Listeria spp. and *Listeria monocytogenes* contamination in ready-to-eat sandwiches collected from vending machines

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Abstract

Ready-to-eat (RTE) food is characterised by a long shelf-life at refrigerated temperature and can be consumed as such, without any treatment. The aim of the work was to evaluate the presence of *Listeria* spp. and *Listeria monocytogenes* in RTEs collected from refrigerated vending machines placed in hospital environment and accessible to the hospitalised patients. In 4 different sampling, 55 RTEs were collected from vending machines of six hospitals located in different areas of Sardinia region. All the samples were characterised by similar manufacturing process, such as the use of modified atmosphere packaging and belonged to 5 different producers. *Listeria* spp. was not countable using the enumeration method in all of the analysed samples. Using the detection method, *Listeria* spp. was recovered from 9 sandwich samples. Interestingly, 3 of these samples (5.5%) made by the manufacturer, were positive for *L. monocytogenes* contamination. The risk related to the *L. monocytogenes* presence in RTEs proportionally increases when food is introduced in *susceptible environments*, such as hospitals and consumed by *susceptible people*. Although the RTEs analysed showed values that complied with the European microbiological criteria for foodstuffs, the availability of these products in a *susceptible* environment should be carefully checked. Therefore, in order to limit the possible exposition to *L. monocytogenes*, more information on the risk related to RTE consumption should be provided to the hospitalised patients.

Introduction

In the last decade products ready for consumption [ready-to-eat (RTE)], with or without preheating, are largely emerged into the world food market. Nowadays these kinds of products represent an important portion of the food market and cover different sector of food production chain, *e.g.* raw material suppliers, packaging equipment, large-scale retail trade, *etc*. The RTE are characterised by a long shelf life at refrigerated temperatures and they are consumed as they are, without undergoing any treatment (for example cooking) (Rocourt, 1996). The prerequisites to ensure the health safety of consumers are represented by the application of good hygienic practices of production, the use of hazard analysis and critical control points systems, training of personnel to the different productive sectors and the adoption of systems of identification and traceability (Reg. EC 178/2002 and 852/2004; European Commission, 2002, 2004). The microbiological quality of the raw materials used in the production of RTE, the management of the various stages of processing, storage and refrigerated transport have a significant importance and can affect the microbiological composition of the end product (Angelidis *et al*., 2006). These products meet the tastes and habits of consumers who appreciate the RTE for speed and ease use. There are different types available on the market: from pasta sauces to fresh toppings, prepared salads (products of IV and V range), from frozen precooked seasoned pasta to sandwich. The RTE today represent a turnover of around 70 billion EUR at the international level, where the USA and Japan are two of the countries with the greater sales value. In Europe, the turnover is around 26 billion euro, and in particular Western Europe has a sales value of around 21 billion euro, which corresponds to 37% of total sales, the highest proportion in the world. UK, Germany and France are the countries that have the highest sales of ready meals in Europe, despite the rapid growth that are showing countries as Norway, Finland, Italy and Spain. In Italy RTE represents the 9% of sales compared to the rest of Europe, a market of about 2 billion of euros, with a high growth potential (http://www.euromonitor. com/). The RTE products may nevertheless present, for their composition and production technology, a high risk for public health and among these products, and the RTE sandwiches are the one with the high risk. The production technology foresees in some cases the assembly of finished products of different origin. The RTEs are in fact represented by a wide range of bakery products (white bread and other types of bread), stuffed with different ingredients, which include meat products, dairy products, fish products, vegetable and seasonings in the form of sauces. The assembly of different raw materials, without any technological stage able to reduce the risk of contamination or to be able to keep it under control, can result in the growth of pathogenic microorganisms that can cause foodborne infections in humans (Erickson, 2010; Gerner-Smidt and Whichard, 2008; Oliver *et al*., 2005). According to the EFSA report of 2008 the prevalence of *Listeria monocytogenes* in RTEs analysed in Slovakia was 8.3%, while in 2010 highest prevalence was recorded in Ireland (1.9%) (EFSA, 2010, 2012). Consumers with the highest risk of contracting iseases after consumption of RTEs, are among those belong to the category [young, old, pregnant, and immunocompromised (YOPI)], consumers elderly, pregnant women and especially hospitalised patients and immunocompromised (Allerberger and Wagner, 2010). This work is part of a larger research project involving the development of a strategy to increase active surveillance against human listeriosis through the development of operational protocols to *L. monocytogenes* search, the collection of biological samples in case of human clinical listeriosis, and evaluation of the immunological profile of patients. The main object of the present work was to assess the *L. monocytogenes* contamination levels in RTEs sold in hospitals. Therefore, RTEs were purchased at vending machines accessible to hospitalised patients and placed close to departments with high risk as obstetrics and gynecology, hematology, infectious diseases etc. In hospital sections in which vending machines were not present, ready-to-eat sandwiches were collect-

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ed from the areas easily accessible, as the hospital halls or passageways between departments.

Materials and Methods

Sampling

Four different sampling were conducted from November 2014 to May 2015. Samples were collected from refrigerated vending machines of six hospitals (A, B, C, D, E, F) located in different areas of the Sardinia region. During each trial different types of RTEs were randomly collected. Sandwiches were made with various ingredients: bread, tomatoes, salad, cheese, ham, tuna, *salame milano*, *mortadella*, mushrooms, olives, mayonnaise. On the total of the samples (n. 55) collected, 18 originated from the hospital A, 21 from the hospital B, 7 from the hospital C and 5 from the hospital D. Only 2 samples were collected from the hospitals E and F, respectively. All the samples were characterised by similar manufacturing process, such as the use of modified atmosphere packaging (MAP) and belonged to 5 different producers. After collection, sandwiches were transported under refrigeration condition to the laboratory of the Department of Veterinary Medicine, University of Sassari (Italy) and analysed until 24 h.

Microbiological analysis

All microbiological analysis were conducted according to international standard methods and included the following parameters: total aerobic mesophilic counts (ISO 4833-1:2013; ISO, 2013), Enterobacteriaceae (ISO 21528-2:2004; ISO, 2004a), *L. monocytogenes* (ISO 11290-1/2:1996/amd 2004; ISO, 2004b, 2004c), *Pseudomonas* spp. (ISO/TS11059:2009; ISO, 2009), *Bacillus cereus* (ISO 7932:2004; ISO, 2004d), yeast, moulds (ISO 6611/IDF 94, 2004; ISO, 2004e), *Salmonella* spp. (ISO 6579:2002/amd 2007; ISO, 2007) and Coagulase Positive Staphylococci (ISO 6888-1/2:1999/amd 2003; ISO, 2003a, 2003b). In addition, the detection of coagulase negative staphylococci was performed using Baird Parker + RPF agar plates (BioMeriéux, Lyon, France). From each sample, two 25 g aliquots were aseptically collected. One aliquot was homogenised with 225 mL of Buffered Peptone Water (Biolife, Milan, Italy), while the other, used for the detection of *L. monocytogenes*, with 225 mL of selective liquid media Fraser Broth Base (Biolife). Following homogenisation, serial decimal dilutions were prepared and streaked on the appropriate culture media agar plates.

Listeria spp., *Listeria monocytogenes* and *Pseudomonas* spp. identification

From samples positive for *Listeria* spp., 5 colonies were picked and submitted to molecular identification. For each strain confirmed as *L. monocytogenes* the major serovars were also detected (Doumith *et al*., 2004). Strains identified as *Listeria* other than *L. monocytogenes* were typed by biochemical identification using API Listeria (BioMeriéux). Similarly, 5 colonies with phenotypic characteristics ascribable to *Pseudomonas* spp. were submitted to molecular identification using specific primers to detect *Pseudomonas* spp. (De Vos *et al*., 1997) and *P. fluorescens* (Scarpellini *et al*., 2004). All the strains were stored at -80°C in Brian Heart Infusion broth (Biolife) with glycerol (20% v/v).

Headspace gas composition

The atmosphere inside the trays was determined with using the Dansensor gas analyser (Dansensor, Ringsted, Denmark). Determinations were conducted piercing the surface of sealed MAP sandwich samples with a sterile needle connected to the Dansensor. Measure of O_2% and CO_2% were directly read on the instrument while N_2 was calculated by difference.

Physico-chemical analysis

The pH was measured using pH meter GLP22 (Crison Instruments, Barcelona, Spain) by insertion of the probe into the product, while water activity (aW) was estimated on the homogenised product using water activity meter Aqualab 4TE (Decagon, Pullman, WA, USA). Two different measurements were performed to calculate the pH of the whole sample (bread and ingredients) and the pH of the ingredients (without bread).

Results and Discussion

Fifty-five sandwich samples were collected and analysed during the study. All the samples were positive for the presence of aerobic mesophilic bacteria. *Enterobacteriaceae* were detected in 27.3% of the samples, while 14.5% of the sandwiches showed yeast and moulds contamination. The highest prevalence was reached for coagulase negative staphylococci, with 45.5% prevalence. None of the analysed sandwiches showed *B. cereus*, *Salmonella* spp. and coagulase positive staphylococci contamination. Mean count values (x±sd) and the contamination range (log_{10} cfu/g) for the target microorganisms are shown in Table 1.

In all the analysed samples, *Listeria* spp. was always below the detection limit (10 cfu/g) of the enumeration method. On the other hand, using the detection method (presence/absence in 25 g of the product), *Listeria* spp. was recovered from 9 sandwich samples collected from 3 different manufactures. In three out of 9 samples, made by the manufacturer, contamination by *L. monocytogenes* was recovered. All the strains identified as *L. monocytogenes* (15/45, 33.3%) belonged to the 4b serovar. Of the remaining strains (30/45, 66.6%), 11 were *L. innocua* (24.4%), 11 *L. seeligeri* (24.4%), 7 were *L. welshimeri* (15.5%) and 1 *L. grayi* (2.2%). *Pseudomonas* spp. was detected in 7 (12.7%) sandwich samples, with a mean contamination value of 3.40±0.44 and range from 2.9-4 log_{10} cfu/g. On 35 isolates, 22 strains were identified as *Pseudomonas* spp. and 11 of these were *P. fluorescens*. Mean values for the gas composition of the headspace were 1.04%±2.48 (x±sd) for O_2 level (range 0-10.5%), 16.36%±3.67 for CO_2 level (range 7.9-25.5%) and 82.60±2.99 for N_2 level (range 74.5-87.7%). pH computed on the whole samples showed a mean value of 5.33±0.32 and ranged

Table 1. Microbiological profile of ready-to-eat sandwiches collected from hospital vending machines.

Samples	Aerobic mesophilic bacteria			Enterobacteriaceae			Yeasts and molds			Coagulase-negative staphylococci		
	N. Positive (%)	Mean±SD	Min-Max	N. Positive (%)	Mean±SD	Min-Max	N. Positive (%)	Mean±SD	Min-Max	N. Positive (%)	Mean±SD	Min-Max
A	14 (100.0)	6.88±1.13	4.39-8.51	7 (50.0)	2.39±1.43	1.00-4.64	nd	nd	nd	13 (92.9)	3.60±0.92	2.30-5.08
B	11 (78.6)	6.21±1.30	3.78-7.81	5 (35.7)	2.15±0.62	1.60-3.10	8 (57.1)	3.28±0.91	2.30-4.87	5 (35.7)	3.19±0.71	2.48-4.15
C	13 (86.7)	6.19±1.06	4.00-7.41	3 (20.0)	1.66±0.22	1.48-1.90	nd	nd	nd	3 (20.0)	3.99±0.99	2.85-4.60
D	12 (85.7)	5.77±1.38	2.77-7.51	nd	nd	nd	nd	nd	nd	4 (33.3)	3.12±1.04	1.57-3.78

SD, standard deviation; nd, not detected.

between 4.65 and 5.91. Similar data were found for the pH of the ingredients with a mean value of 5.26±0.34 and range from 4.49 to 5.95. a_w was 0.964±0.018 (x±sd) and ranged between 0.915 and 0.994.

Conclusions

RTE food, is generally susceptible to *L. monocytogenes* contamination.As demonstrated from several studies, the risk related to the presence of the pathogen in the processing environment is high, since these products are consumed as such (Dawson *et al.*, 2006; Little *et al.,* 2008; Ibba *et al.*, 2013). Furthermore, considering the criteria on physicochemical characteristics (pH and a_w) established by Regulation (EC) 2073/2005 (European Commission, 2005), all the analysed sandwiches were able to support the growth of *L. monocytogenes*. The risk due to the *L. monocytogenes* presence in RTE sandwiches proportionally increases when food is introduced in *susceptible environments*, such as hospitals and could be consumed by *susceptible people*, *i.e.* YOPI.

Previous investigations demonstrated that listeriosis cases were associated with the consumption of sandwiches contaminated by *L. monocytogenes*. In the current study, the prevalence of *L. monocytogenes* in the analysed samples was lower than in other studies (Little *et al*., 2009; Pesavento *et al.*, 2010). Although the RTE sandwiches examined showed a contamination level that complied with the microbiological criteria for foodstuffs (Regulation EC 2073/2005; European Commission, 2005), the availability of these products in a hospital environment should be carefully checked. Moreover, the presence of *Listeria* spp. other than *L. monocytogenes* in nine sandwich samples is worth noting, since finding *Listeria* spp. could indicate the potential presence of *L. monocytogenes*. Therefore, in order to limit the possible exposition to *L. monocytogenes*, more information on the risk related to the consumption of RTE sandwiches should be provided to the hospitalised patients.

References

Allerberger F, Wagner M, 2010. Listeriosis: a resurgent foodborne infection. Clin Microbiol Infect 16:16-23.

Angelidis AS, Chronis EN, Papageorgiou DK, Kazakis II, Arsenoglou KC, Stathopoulos GA, 2006. Non-lactic acid contaminating flora in ready-to-eat foods: a potential food-quality index. Food Microbiol 23:95-100.

Dawson SJ, Evans MR, Willby D, Bardwell J, Chamberlain N, Lewis DA, 2006. Listeria outbreak associated with sandwich consumption from a hospital retail shop, United Kingdom. Euro Surveillance 11:89-91.

De Vos D, Lim A Jr, Pirnay JP, Struelens M, Vandenvelde C, Duinslaeger L, Vanderkelen A, Cornelis P, 1997. Direct detection of Pseudomonas aeruginosa in clinical samples such as skin biopsy specimens and expectorations by multiplex PCR based on two outer membrane lipoprotein genes, oprI and oprL. J Clin Microbiol 35: 1295-9.

Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P, 2004. Differentiation of the major Listeria monocytogenes serovars by multiplex PCR. J Clin Microbiol 42:3819-22.

EFSA, 2010. The Community summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. EFSA J 1496:1-288.

EFSA, 2012. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. EFSA J 10:1-442.

Erickson MC, 2010. Microbial risks associated with cabbage, carrots, celery, onions, and deli salads made with these produce items. Compr Rev Food Sci F 9:602-19.

European Commission, 2002. Regulation of the European Parliament and the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, 178/2002/EC. In: Official Journal, L 31, 01/02/2002.

European Commission, 2004. Regulation of the European Parliament and the Council of 29 April 2004 on the hygiene of foodstuffs, 852/2004/EC. In: Official Journal, L 139, 30/04/2004.

European Commission, 2005. Regulation of the European Commission of 15 November 2005 on microbiological criteria for foodstuffs, 2073/2005/EC. In: Official Journal, L 338/1, 22/12/2005.

Gerner-Smidt P, Whichard JM, 2008. Foodborne disease trends and reports. Foodborne Pathog Dis 5:551-4.

Ibba M, Cossu F, Spanu V, Virdis S, Spanu C, Scarano C, De Santis EPL, 2013. Listeria monocytogenes contamination in dairy plants:Evaluation of listeria monocytogenes environmental contamination in two cheese-making plants using sheeps milk. Ital J Food Safety 2:109-12.

ISO, 2003a. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 1: technique using Baird-Parker agar medium. Amendment 1: inclusion of precision data. ISO 6888-1:1999/Amd.1:2003. International Organization for Standardization, Geneva, Switzerland.

ISO, 2003b. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 2: technique using rabbit plasma fibrinogen agar medium. Amendment 1: inclusion of precision data. ISO 6888-2:1999/Amd.1:2003. International Organization for Standardization, Geneva, Switzerland.

ISO, 2004a. Microbiology of food and animal feeding stuffs. Horizontal methods for the detection and enumeration of Enterobacteriaceae. Part 2: Colony-count method. ISO Norm 21528-2:2004. International Standardization Organization, Geneva, Switzerland.

ISO, 2004b. Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of Listeria monocytogenes. Part 1: detection method. ISO Norm 11290-1:1996/Amd 2004. International Standardization Organization, Geneva, Switzerland.

ISO, 2004c. Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of Listeria monocytogenes. Part 2: enumeration method. ISO Norm 11290-2:1996/Amd 2004. International Standardization Organization, Geneva, Switzerland.

ISO, 2004d. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of presumptive Bacillus cereus and colony-count technique at 30°C. ISO Norm 7932:2004. International Standardization Organization, Geneva, Switzerland.

ISO, 2004e. Milk and milk products, Enumeration of colony forming units of yeast and moulds. Colony count technique at 25°C. ISO Norm 6611:2004. International Standardization Organization, Geneva, Switzerland.

ISO 2007. Annex D: detection of Salmonella spp. in animal faeces and in environmental samples from the primary production stage. ISO Norm 6579:2002/Amd 1:2007. International Organisation for Standardization, Geneva, Switzerland.

ISO, 2009. Milk and milk products. Method for the enumeration of Pseudomonas spp. ISO/TS Norm 11059:2009 (IDF/RM 225:2009). International Standardization Organization, Geneva, Switzerland.

ISO, 2013. Microbiology of the food chain — Horizontal method for the enumeration of

microorganisms. Part 1: Colony count at 30 degrees C by the pour plate technique. ISO Norm 4833-1:2013. International Standardization Organization, Geneva, Switzerland.

Little CL, Barrett NJ, Grant K, McLauchlin J, 2008. Microbiological safety of sandwiches from hospitals and other health care establishments in the United Kingdom with a focus on Listeria monocytogenes and other Listeria species. J Food Protect 71:309-18.

Little CL, Sagoo SK, Gillespie IA, Grant K, McLauchlin J, 2009. Prevalence and level of Listeria monocytogenes and other Listeria species in selected retail ready-to-eat foods in the United Kingdom. J Food Protect 72:1869-77.

Oliver SP, Jayarao BM, Almeida RA, 2005. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. Foodborne Pathog Dis 2:115-29.

Pesavento G, Ducci B, Nieri D, Comodo N, Lo Nostro A, 2010. Prevalence and antibiotic susceptibility of Listeria spp. isolated from raw meat and retail foods. Food Control 21:708-13.

Rocourt J, 1996. Risk factors for listeriosis. Food Control 7:192-202.

Scarpellini M, Franzetti L, Galli A, 2004. Development of PCR assay to identify Pseudomonas fluorescens and its biotype. FEMS Microbiol Lett 236:257-60.