Meat Science 97 (2014) 443–450

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Presence and molecular characterization of the major serovars of *Listeria monocytogenes* in ten Sardinian fermented sausage processing plants

Domenico Meloni *, Simonetta Gianna Consolati, Roberta Mazza, Anna Mureddu, Federica Fois, Francesca Piras, Rina Mazzette

Department of Veterinary Medicine, University of Sassari, Via Vienna 2, 07100 Sassari, Italy

ARTICLE INFO

Article history:
Received 16 July 2013
Received in revised form 11 February 2014
Accepted 17 February 2014
Available online 23 February 2014

Keywords:
Listeria monocytogenes
Fermented sausage
Serovar
Pathogenic profile
Adhesion ability
Pulsotype

ABSTRACT

The aim of the present study was to investigate the occurrence of *Listeria monocytogenes* in ten Sardinian fermented sausage processing plants. A total of 230 samples were collected and 40 *L. monocytogenes* isolates were obtained and subjected to serotyping and investigated for the presence of ten virulence-associated genes using multiplex PCR assays. The isolates were further subjected to PFGE and investigated for their adhesion abilities in polystyrene microtiter plates. *L. monocytogenes* was found in 6% of food contact surfaces, in sausages at the end of acidification (3%) and ripening (8%). Serotyping revealed the presence of four serovars: 1/2c (37.5%), 1/2b (27.5%), 4b (22.5%) and 1/2a (12.5%). All virulence-associated genes were detected in 67.5% of the isolates. Isolates from processing environment, semi-processed and finished products showed high pulsotype diversity and the majority of isolates presented weak adhesion capability. The detection of the pathogen in fermented sausages confirms the ability of *L. monocytogenes* to overcome the hurdles of the manufacturing process.

1. Introduction

Listeria monocytogenes has been isolated from various ready-to-eat (RTE) food products including meat products (EFSA, 2011). RTE fermented dry and semi-dry sausages have rarely been implicated in foodborne illness. Nevertheless, pH and a_w of these products are often within the limits for growth of this pathogen (Hospital, Hierro, & Fernández, 2012). Therefore, contamination of fermented sausages by *L. monocytogenes* would render their consumption a potential public health risk (Levine, Rose, Green, Ransom, & Hill, 2001). The raw materials used for the production of fermented meat products may be contaminated by *L. monocytogenes* from the slaughterhouse environment, during the production process or in the post-processing stages (Colak, Hampikyan, Ulusoy, & Bingol, 2007).

L. monocytogenes is able to survive in fermented meat products due to its high tolerance to environmental stress factors, including low pH conditions and high salt concentration (Farber & Peterkin, 1991). According to EC Reg. 2073/2005 on microbiological criteria for foodstuffs (European Commission, 2005) the growth of *L. monocytogenes* is not favored in RTE foods with pH ≤ 4.4 or $a_w \leq 0.92$ or pH ≤ 5.0 and $a_w \leq 0.94$. For RTE foods that accomplish these conditions a food safety criterion of 100 CFU/g of *L. monocytogenes* throughout shelf-life has been established. Many Mediterranean-style dry fermented sausages could be included in this category, although there is great variability depending on the local traditions that influence fermentation and ripening (Hospital et al., 2012).

The presence of *L. monocytogenes* in processed pork meat products has been sporadically reported. Nevertheless previous surveys focusing on fermented meat products at the end of ripening showed a prevalence of 10% in France (Thévenot, Delignette-Muller, Christieans, & Vernozy-Roland, 2005) and Chile (Cordano & Rocourt, 2001) and from 13 to 40% in Italy (Cantoni, Aubert, Valenti, Comi, & Aubert, 1989; De Cesare, Mioni, & Manfreda, 2007; Meloni et al., 2009). Fermented sausages ("Salsiccia Sarda") are the primary product of the meat supply chain in Sardinia (Italy) and are typical Mediterranean-style naturally fermented sausages (Greco, Mazzette, De Santis, Corona, & Cosseddu, 2005). The results of a former survey showed the presence of *L. monocytogenes* in four "Salsiccia Sarda" processing plants (overall prevalence in the environments: 15%). The occurrence was 37% in ground meat and 80% in sausages at the end of ripening. These products did show contamination levels always lower than 100 CFU/g (Meloni et al., 2012).

The objectives of the present study were to evaluate the presence of *L. monocytogenes* in ten Italian fermented sausage processing plants and their products, to characterize the *L. monocytogenes* isolates using phenotypic and molecular methods and to investigate their distribution and adhesion abilities in the processing plants.

* Corresponding author. Tel.: +39 079 229 570; fax: +39 079 229 458.
E-mail address: dmeloni@uniss.it (D. Meloni).

http://dx.doi.org/10.1016/j.meatsci.2014.02.012
0309-1740/

2. Materials and methods

2.1. Sampling

Over a one-year period, a survey was carried out in ten fermented sausage processing plants (labeled as A to L) located in Sardinia (Italy), in which raw materials from Sardinia or imported from other Italian regions and European Union countries are processed. The processing plants included in the survey were representative of the regional fermented sausage production. Some characteristics of the plants are summarized in Table 1. Multiple sampling days were programmed in every processing plant in order to collect a total of 230 samples from environments, raw, semi-processed and finished products. A total of 100 environmental samples were collected, consisting of 50 swabbed non-food-contact surfaces (NFCS) and 50 food-contact surfaces (FCS). Environmental sampling was performed in each of the ten processing plants at the end of the sampling day, before cleaning and disinfection. NFCS and FCS were sampled during the production stages by swabbing with sterile gauze pads rehydrated with 10 ml of neutralizing buffer (Solar-cult sampling kit, Biogenetics, Padua, Italy). Sampling locations were chosen to represent those most likely to harbor *L. monocytogenes* (Barros et al., 2007; López et al., 2008): for NFCS e.g. walls and floor drains in the ground meat store rooms, drying and ripening rooms, processing and packaging/shipment rooms. For FCS e.g. work tables, trolleys, mincing, mixing and stuffing machines. The area of environmental sampling varied depending on the sampling location: on the walls, work tables and trolleys, a sterile template to delineate a swabbed area of 100 cm^2 was used. A total of 130 samples of 50 different batches (5 batches from each processing plant) consisting of raw materials, semifinished and finished products were collected: 50 ground meat samples, 30 sausages at the end of acidification (after 5 days from stuffing) and 50 sausages at the end of ripening (after 20 days from stuffing). Samples of sausages at the end of acidification were obtained only from six processing plants (A, B, D, E, I, L). All the items identified with a univocal code and placed in sterile bags (kept in ice boxes at 3 °C) were transported to the laboratory of the Department of Veterinary Medicine in Sassari and analyzed on the same day.

2.2. Physico-chemical analysis

For all the samples of ground meat, sausages at the end of acidification and ripening the pH and water activity (a_w) were determined. The measurement of pH was carried out by inserting the pin electrode of a pH-meter GLP 22 (Crison Instruments SA, Barcelona, Spain) directly into each sample. Water activity (a_w) was determined using a water activity meter Aqualab 4 TE (Decagon, Pullman, WA, USA).

2.3. Detection and enumeration of L. monocytogenes

The detection and enumeration of *L. monocytogenes* were carried out according to the international standard methods ISO 11290-1:1996-Amd 1/2004 (Anonymous, 2004a) and 11290-2:1998-Amd 1/2004 (Anonymous, 2004b). The swabbed samples and sterile 25 g aliquots of ground meat, sausages at the end of acidification and ripening were homogenized 1/10 with sterile Fraser broth base (Biolife, Milan, Italy) in a Stomacher Lab Blender 400 (Seward Medical, London, UK) for 2 min. The homogenates were incubated at 20 °C for 1 h. For the enumeration of *L. monocytogenes*, a volume of 1-ml samples of each inoculum were streaked onto three ALOA 90-mm plates (Biolife) and were incubated at 37 °C for 48 h. Enumeration of *L. monocytogenes* was not carried out in environmental samples. For primary enrichments, the homogenates were supplemented by Fraser half selective supplement (Biolife) and were incubated at 30 °C for 24 h. For secondary enrichment, 0.1-ml aliquots of the primary enrichment were transferred into 10-ml tubes containing Fraser broth (Biolife) supplemented by Fraser selective supplement (Biolife) and were incubated at 37 °C for 48 h. After incubation, aliquots of 0.1-ml from primary and secondary enrichments were simultaneously streaked onto Oxford (Oxoid, Milan, Italy) and ALOA plates and incubated, respectively, at 30 and 37 °C for 48 h. From each positive sample plate of the primary and secondary enrichment, five colonies presumed to be *Listeria* spp. were streaked onto tryptone soya yeast extract agar (TSYEA) plates (Biolife) and incubated at 37 °C for 24 h. Those putative *Listeria* spp. colonies were submitted to Gram stain, catalase, and oxidase tests. Hemolytic activity and CAMP tests on sheep blood agar were performed for confirmation of *L. monocytogenes* isolates. Biochemical characterization of all the isolates was performed using the API *Listeria* identification system (bioMerieux, Marcy l'Etoile, France). After identification, *L. monocytogenes* isolates were stored at −80 °C in Brain Heart Infusion (BHI) broth (Biolife) with glycerol (15% v/v).

2.4. Characterization of the isolates

2.4.1. Serotyping

Isolates were subjected to a multiplex PCR-based serotyping assay to identify the five serogroups targeting the genes *lmo0737*, *lmo1118*, ORF2819, ORF2110 and *prs* (Doumith, Buchrieser, Glaser, Jacquet, & Martin, 2004). Each serogroup represents more than one serovar: serogroup IIa (serovars 1/2a and 3a), serogroup IIb (serovars 1/2b, 3b, and 7), serogroup IIc (serovars 1/2c and 3c), serogroup IVb (serovars 4b, 4d, and 4e), and serogroup IVa (serovars 4a and 4c). The concentration of each primer (Roche diagnostics, Milan, Italy) is reported in Table 2. The multiplex PCR products were resolved by electrophoresis on 1.5% agarose gel in 1 × TAE (Invitrogen, Carlsbad, CA, USA) and stained

Table 1
Some characteristics of the Sardinian processing plants which manufactured fermented sausages from raw ground pork meat.

Plants	Tons/year	Processing line complexity	Origin of the ground meat	Characteristics of the ground meat	Ripening period (fermented sausages)
A	50–100	++[a]	Regional/National	Fresh	15 days
B	>500	++++[c]	National/European	Fresh	7–15 days
C	300–500	+++[b]	National	Fresh	15 days
D	300–500	+++	National/European	Fresh	15 days
E	300–500	+++	National/European	Fresh	15 days
F	50–100	++	Regional	Fresh	15 days
G	300–500	+++	National	Fresh	15 days
H	300–500	+++	National	Fresh	15 days
I	300–500	+++	Regional/National	Fresh	15 days
L	300–500	+++	Regional/National	Fresh	15 days

[a] Simple processing line.
[b] Complex processing line.
[c] More complex processing line.