



Prevalence of *Listeria* spp. and characterization of *Listeria monocytogenes* isolated from food products in Tetouan, Morocco



Nadia Amajoud ^{a,*}, Alexandre Leclercq ^b, Jose M. Soriano ^{c,d}, Hélène Bracq-Dieye ^b, Mohammed El Maadoudi ^e, Nadia Skalli Senhaji ^a, Ayoub Kounoun ^a, Alexandra Moura ^b, Marc Lecuit ^{b,f}, Jamal Abrini ^a

^a Laboratory of Biology and Health, Department of Biology, Faculty of Sciences of Tetouan, Abd Al Malek Essaadi University, Morocco

^b Institut Pasteur, Biology of Infection Unit, INSERM U1117, World Health Organization Collaborating Centre and National Reference Centre *Listeria*, Paris, France

^c Gastrolab, Research Group in Food Sciences Based on the Evidence and Experimentation, Institute of Material Science, Scientific Park, University of Valencia, Paterna, Spain

^d Joint Research Unit on Endocrinology, Nutrition and Clinical Dietetics, University of Valencia-Medical Research Institute Hospital La Fe, Valencia, Spain

^e Regional Analysis Laboratory and Research ONSSA, Tangier, Morocco

^f Paris Descartes University, Necker-Enfants Malades University Hospital, Division of Infectious Diseases and Tropical Medicine, Paris, France

ARTICLE INFO

Article history:

Received 5 June 2017

Received in revised form

18 August 2017

Accepted 19 August 2017

Available online 25 August 2017

Keywords:

Listeria

PCR-Serogrouping

PFGE

cgMLST

Morocco

ABSTRACT

A total of 1096 food samples were purchased in Tetouan, North-Western of Morocco, to examine the presence of *Listeria* spp. Eighty (7.3%) of the tested samples were found positive for the presence of *Listeria* spp., while *L. monocytogenes* was detected in 16 (1.5%) samples. *L. monocytogenes* isolates belonged to clonal complexes CC2 (PCR serogroup IVb) and CC199 (PCR serogroup IIa) and to 8 different combined *AscI/ApaI* pulsed-field gel electrophoresis profiles. Core genome multilocus sequence typing (cgMLST) allowed to distinguish 12 different cgMLST types and revealed the presence of 3 clusters of closely-related isolates from different samples, suggesting the existence of common sources of contamination. Isolates showed no resistance to the reference antibiotics used for the treatment of listeriosis. This study underlines the circulation of *L. monocytogenes* strains of clonal complex CC2 in Morocco and highlights the importance of microbiological surveillance in order to minimize consumers' exposure to this foodborne pathogen.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Listeria are Gram-positive ubiquitous bacteria, widely distributed in different environments and areas (Hamon, Bierne, & Cossart, 2006). There are currently seventeen species in the genus *Listeria* grouped into *Listeria sensu stricto* (*L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri*, *L. marthii*) and *Listeria sensu lato* (*L. fleischmannii*, *L. grayi*, *L. rocourtaie*, *L. weihenstephanensis*, *L. floridensis*, *L. aquatica*, *L. cornellensis*, *L. riparia*, *L. grandensis*, *L. booriae*, *L. newyorkensis*) (Orsi & Wiedmann, 2016). Among these species, *L. monocytogenes* is the causative agent of listeriosis, one of the most severe foodborne infections (Maertens de Noordhout et al., 2014). *L. monocytogenes* can resist

disinfectants, form biofilms and survive or multiply under extreme physicochemical characteristics, such as dry environments, different temperatures, a wide range of pH and high salt concentrations. All these conditions promote the survival and proliferation of *L. monocytogenes* in a large variety of food matrices including unpasteurized dairy products, meat products, seafood, vegetables, and in food industry environments. The contamination of food products with this pathogen can occur during production, packaging, transport, and storage (Carpentier & Cerf, 2011).

Human listeriosis can manifest as septicemia, meningoencephalitis, abortion or neonatal infection with high case fatality rate (20–30%) even in the absence of resistance to reference antibiotic treatment (Charlier et al., 2017; Morvan et al., 2010). In Morocco, the incidence of human listeriosis remains unknown due to the absence of epidemiological surveillance (Cohen, Ennaji, Hassar, & Karib, 2006; Maertens de Noordhout et al., 2014), and so far only one case of a neonatal listeriosis has been reported

* Corresponding author.

E-mail address: amajoud@yahoo.fr (N. Amajoud).

(Benabdejlil, Chergi, Kouach, Massaoui, & Dehayni, 2015; Benomar, Nejari, & Lahbabi, 2000).

Despite the lack of surveillance for *Listeria* infections in Morocco, several studies reporting the prevalence of *L. monocytogenes* in food have been performed in the center of the country to estimate consumers' exposure to this pathogen. In Casablanca, for instance, *L. monocytogenes* was present in 14.4% of red meat products collected in 1998 (Kriem, El Marrakchi, & Hamama, 1998), absent in 156 red meat and offal samples collected between 2002 and 2004 (Cohen et al., 2006), and present in 2.3% of 426 poultry and red meat samples collected in 2008 (Ennaji, Timinouni, Ennaji, Hassar, & Cohen, 2008). A study in the city of Rabat showed increasing prevalence of *L. monocytogenes* in various foodstuffs ranging from 0.8% in 2009 to 4.1% in 2011, with the highest prevalence (13.8%) in poultry products in 2010 (El Habib, Ennaji, El Ouardi, & Senouci, 2014). Another study has reported a prevalence of *L. monocytogenes* of 3.7% in chicken meat samples collected in Rabat in 2011–2012 (Khallaf, Ameer, Boraam, Senouci, & Ennaji, 2016). In the Kenitra region, *L. monocytogenes* was detected in 0.8% of raw milk samples (Hadrya et al., 2012), whereas in the region of Fez, the prevalence of *L. monocytogenes* was 5.9% in raw milk and traditional dairy products (El Marnissi, Benani, Cohen, El Ouali Lalami, & Belkhou, 2013). Nevertheless, the prevalence of *L. monocytogenes* in food items in Northern Morocco remains unknown.

Thus, the aims of this study were: i) to evaluate the presence of *L. monocytogenes* and other *Listeria* spp. in different food matrices collected in Tetouan (North-Western of Morocco), ii) to characterize the antibiotic susceptibility profiles of *L. monocytogenes* isolates, and iii) to type *L. monocytogenes* isolates by PCR serogrouping, pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and core genome MLST (cgMLST).

2. Materials and methods

2.1. Sample collection and bacterial isolation

A total of 1096 food samples, including dairy products ($n = 404$), bovine meat products ($n = 258$), pastry ($n = 162$), salads ($n = 143$), poultry meat products ($n = 103$), chickpea flour cooked with eggs sold in the street ($n = 20$) and mayonnaises ($n = 6$), were collected from January 2009 to August 2015. The samples were obtained from different food outlets in Tetouan (North-Western of Morocco). All samples were immediately transported to the laboratory at 5 ± 3 °C and were microbiologically examined within 1–2 h after sampling.

Isolation and identification of *Listeria* spp. were performed according to the official method for the detection of *L. monocytogenes* (ISO, 1996). Biochemical identification was performed using the API-*Listeria* system (bioMérieux, Marcy l'Etoile, France) (Bille et al., 1992). *Listeria* strains were stored at -80 °C in Brain Heart Infusion (Biokar, Beauvais, France) with 20% glycerol until further use.

2.2. PCR serogrouping

PCR serogrouping was determined by multiplex PCR targeting *lmo0737*, *ORF2819*, *ORF2110*, *lmo1118*, and *prs* gene regions, using primers and conditions as previously described by Doumith, Buchrieser, Glaser, Jacquet, and Martin (2004). PCR products were resolved on a 2% agarose gel containing 0.5 µg/ml of ethidium bromide (Eurobio, Courtaboeuf, France) and documented using the Bio-Rad Gel Doc 2000™ imaging system (Bio-Rad Laboratories, Milan, Italy). Electrophoresis profiles were interpreted according to Doumith et al. (2004) and Leclercq et al. (2011).

2.3. PFGE *Ascl/Apal* typing

L. monocytogenes PFGE fingerprints were obtained with the restriction enzymes *Ascl* (New England BioLabs, Massachusetts, Ipswich, USA) and *Apal* (MBI Fermentas, Burlington, Canada), as previously described by Graves and Swaminathan (2001) and Martin, Jacquet, Goulet, Vaillant, De Valk, and Participants in the PulseNet Europe Feasibility S (2006). The combined *Ascl/Apal* PFGE pulsotypes were analyzed using BioNumerics software version 6.6 (Applied Maths, Kortrijk, Belgium). Bands automatically assigned by the computer were checked visually and corrected manually when necessary. Cluster analysis of the individual or combined PFGE pulsotypes was performed using the unweighted pair group method with average linkage (UPGMA) method. The similarities of the banding patterns were estimated based on Dice's coefficient (Dice, 1945). The discriminatory power was determined by calculating the discrimination index (D) based on Simpson's index of diversity (Hunter & Gaston, 1988). Two PFGE patterns were considered to be similar if they differed by less than two bands (Martin et al., 2006).

2.4. MLST and cgMLST molecular typing

L. monocytogenes genome sequences were obtained after DNA extraction (DNeasy Blood & Tissue kit, Qiagen, Denmark), library preparation (Nextera XT DNA Sample kit, Illumina, California, USA) and sequencing using NextSeq 500 (2×150 bp) platform (Illumina, California, USA), according to the manufacturer's protocol. Draft assemblies were obtained using CLC Assembly Cell 4.3.0. (Qiagen, Hilden, Germany), as previously described (Moura et al., 2017). MLST and cgMLST profiles were obtained from genomic data as described previously (Moura et al., 2016; Ragon et al., 2008). cgMLST types were defined using the profile similarity cut-off of 99.6% (i.e. isolates sharing 7 or less allelic differences on 1748 alleles were considered the same type), as previously described (Moura et al., 2016). cgMLST clusters were defined as groups of isolates sharing the same cgMLST type.

2.5. Antimicrobial susceptibility testing

L. monocytogenes isolates were tested for their antimicrobial susceptibility using the agar disc diffusion method following the guidelines of the French committee on antimicrobial susceptibility testing (French Society of Microbiology [SFM], 2016; Morvan et al., 2010). Inoculum concentration was standardized by turbidimetry (Densimat photometer, bioMérieux). Mueller-Hinton agar plates (bioRad, Marne-la-Coquette, France) were inoculated with $1.0-2.0 \times 10^8$ cfu/ml and incubated at 35 °C for 24 h. The following discs (Oxoid Ltd., Hampshire, United Kingdom) of antimicrobial agents were used in this study: amikacin (30 µg), amoxicillin (25 µg), ampicillin (10 µg), cefotaxime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), fosfomicin (50 µg), fusidic acid (10 µg), gentamicin (15 µg), imipenem (10 µg), kanamycin (30 µg), levofloxacin (5 µg), lincomycin (15 µg), moxifloxacin (5 µg), nalidixic acid (30 µg), penicillin G (6 µg), rifampicin (30 µg), streptomycin (10 µg), sulfamethoxazole (200 µg), tetracycline (30 µg), trimethoprim (5 µg), tobramycin (30 µg), and vancomycin (30 µg). The diameters of growth inhibition zones were measured with Scan 4000 (Interscience, Saint Nom, France). Strains were classified as susceptible, intermediately resistant (reduced susceptibility), or resistant by using the breakpoints recommended by the French Committee on antimicrobial susceptibility testing (French Society of Microbiology, 2016). *Streptococcus pneumoniae* ATCC 49619 and *Escherichia coli* ATCC 25922 were used as the quality control strains (French Society of Microbiology, 2016).

Download English Version:

<https://daneshyari.com/en/article/5767330>

Download Persian Version:

<https://daneshyari.com/article/5767330>

[Daneshyari.com](https://daneshyari.com)