

# A first molecular characterization of *Listeria monocytogenes* isolates circulating in humans from 2009 to 2014 in the Italian Veneto region

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## SUMMARY

Listeriosis is a disease usually associated with the consumption of low-processed ready-to-eat food products contaminated by *Listeria monocytogenes*. In Italy, listeriosis has an incidence of 0.19-0.27 cases per 100000 persons. Since detailed information concerning the molecular characterization of listeriosis in the Italian Veneto region is currently lacking, we analyzed 36 *L. monocytogenes* clinical isolates collected between 2009 and 2014. Results show that the serotype 1/2a was the most represented among the tested samples. No antimicrobial resistance was detected in selected isolates representing the main pulsotypes.

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Gram-positive *Listeria monocytogenes* (*Lm*) is the etiologic agent of the foodborne disease listeriosis. Transmission occurs through contaminated food, such as ready-to-eat foods, soft cheese, raw fruits and vegetables. Although *Lm* may cause asymptomatic infections or self-limiting febrile gastroenteritis in healthy people, those with altered immunity such as pregnant women, neonates, elderly and immunocompromised patients may develop severe invasive infections characterized by a fatality rate of 20-30% (Todd and Noterman, 2011).

Listeriosis is a communicable disease in the European Union (EU), and in Italy the annual incidence is 0.19-0.27 per 100000 inhabitants per year, a lower rate than that reported in the EU (Zolin *et al.*, 2017). However, the incidence of listeriosis is increasing in both the EU and in Italy (European Food Safety Authority report, 2017). The different notification rates of listeriosis from the various Italian Regions does not guarantee a complete national coverage. In addition, very poor information on the molecular characterization of *Lm* from cases of listeriosis is available (Amato *et al.*, 2015; Eurosurveillance editorial team, 2015). This study analyzed 36 *Lm* clinical isolates from human samples collected during a surveillance activity coordinated by the Regional Reference Centre between 2009 and 2014 in Veneto region.

*Lm* was isolated from blood cultures and cerebrospinal

fluid (CSF) in 23 and 4 patients, respectively (in one case *Lm* was isolated simultaneously in both blood and CSF - samples 25 and 26 of this study), and once from pus. For eight patients the *Lm* isolates were directly submitted from regional Hospitals. More than 66% of cases occurred in patients older than 65 years and gender distribution revealed an equal number of cases occurring in women (17) and men (17). For two samples, data on patients were not available. *Lm* isolates were stored at -80°C until processing.

At the beginning of the study all the *Lm* isolates were confirmed by a MALDI-TOF spectrometry-based instrument (VITEK® MS, Biomérieux). A multiplex-PCR assay, combined with an additional PCR that amplifies the flagellin gene (*flaA*), was used for the prediction of the serovar groups including the main *Lm* serotypes involved in human listeriosis (Borucki *et al.*, 2003; Doumith *et al.*, 2004). Results showed that the serovar group, including serotypes 1/2a and 3a, is the main cause of listeriosis in Veneto (55%), followed by the serovar group including the serotypes 1/2b, 3b (25%), the serovar group comprising the serotypes 4b, 4d, 4e (16%), and the serovar group with the serotypes 1/2c, 3c (3%). In a previous study reporting data on *Lm* strains circulating in Italy between 2000 and 2010, the serotypes 1/2a, 4b, 1/2b represented 92% of the total cases (Pontello *et al.*, 2012). The temporal distribution of the serovar groups is reported in Figure 1.

To further characterize the *Lm* isolates, the gold standard assay, i.e. pulsed-field gel electrophoresis (PFGE) according to the PulseNet protocol with the *ApaI* enzyme (Schjørring *et al.*, 2016) was performed. The Bionumeric 7.5 software (Applied Maths NV) was used to analyze the PFGE fingerprints. Applying the UPGMA clustering algorithm to the distances for 36 samples results in the dendrogram shown in Figure 2, these samples grouped together according to the PCR assay. Results showed several pulsotypes suggesting different origins of the *Lm* involved in

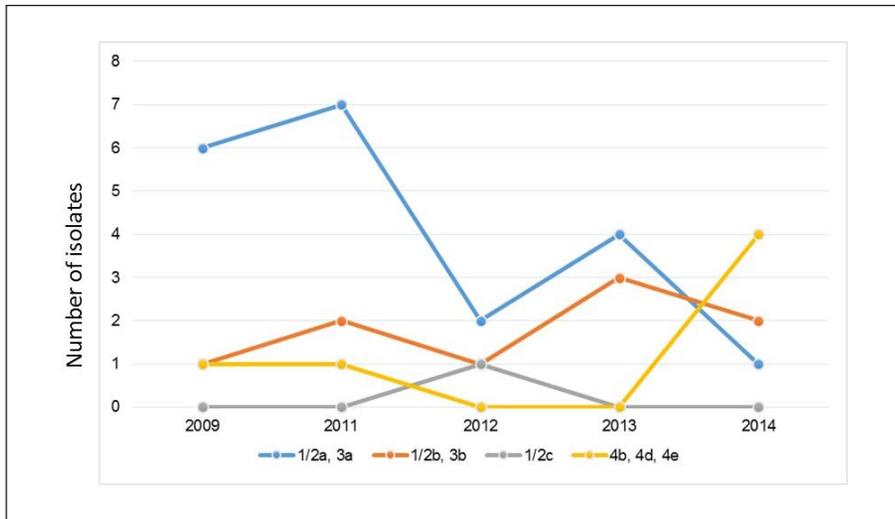
### Key words:

*Listeria monocytogenes*, Listeriosis, Pulsed-field gel electrophoresis (PFGE), Genotyping, Antimicrobial resistance.

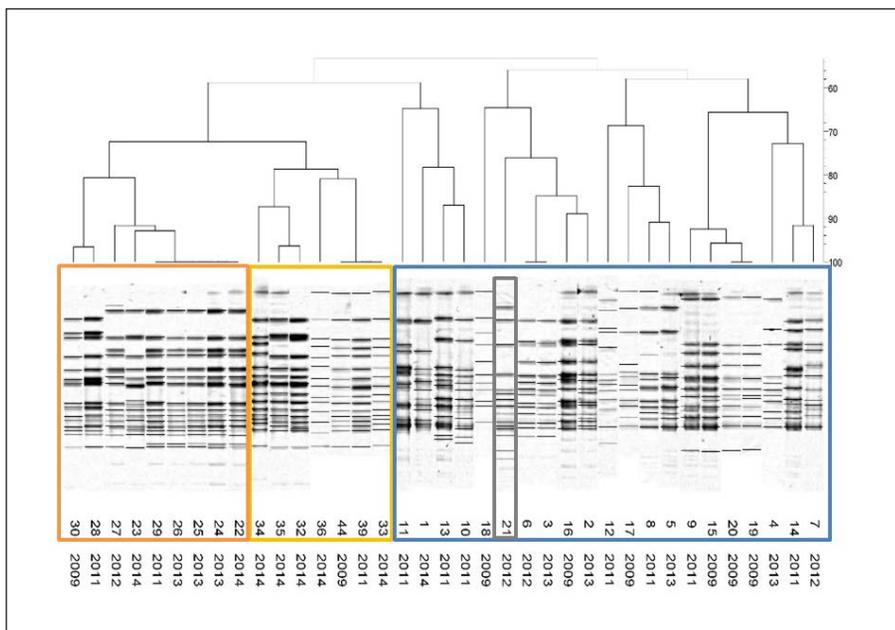
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**Figure 1** - Temporal distribution of the *Listeria monocytogenes* serovar groups determined by PCR.



**Figure 2** - PFGE profiles of *Listeria monocytogenes* strains isolated from human samples. Samples belonging to the different serovar groups are indicated with colored lines corresponding to those reported in figure 1.

the clinical cases. The most prevalent pulsotype clustered three isolates (samples 22, 24, 25-26) that were temporally and geographically related to the provinces of Treviso and Vicenza. It has also been shown that one pulsotype (samples 19 and 20) could be attributed to an event of maternal-foetal transmission, considering that the two samples were isolated from clinical samples collected in the same hospital from a mother and her newborn. This pulsotype was also detected in sample 15 collected nine months before from an elderly woman, but no relation was found with samples 19 and 20.

Finally, 18 isolates representative of the different pulsotypes were randomly selected and tested for their susceptibility to the following antimicrobials: ampicillin, linezolid, penicillin, ceftriaxone, erythromycin, cefotaxime, meropenem, levofloxacin, clindamycin, doxycycline, teicoplanin, daptomycin, trimethoprim/sulfamethoxazole, moxifloxacin, vancomycin, using the Sensititre ARIS® 2X system (Thermo Fisher Scientific) and following the European

Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. All the selected *Lm* strains analyzed were susceptible to the tested antimicrobials; indeed, only a minority of clinical antibiotic-resistant *Lm* strains have been described in the literature so far (Camargo *et al.*, 2017).

Overall, genotyping with PFGE did not show a particular relation between the collected isolates, suggesting multiple origins for *Lm* strains involved in infections that required admission to hospitals. Furthermore, antimicrobial resistance was not detected in the selected isolates representing the different pulsotypes. However, implementation of surveillance with molecular typing of *Lm* isolated from food and humans is needed for a rapid identification and control of outbreaks, and to trace the origin of infection. Furthermore, improvements to the genotyping approach, i.e. PFGE combined with other molecular techniques (e.g. multilocus sequence typing) could represent a strategy for an in-depth genetic characterization of *Lm* isolates.

### Competing interests

The authors declare that there is no conflict of interest regarding the publication of this article.

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