



Listeria monocytogenes National Reference Laboratory

> Whole Genome Sequencing of microbial pathogens: database and bioinformatic analysis National Reference Centre

# Identification of persistent *Listeria monocytogenes* in food processing plants by using WGS can lead to modify cleaning and sanitation procedures

Antoci S<sup>1</sup>, Acciari VA<sup>1</sup>, Cammà C<sup>2</sup>, Calistri P<sup>3</sup>, Di Pasquale A<sup>2</sup>, Pomilio F<sup>1</sup>, Centorotola G<sup>1</sup>, Cito F<sup>3</sup>, Curini V<sup>2</sup>,
Del Matto I<sup>1</sup>, Di Domenico M<sup>2</sup>, Di Marzio V<sup>1</sup>, Iannetti S<sup>3</sup>, Marfoglia C<sup>1</sup>, Rossi F<sup>1</sup>, Torresi M<sup>1</sup>, Rinaldi A<sup>2</sup>
<sup>1</sup> National Reference Laboratory for *Listeria monocytogenes*, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'.
<sup>2</sup> National Reference Centre for Whole Genome Sequencing of microbial pathogens: database and bioinformatic analysis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'.

<sup>3</sup> National Reference Centre for Veterinary Epidemiology, Programming, Information and Risk Analysis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'.



## Introduction

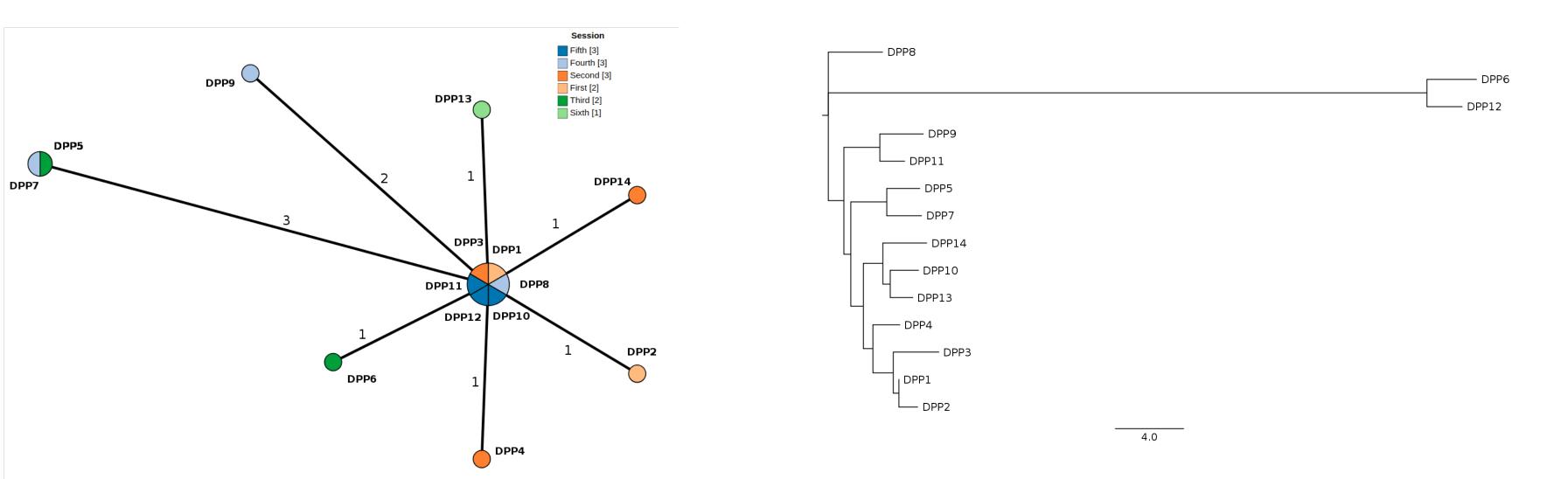
The presence of *Listeria monocytogenes* in food plants is a matter of great concern since the ability of this organism to colonize and persist in food processing plants increases the risk of food contamination. To date there is no agreement on "persistence", nevertheless, strains definition of the repeatedly isolated over time in the same plant are considered persistent (Pasquali et al., 2018). The reasons beyond the persistence of some strains of *L. monocytogenes* in food processing plants are also still on debate. It has been proposed that the persistence is due to opportunistic colonization of harbourage sites. Alternatively, persistent strains have been linked to their ability to produce biofilms, and recently also to the resistance to quaternary ammonium compounds (QAC) generally used for sanitation (Cherifi et al., 2018). In this study, we investigated the prevalence and the persistence of *L. monocytogenes* strains along the production plants of one dairy and one meat processing plant in Central Italy.

#### **Results**

A total of 15 out of 190 (8%) environmental samples were positive for *L. monocytogenes* in the dairy processing plant, and 28 out of 200 (14%) in the meat processing plant. Isolates from dairy plant were serotyped as 1/2c, whereas in meat processing plant three different serotypes 1/2a, 1/2b, 1/2c were found. As result of the cgMLST analyses, a single cluster was observed in the dairy processing plant, represented by detected pulsotypes (GX6A12.0002-GX6A16.0007, two GX6A12.0001-GX6A16.0007) and a single sequence type (ST9). The isolates of this cluster were always found in two different refrigerating rooms of the plant during sampling sessions (Figure 1). SNPs analysis confirmed high similarity among the isolates of the cluster (max distance 11 SNPs), except for samples DPP6 and DPP12 for which the distance observed was about 45 SNPs compared to the other isolates (Figure 2). In meat processing plant, cgMLST analysis highlighted two main clusters (Figure 3) in almost all sampling sessions and in all plant production areas, even after sanitation procedures. Cluster 1 includes 13 strains attributable to single pulsotype (GX6A12.0349-GX6A16.0255) and belonging to a novel sequence type never described before, while Cluster 2, composed by 11 strains, was represented by different pulsotypes (GX6A12.0104-GX6A16.0002, GX6A12.0140-GX6A16.0002, GX6A12.0103-GX6A16.0002) but a single sequence type (ST121). No metal and detergent resistant genes were found in the persistent strains of the dairy plant nor in the strains belonging to the Cluster 1 of the meat plant. Differently, all the 11 strains of the meat plant Cluster 2 harbor the transposon Tn6188 responsible for tolerance to benzalkonium chloride.

## **Materials and methods**

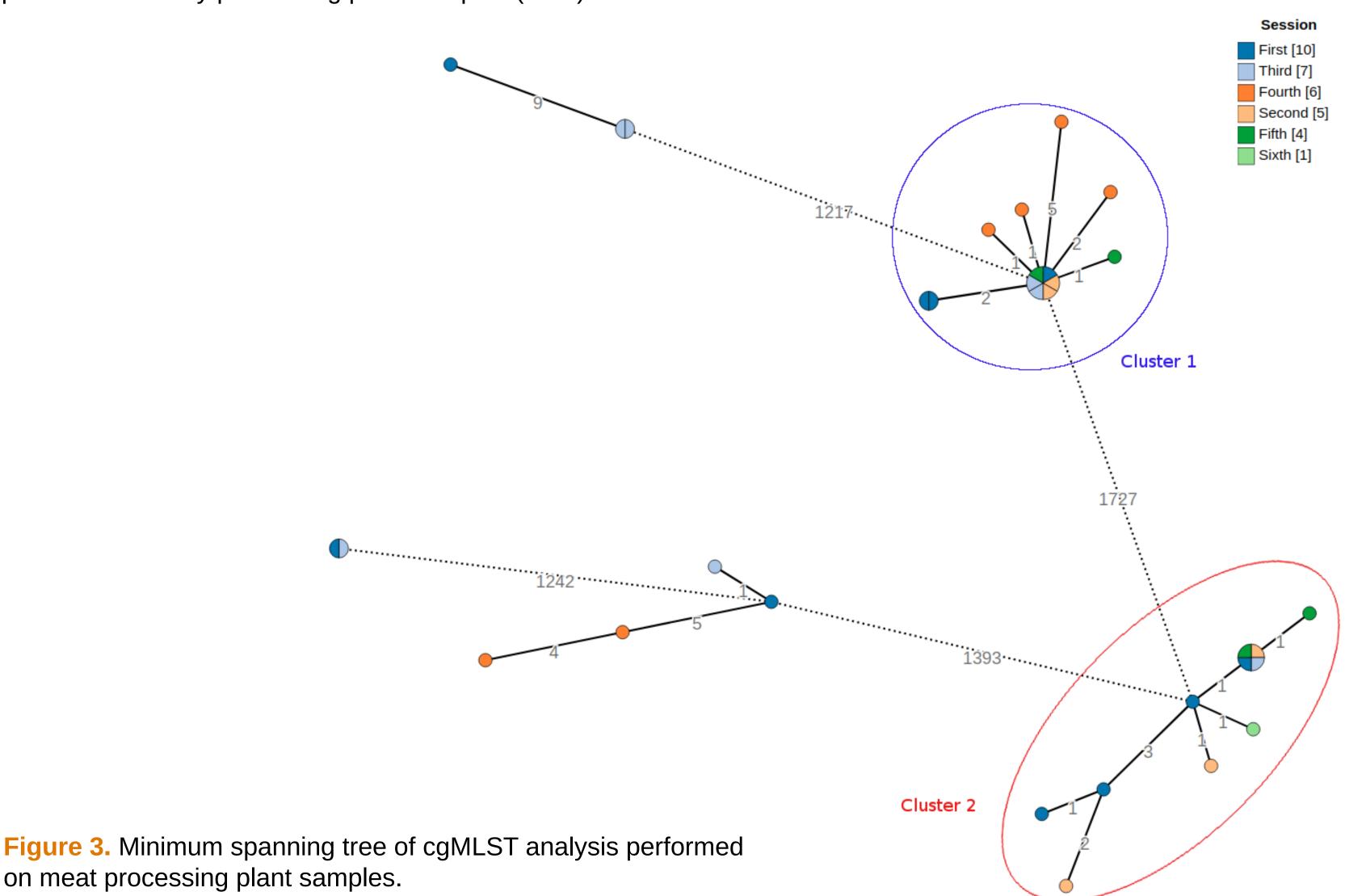
Sampling was performed in 6 sessions, from March 2017 to May 2018, and 30-35 environmental samples were collected per session in each plant, from food-contact and non food-contact surfaces across different production areas. *L. monocytogenes* isolates were characterized by serotyping and PFGE according to the PulseNet protocol. DNA was also subjected to Next Generation Sequencing (NGS) using NextSeq 500 Illumina platform in standard 150 bp paired-end reads. Quality control was performed by FastQC and trimming was carried out by Trimmomatic (Bolger et al., 2014). A *de novo* assembly was performed by Spades 3.11 (Nurk et al., 2013) and *in silico* MLST 7 loci was defined. The cgMLST analysis was carried out by chewBBACA (Silvia et al., 2018) using the Pasteur Institute scheme for *L. monocytogenes* and for a higher resolution SNPs analysis was performed using kSNP3 (Shea et al., 2015). Finally, contigs from persistent strains were uploaded to the Pasteur Institute platform (https://bigsdb.pasteur.fr) and queried for "Metal and Detergent Resistance" loci.



**Figure 1.** Minimum spanning tree of cgMLST analysis perfomed on dairy processing plant samples (DPP).

**Figure 2.** Neighbor joining tree of SNPs analysis perfomed on dairy processing plant samples (DPP).

## **Discussion and Conclusions**



These results show that persistence is a constant evidence into both food producing plants investigated. CgMLST and SNPs analysis improved the identification of the clusters, being able to characterize the strains with high resolution. The 11 strains of the meat plant Cluster 2 containing Tn6188, belonged to the ST121. This is one of the most prevalent STs worldwide and it has been isolated as a persistent type in food and food-associated environments (in particular in meat and meat products) in different countries (Ortiz et al. 2016). The presence of the Tn6188 responsible for tolerance to benzalkonium chloride could explain the persistence features of the Cluster 2 isolated in the meat plant. Conversely, the reason why the strains of the dairy plant and those of the Cluster 1 of the meat plant can persist in the food processing environment still needs elucidation. In conclusion, genomics may give a crucial help to put in place different cleaning procedure in case of persistent strains. Further investigation will include the assessment of biofilm formation ability and phenotypic resistance to quaternary ammonium compounds of the persistent *L. monocytogenes* strains. Based on the results of the sampling, cleaning and sanitation procedures have been modified in both dairy and meat plants. The ability to differentiate between sporadic and persistent strains using WGS data highlighted in this study is a key element for risk management actions to avoid recurrent food contamination.

#### **Acknowlegments**

This work was founded by the Italian Ministry of Health (IZS AM 02/14 RC)

#### **References**

• Bolger, A. M.et al. (2014). Trimmomatic: A flexible trimmer for Illumina Sequence Data. Bioinformatics, btu170.

• Nurk S. et al. (2013) Assembling Genomes and Mini-metagenomes from Highly Chimeric Reads. In: Deng M., Jiang R., Sun F., Zhang X. (eds) Research in Computational Molecular Biology. RECOMB 2013. Lecture Notes in Computer Science, vol 7821. Springer, Berlin, Heidelberg

- Silva M, et al. chewBBACA: A complete suite for gene-by-gene schema creation and strain identification[published online ahead of print, 2018 Mar 15]. Microb Genom. 2018;4(3):e000166. doi:10.1099/mgen.0.000166
- Shea N et al. kSNP3.0: SNP detection and phylogenetic analysis of genomes without genome alignment or reference genome, Bioinformatics, Volume 31, Issue 17, 1 September 2015, Pages 2877–2878
- Cherifi T et al. Genomic characterization of Listeria monocytogenes isolates reveals that their persistence in a pig slaughterhouse is linked to the presence of benzalkonium chloride resistance genes. BMC Microbiology (2018) 18:220
- Pasquali F et al. Listeria monocytogenes Sequence Types 121 and 14 Repeatedly Isolated Within One Year of Sampling in a Rabbit Meat Processing Plant: Persistence and Ecophysiology Frontiers in Microbiology March 2018 | Volume 9 | Article 596
- Ortiz S et al. The Connection between Persistent, Disinfectant-Resistant Listeria monocytogenes Strains from Two Geographically Separate Iberian Pork Processing Plants: Evidence from Comparative Genome Analysis. Applied and Environmental Microbiology January 2016 Volume 82 Number 1