Apulia city, southern Italy.

**Methods:** The study was carried out from January to June 2014 in accordance to Regulation (EC) No. 852/2004, and ISO 11290-1:1996/Amd.1:2004 methods.

**Results:** Listeria spp. was isolated in 8.3% of the samples: L. monocytogenes was identified with the highest prevalence in potato gateau (66.6%), followed by rice dishes (11.1%), Listeria innocua was isolated from potato purea (11.1%) and cooked vegetables (11.1%).

**Conclusions:** These preliminary results confirm the diffusion of the microorganism in ready-to-eat products; therefore, strategies aimed at protecting the consumers should be adopted. First of all, correct hygiene procedures should be followed and then microbiological tests should be implemented in order to early detect Listeria spp. (not only LM) contamination in cooked foods.

**Introduction**

Worldwide, foodborne diseases are currently listed among the main problems of public health and *Listeria monocytogenes* (LM) has been recognized as a relevant emerging food-borne pathogen. LM is a ubiquitous microorganism capable of adapting to a wide range of environmental conditions; it can be commonly found in soil, decaying vegetation, and water (1). LM is also detected in food products, where it is able to multiply at temperatures as low as 2 to 4 °C, low pH and high salt concentration. The main method of transmission to humans is believed to be by contaminated food consumption: in fact, in 99% of cases listeriosis is due to foodborne transmission (2). In particular, the European Centre for Disease Prevention and Control (ECDC) demonstrates its widespread presence in various ready-to-eat foods (1). Although

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Listeriosis is considered a rare disease, in the Europe Union about 1,470 human cases were reported in 2011, with a mortality rate of 12.7% (3).

Therefore, many countries have introduced legislation to control the incidence of listeriosis, being the microbiological monitoring of LM one of the most effective. In the European Union, the Microbiological Criteria Regulation (4, 5) requires regular testing for ready-to-eat foods. In particular, ready-to-eat foods intended for infants and for special medical purposes impose absence in 25 g; ready-to-eat foods able and unable to support the growth of LM, other than those intended for infants and for special medical purposes, impose limits of 100 cfu/g (4). A regular microbiological monitoring is not required in normal circumstances for ready-to-eat foods which are heat treated in their final package, for fresh vegetables and fruits and chocolate products (4).

The present article reports the frequency of LM in cooked ready-to-eat foods from a large catering service in Apulia, Italy.

Material and methods

This study was carried out from January to June 2014 in according to Regulation (EC) No. 852/2004 (6).

The cooked ready to eat foods were sampled from a catering service, each sample weighed at least 100 g. The cooked foods were defined ready-to-eat when intended by the producer or the manufacturer for direct human consumption without any other processing (5). Tests for presence of LM were performed according to ISO 11290-1:1996/Amd.1:2004 methods (7). All food samples were submitted to a pre-enrichment culture in a liquid medium Half-Fraser broth and incubated for a further 24 hours at 35°C.

The selective enrichment culture was inoculated onto selective chromogenic medium Agar Listeria according to Ottaviani and Agosti (ALOA) and incubated at 35°C for 24-48 hours. LM identification was performed on colony morphology, sugar fermentation and haemolytic properties.

For identification and confirmation of LM were used Gram staining and biochemical tests such as Catalase, Oxidase, Nitrate reduction, Methyl Red/Voges-Proskauer, hydrolysis of Esculin, Citrate test, fermentation of glucose, mannitol, α-methyl-D-mamoside and rhamnose, motility at 25°C and 35°C, gas production, β-hemolytic activity and CAMP tests (8). Strains of suspected Listeria non-monocytogenes were identified by biochemical tests (Api Listeria, BioMérieux, Italy). In our study, we considered the food suitable to human consumption if LM resulted absent in 25 g of ready-to-eat foods. Categorical data are expressed as number and percentage and compared using the Fisher’s exact tests. Statistical analyses were carried out with GraphPad Prism version 5.0 for Windows (San Diego, CA, USA), and the statistical significance was defined as p<0.05.

Results

Overall, 108 ready-to-eat food samples were examined: 28 pasta and 16 rice dishes, 22 from meat and 12 eggs products, 22 potatoes and 8 mixed cooked vegetables (onions, carrots, spinach) (Table 1).

Listeria spp was detected in 9 products (9/108; 8.3%). In particular, LM was detected in 7 samples (7/9; 77.7%), with highest prevalence in potato gateau (6/9; 66.6%), followed by rice dishes (1/9; 11.1%), and Listeria innocua was isolated from potato purea (1/9; 11.1%) and cooked vegetables (1/9; 11.1%).
Foods made with potatoes seemed more often involved than those made with rice; but the difference resulted not statistically significant (p=0.203).

Discussion

Despite an increased attention to the control of food products, the burden of food-borne-diseases is still of concern. In particular, ready-to-eat foods with a prolonged shelf-life under refrigeration are at risk for LM contamination. The ECDC reports that in the period 2008-2012 the trend of listeriosis has been rather stable with slight annual fluctuation due to a seasonal peak (1). The consumption of contaminated food like non-pasteurized milk products, meat and salmon products can be responsible of the infection mainly in immunocompromised patients, pregnant woman, newborns. In particular, the pregnant woman are twenty time more susceptible to the listeriosis because it can cause miscarriage, infection to fetus and neonatal death (1). In fact, even if actually LM is considered as a serious foodborne pathogen, with a case-fatality rate of up to 50% (9-11), these data often result underestimated because of the absence of specific surveillance programs.

In our preliminary study *Listeria* spp was isolated in about 8% of selected samples with a frequency of LM and *L. innocua* amounting at 77.7% and 22.2% respectively; the more involved foods were rice and potato products. The contamination of these foods could be correlated to the use of baked ham, because this was the only ingredient common to the two dishes. The practice of salting foods, performed at low temperature to promote a better penetration of the salt, is aimed at reducing the microbial contamination; but, some bacteria are resistant even in these conditions. Unfortunately, it was not possible to confirm our hypothesis; but, a further study is ongoing with this aim.

The results obtained in our study confirm the presence of both LM and other species in cooked foods in according to other findings where these species resulted most frequently recovered (12, 13). *Listeria innocua* is widespread in the environment and in food and is generally known as no pathogen microorganism, even if rare cases of human infections are described in literature: a ventriculoperitoneal shunt infection by *L. innocua* in a cerebrospinal fluid of a 9-month-old baby (14); a fatal case of bacteremia in a 62-year-old patient (15), and a case of meningitis in a 72-year-old woman (16).

### Table 1 - *Listeria* spp in ready-to-eat food samples from a catering service

<table>
<thead>
<tr>
<th>Read-to-eat food samples (No.)</th>
<th>Isolates No.</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>L. monocytogenes</em></td>
<td><em>L. innocua</em></td>
</tr>
<tr>
<td>Rice (16)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pasta (28)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Meat products (22)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Eggs products (12)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gateau/purea potatoes (22)</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Cooked vegetables (8)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total (108)</td>
<td>7</td>
<td>2</td>
</tr>
</tbody>
</table>
It is important to underline that literature data report that *Listeria innocua* presence could mask LM and to lead to a false negative result for the research of LM (17-20). Probably this fact could be attributed to a more rapid growth advantage of *L. innocua* vs *L. monocytogenes* or to inhibitory interspecies interactions attributed to the production of bacteriocin like agents. Consequently, it is important to investigate further for LM in case of isolation of *Listeria innocua*.

**Conclusion**

In addition to monitoring food safety considering that Good Manufacturing Practices, appropriate hygiene programs throughout the food production chain are always required, in order to avoid the contamination or inhibit the growth of LM in foods (20). Moreover, our study underlines the importance of microbial investigation in food stuffs.

These preliminary investigations lead to consider the necessity to estimate the diffusion of LM in ready-to-eat products and because of the antagonistic role of *Listeria innocua* strains vs LM during culture investigations it is necessary to focus attention also to other *Listeria* species. In this way it is possible to increase the precautionary measures for consumer protection.

**References**

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