Listeria monocytogenes and other Listeria species in raw milk and sausage in East Algeria

L. Benhalima*1, T. Merad2, M. Bensouilah1 and R. Ouzrout3

Department of Biology, Faculté des Sciences
de la Nature et de la Vie et Sciences de la Terre et de l’Univers, Université 8 Mai 1945 Guelma, Algeria.

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ABSTRACT

Listeria monocytogenes is a pathogenic bacterium that can cause Listerialis in humans. The aim of this study is to isolate and enumerate L. monocytogenes and other Listeria species from raw milk and sausage samples collected in East Algeria. A total of 87 food samples were analyzed according to ISO 11290-1 and ISO 11290-2 methods. Of the samples examined, 10.34% were found to be positive for Listeria spp. Three species of Listeria were detected, in which L. innocua was the most commonly recovered species (66.67%) followed by L. seeligeri (22.22%) and L. monocytogenes (11.11%). The count for L. innocua ranged from 1.95 to 3.13 log10(CFU g-1 or CFU ml-1), against 1.65 to 2.48 log10(CFU g-1 or CFU ml-1) for L. seeligeri. L. monocytogenes contaminated sausage sample had enumeration results of 1.65 log10(CFU g-1). The presence of Listeria in milk and sausage samples reflects the no control of hygienic practices.

Key words: Detection, Enumeration, Listeria monocytogenes, Listeria spp, Raw milk, Sausage.

INTRODUCTION

Listeria monocytogenes is an opportunistic intracellular pathogen that has become an important cause of human foodborne infections worldwide (Liu, 2006). Foodborne L. monocytogenes causes large outbreaks of Listeriosis (Panda and Garg, 2003), with a high fatality rates (20-30%) compared with other foodborne microbial pathogens (FAO/WHO, 2005). The widespread distribution of L. monocytogenes and other Listeria spp. in nature and an association with domestic livestock makes their occasional presence on raw milk and raw meats almost unavoidable (Sarangi and Panda, 2012). Contamination of milk and sausages with L. monocytogenes poses a special threat to public health because of the organism’s ability to grow at refrigeration temperatures, low pH, low water activity, high salt concentration, and its pathogenicity within certain segments of the population (Jadhav et al. 2012).

Milk is the primary source of animal protein in the Algerian consumer-diet; with globalization, sausages have become recognized as an important meat product. The contamination of milk and meat products such as beef and sausages don’t affect the quality but also causes foodborne illness (Saludes et al. 2015; Singh et al. 2018). To our knowledge, there is rare published data covering variety sources of food samples for isolation and detection of Listeria in Algeria. The objectives of the present research were: (i) to assess the use of ISO methods in the detection and enumeration of Listeria spp. in food products, (ii) to obtain additional information about rates of L. monocytogenes and other Listeria species in raw milk and sausage in Algeria focusing on their prevalence and phenotypic characteristics.

MATERIALS AND METHODS

A total of 87 samples were studied which included raw milk-42 and sausage-45. The raw milk analyzed for Listeria was obtained from farm bulk tanks in two different areas of East Algeria: El Taref and Annaba cities. Sausage samples were obtained from various butchers. Yet, all sausages are prepared with the same ingredients (beef meat and fat, sugar, starter, spices) and manufactured with the same technology (fermentation and drying). All samples were maintained at 4°C during shipment and storage. Analysis was begun within one hour of sampling.

Fraser half concentration enrichment broth (½Fraser) and Fraser broth (Oxoid, Dardilly, France), were used, as the primary enrichment broth and a secondary enrichment medium respectively, according to the EN ISO 11290 for the detection of Listeria (ISO, 2004a). Fraser mediums were incubated for 48 ± 2 hours to permit the development of the black color. Each inoculated tube was compared to an inoculated control against a white background. Two esculin based Listeria selective agars were used: Palcam medium (AES, Combourg, France) and Oxford agar (Lab UK). Five typical colonies from each esculin based agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-agar were selected and streaked for purity to Trypticase Soy-agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-Agar were selected and streaked for purity to Trypticase Soy-

*Corresponding author’s e-mail: lamia-kos1@hotmail.fr, benhalima.lamia@univ-guelma.dz
1Department of Biology, Université 8 Mai 1945 Guelma, Algeria.
2Department of Marine Biology, Université Badji Mokhtar Annaba, Algeria. merad.tarek@univ-annaba.dz
3Department of Biochemistry, Université Badji Mokhtar Annaba, Algeria. bensouilah_mourad@yahoo.fr
Yeast Extract (Sigma, Germany) (TSAYE) (ISO, 2004a). After incubation, *Listeria* colonies on TSAYA were examined by the Henry Method of oblique lighting (Hitchins, 2003).

Bacteria were confirmed as *Listeria* biochemically, by Gram stain, motility at 25°C, catalase production, oxidase test, nitrate reduction, urease production, indole production, methyl red-Voges-Proskauer (MR-VP) reactions, fermentation of sugars (rhamnose, xylose and mannotol), β-haemolysis, CAMP (Christie, Atkins, and Munch-Peterson) test and API *Listeria* (bioMerieux, 10300, Lyon, France) (Sarangi and Panda, 2012).

Viable counts of bacteria were determined according to ISO 11290-2 methods (ISO, 2004b). The colony-forming units per gram or per liter (CFU g⁻¹ or CFU l⁻¹) were calculated and transformed to log₁₀ values prior to further analysis.

The data obtained were analyzed using SPSS 25.0 software. The prevalence of different species, the percentages of false-positive results in relation to food, enrichment broths and selective media and the detection of *Listeria* on agar after the primary and secondary enrichments were compared using the chi-square test (p ≤ 0.05). The enumerations obtained were compared using an analysis of variance.

**RESULTS AND DISCUSSION**

**Prevalence of *Listeria* spp**: Out of 87 samples analyzed, *Listeria* spp. was recovered from 9 samples (Table 1). On biochemical characterization, *Listeria* spp. were identified as *L. innocua*, *L. seeligeri* and *L. monocytogenes* with prevalence rate of 66.67%, 22.22% and 11.11%, respectively (χ² = 7.6999, p = 0.053). This finding was in agreement with other studies (Jamali et al., 2013; Belabed et al., 2016). *L. innocua* is an indicator of the presence of *L. monocytogenes*. Furthermore, it has been used as a surrogate for the study of *L. monocytogenes* in a variety of food systems (Milillo et al., 2012). *L. seeligeri* is periodically isolated from food products (Dailey et al., 2015), it is a non-pathogenic bacterium, but it also carries a virulence gene cluster like *L. monocytogenes* and *L. ivanovii* (Muller et al., 2010). The identification of non-pathogenic *Listeria* species in current study was important. These non-pathogenic species have been found to cause disease in both immunocompetent and immunocompromised individuals (Usman et al., 2016).

*L. monocytogenes* was not detected in any of the raw milk samples. The non-pathogenic *Listeria* species can impose negative impact on growth of *L. monocytogenes* via competition for nutrients. Indeed, *L. monocytogenes* growth was reported to be strongly inhibited due to the presence of other *Listeria* species, e.g. *L. innocua* and/or *L. welshimeri* (Dailey et al., 2015). In contrast to our findings, Lebres et al. (2004) and Boubendir et al. (2011) reported a prevalence of 33.3% and 5.76%, respectively, of *L. monocytogenes* in bovine raw milk produced in the North Eastern Algeria. The contamination sources of the other *Listeria* species in raw milk samples can be related to the non-respect of good practices of production and milking, storage and transport, and infected cows in dairy farms (Sarangi and Panda, 2012; Singh and Gupta, 2015; Şahlbaba et al., 2018).

In the present study, sausage samples were contaminated by *Listeria* to an average of 6.67% (Table 1). This contamination rate could be correlated with the complexity of processing line, contaminated raw meat, combination of cross contaminations and poor hygiene (Azevedo et al., 2005). Only one pathogenic *L. monocytogenes* was recovered from 45 sausage samples. Sausages generally offer barriers, known as hurdles, for the growth of opportunistic pathogenic bacteria. These hurdles comprise the low pH, high salt level, presence of organic acids and nitrite, low aw, competition with the resident microbiota, addition of spices, herbs and smoke (Työppönen et al., 2003). Even though, *L. monocytogenes* were found to persist and eventually grow in dry fermented sausages (Talon et al., 2008). These results concurred with those reported by Çon et al. (2001), who are found 16% of sausage samples were positive for *L. monocytogenes*.

**Characterization of isolates**: Thirteen of the 87 products analyzed were positive after the first enrichment (24 h), whereas, only 6 new products were positive after the second enrichment (48 h) (p < 0.001). The percentage of false-positive is shown in relation to food, enrichment broths and selective media in Table 2. The first enrichment enabled detection of more *Listeria* positive samples than did the second enrichment, as also reported by Warburton et al. (1991). The presence of competing flora in products can affect the isolation of *Listeria* and it is therefore preferable.

| Table 1: Prevalence of *Listeria* species isolated from analyzed samples. |
|--------------------------|------------------|-----------------|
|                          | Raw milk | Sausage | total |
| Number of analyzed samples | 42       | 45     | 87     |
| Positive samples (%)    | 14.29    | 6.67   | 10.34  |
| *L. monocytogenes* (%)  | 0        | 33.33  | 11.11  |
| *L. innocua* (%)        | 66.67    | 66.67  | 66.67a |
| *L. seeligeri* (%)      | 33.33    | 0      | 22.22  |

a: χ² = 15.0867, p = 0.002.

| Table 2: Percentage of false-positive results in relation to food, enrichment broths and selective media. |
|--------------------------|------------------|-----------------|------------------|
|                          | Primary | Secondary | Oxford | Palcam |
| Food type                | enrichment | enrichment |  |  |
| Raw milk                 | 7.14    | 9.52    | 4.76  | 2.22  |
| Sausage                  | 2.22    | 13.33   | 2.22  | 0     |
| All                      | 4.6     | 11.49   | 3.45  | 1.15  |

*: Significant difference (p < 0.001), b: no significant difference (p>0.05).
The concentrations of Listeria species were generally between 1 and 2 log$_{10}$ (CFU g$^{-1}$ or CFU ml$^{-1}$) ($p < 0.05$; analysis of variance), which is the maximum limit given by the International Commission on Microbiological Specification of Foods (ICMSF, 1996) (Table 4). This 2 log$_{10}$ (CFU g$^{-1}$ or CFU ml$^{-1}$) limit was exceeded in 4.44% and 9.52% of sausage and raw milk samples, respectively. L. monocytogenes-positive sausage sample was estimated at 1.65 log$_{10}$ (CFU g$^{-1}$). The food safety of sausage contaminated with L. monocytogenes could not be guaranteed for at risk population when consuming such product. Importantly, about 1–2 log$_{10}$ CFU of L. monocytogenes are capable of causing listeriosis, although infective doses required for causing listeriosis mainly depend on strains and susceptibility of the individuals (Cotter et Hill, 2003). According to the European Commission Regulation (EC) No. 1441/2007 on microbiological criteria for foodstuffs L. monocytogenes must not exceed the limit of 1 log$_{10}$ CFU, corresponding to Food Safety Objective, throughout the shelf-life of the product for non risk group population, while for the immune-compromised individuals and neonates must be absent (EC, 2007). Moreover, Hitchins (2003) concluded that low levels of L. monocytogenes ($= 1$ log$_{10}$ CFU g$^{-1}$) were too frequent to be responsible for listeriosis in susceptible individuals. L. monocytogenes levels above 1 log$_{10}$ CFU g$^{-1}$ may be reached after in-food growth. Therefore risk management efforts should be focused on those food commodity types where L. monocytogenes can multiply.

**CONCLUSION**

Screening of 87 raw milk and sausage samples from East of Algeria, revealed the isolation of 9 Listeria species. L. innocua was founded the most prevalent species, which is followed by L. seeligeri and L. monocytogenes. The highest prevalence of Listeria was found in raw milk. The concentration of L. monocytogenes highlights the risk of sausage consumption. These results suggest that hygienic conditions should still be enforced in order to minimize the count of Listeria in foods.
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