Ann Ig 2015; 27: 590-594 doi:10.7416/ai.2015.2050

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

Giuseppina Caggiano[1], Osvalda De Giglio[1], Grazia Lovero[1], Serafina Rutigliano[1], Giusy Diella[1], Stella Balbino[1], Christian Napoli[1], Maria Teresa Montagna[1]

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 according 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

[1] *Department of Biomedical Science and Human Oncology, Hygiene Section, University of Bari "Aldo Moro", Bari, Italy*

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 at 30° C for 24 hours. The pre-enrichment culture was than sub-cultured into selective enrichment media, 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%).

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

Read-to-eat food samples (No.)	Isolates No.		Total No. (%)
	L. monocytogenes	*L. innocua*	
Rice (16)	1	0	1 (6.2)
Pasta (28)	0	0	0
Meat products (22)	0	0	0
Eggs products (12)	0	0	0
Gateau/purea potatoes (22)	6	1	7 (31.8)
Cooked vegetables (8)	0	1	1 (12.2)
Total (108)	7	2	9 (8.3)

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).

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.

Riassunto

Listeria monocytogenes ***in cibi cotti provenienti da un servizio di refezione della Puglia***

Obiettivi: Nel corso degli ultimi anni *Listeria monocytogenes* (LM) è stato riconosciuto quale patogeno emergente correlato al consumo di alimenti. Il Centro Europeo per la Prevenzione e il Controllo delle Malattie (ECDC) ne documenta la diffusione in vari alimenti pronti al consumo. Nel presente studio è stata valutata la prevalenza di LM in cibi cotti pronti al consumo distribuiti da un servizio mensa di una città del sud Italia.

Metodi: Lo studio è stato condotto da gennaio a giugno 2014, in accordo con il Regolamento (CE) n. 852/2004 e la ISO 11290-1: 1996/modifica 1: 2004.

Risultati: *Listeria* spp è stata identificata nell'8,.3% dei campioni; in particolare, LM è stata rilevata con la prevalenza più alta nel gateau di patate (66,.6%), e nel riso (11,1%), *Listeria innocua* è stata isolata da purea di patate (11,1%) e verdure cotte (11,1%).

Conclusioni: I dati del presente studio confermano l'importanza di valutare la diffusione di *Listeria* spp in cibi pronti al consumo e di focalizzare l'attenzione anche su altre specie di *Listeria*, sottolineando la necessità di aumentare le misure preventive per la tutela dei consumatori e di stabilire adeguate strategie di intervento.

References

1. European Centre for Disease Prevention and Control (ECDC). Annual epidemiological report 2014. Food and waterborne diseases and zoonoses. Stockholm: ECDC; 2014. Available at: ecdc.europa.eu [Last access on April 22, 2015].
2. Scallan E, Hoekstra RM, Angulo FJ et al. Foodborne illness acquired in the United States–major pathogens. Emerg Infect Dis 2011; **17**: 7-15.
3. www.efsa.europa.eu/en/topics/topic/listeria.htm, last update 28 January 2015 [Last access on April 22, 2015].
4. Commission Regulation (EC) No. 1441/2007 of 5 December 2007 amending Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs (OJ L 322/12, 7.12.2007).
5. Commission Regulation (EC) No. 2073/2005 of 15 november 2005 on microbiological criteria for foodstuffs (OJ L 338/1, 22.12.2005)
6. Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs (OJ L 139, 30.4.2004, p. 1–54).
7. ISO 11290-1:1996/Amd 1:2004. Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 1: Detection method. AMENDMENT 1: Modification of the isolation media and the haemolysis test, and inclusion of precision data.
8. Gasanov U, Hughes D, Hansbro PM. Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: a review. Fems Microbiol Rev 2005; **29**: 851-75.
9. Rocourt J, Bille J. Foodborne listeriosis. World Health Stat Q 1997; **50**(1-2): 67-73.
10. Mead PS, Slutsker L, Griffin PM, Tauxe RV. Food-

related illness and death in the United States reply to Dr. Hedberg. Emerg Infect Dis 1999; **5**(6): 841-2.
11. Vazquez-Boland JA, Kuhn M, Berche P et al. *Listeria* pathogenesis and molecular virulence determinants. Clin Microbiol Rev 2001; **14**(3): 584.
12. Farber JM, Peterkin PI. *Listeria monocytogenes*, a food-borne pathogen. Microbiol Rev 1991; **55**(3): 476-511.
13. Guerra MM, McLauchlin JM, Bernardo FA. *Listeria* in ready to eat unprocessed foods produced in Portugal. Food Microbiol 2001; **18**: 423-429.
14. Karli A, Sensoy G, Unal N et al. Ventriculoperitoneal shunt infection with *Listeria innocua*. Pediatr Int 2014; **56**(4): 621-3.
15. Perrin M, Bemer M, Delamare C. Fatal case of *Listeria innocua* bacteremia. J Clin Microbiol 2003; **41** (11): 5308-9.
16. Favaro M, Sarmati L, Sancesario G and Fontana C. First case of *Listeria innocua* meningitis in a patient on steroids and eternecept. JMM Case Reports 2014.
17. Cornu M, Kalmokoff M, Flandrois JP. Modelling the competitive growth of *Listeria monocytogenes* and *Listeria innocua* in enrichment broths. Int J Food Microbiol 2002; **73**: 261-74.
18. Curiale MS, Lewus C. Detection of *Listeria monocytogenes* in samples containing *Listeria innocua*. J Food Prot 1994; **57**: 1048-51.
19. Zitz U, Zunabovic M, Domig KJ, Wilrich PT, Kneifel A. Reduced detectability of *Listeria monocytogenes* in the presence of *Listeria innocua*. Food Prot 2011; **74**: 1282-7. Available at: dx.doi.org/10.4315/0362-028X.JFP 5-11-045 [Last access on April 22, 2015].
20. European Food Safety Authority (EFSA), Parma, Italy. Scientific Report of EFSA. Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods in the EU, 2010-2011. Part A: *Listeria monocytogenes* prevalence estimates. EFSA J 2013; **11**(6): 3241.

Corresponding author: Dr. Giusepina Caggiano, Department of Biomedical Sciences and Human Oncology, University of Bari "Aldo Moro", Piazza Giulio Cesare 11, 70124 Bari, Italy
e-mail: giuseppina.caggiano@uniba.it