Comprehensive typing and genetic analysis of *L. monocytogenes* isolates: implication for food safety and antibiotic resistance surveillance

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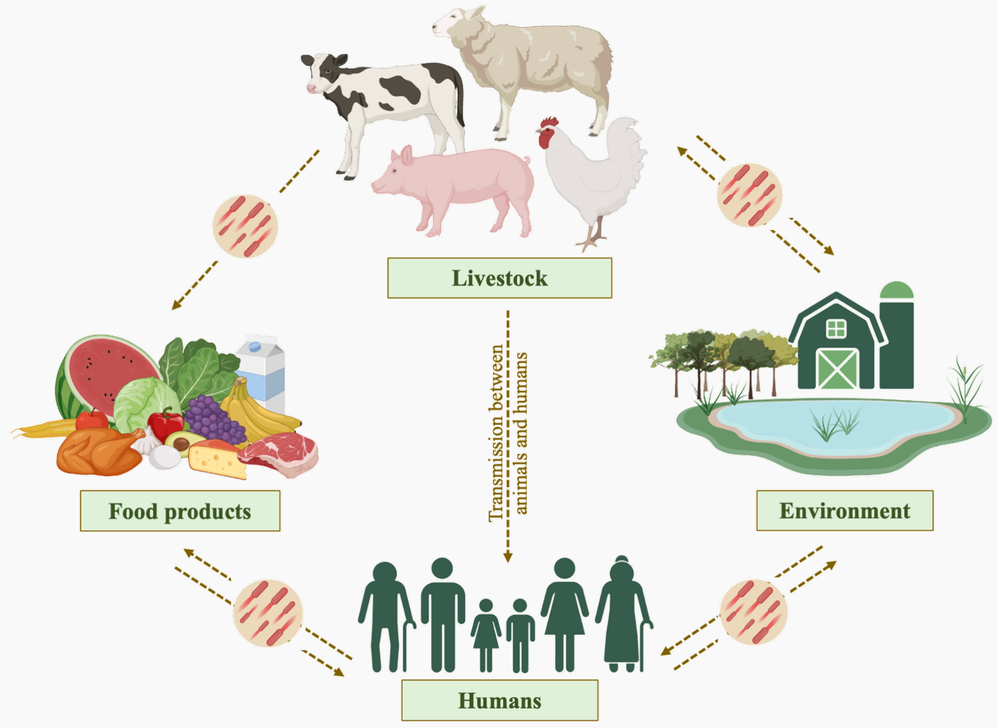


Figure 1. Potential routes for the transmission of *L. monocytogenes*, a foodborne pathogen

Background: Challenges posed by antibiotic resistance in *L. monocytogenes*

Listeria species are commonly found in various environments and contaminated food, with livestock serving as a significant source of foodborne pathogens. Among these species, *Listeria monocytogenes (L. monocytogenes)* is particularly noteworthy as it can affect both livestock and humans. Antibiotics are frequently used in food animals for disease treatment and prevention on a large scale. This practice can lead to the selection of antibiotic-resistant bacterial strains, which can then spread to humans through the food chain. Consequently, *L. monocytogenes*, a ubiquitous foodborne pathogen, has been associated with global outbreaks of foodborne illnesses.

To address this concern, the aim of the study was to conduct comprehensive typing and genetic analysis of 13 *L. monocytogenes* isolates obtained from food and food-processing environments.

Comprehensive typing and genetic analysis of *L. monocytogenes* isolates

Among the 13 *L. monocytogenes* isolates, eight sequence types (ST) were identified: two isolates were identified as belonging to ST9; one as ST155; four as ST3, two as ST121, one as ST8; one as ST87; one as ST1; and one new ST belonging to CC121. Core-genome clustering analysis of *L. monocytogenes* was made to assess the genetic relatedness among the isolates.

The core genome Multilocus Sequence Typing (cgMLST) analysis revealed three genetic clusters of high closely related isolates (≤7 allelic differences (ADs)): cluster 1. Regarding *L. monocytogenes* typing, ST3 was the most prevalent among the isolates, found in 4 isolates, followed by ST9 and ST121. Some of these isolates, like ST1, ST9 and ST87, were previously associated with human clinical cases.

Table 1. Sequence types identified in *L. monocytogenes* isolates

Samples	MLST (7 Loci)	cgMLST (1748 Loci)
1	New ST (CC121)	
2	ST9	
3	ST155	
4	ST3	Cluster_1(≤7 ADs)
6	ST9	
7	ST3	Cluster_1(≤7 ADs)
8	ST3	Cluster_1(≤7 ADs)
10	ST121	
11	ST8	
12	ST3	
13	ST87	
14	ST121	
15	ST1	

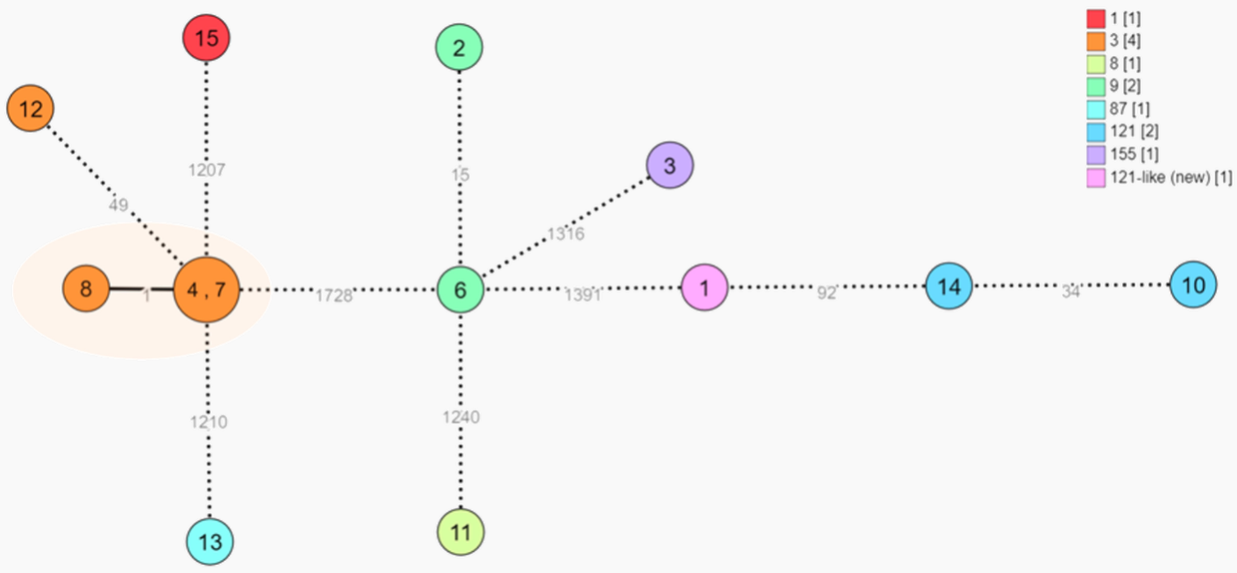
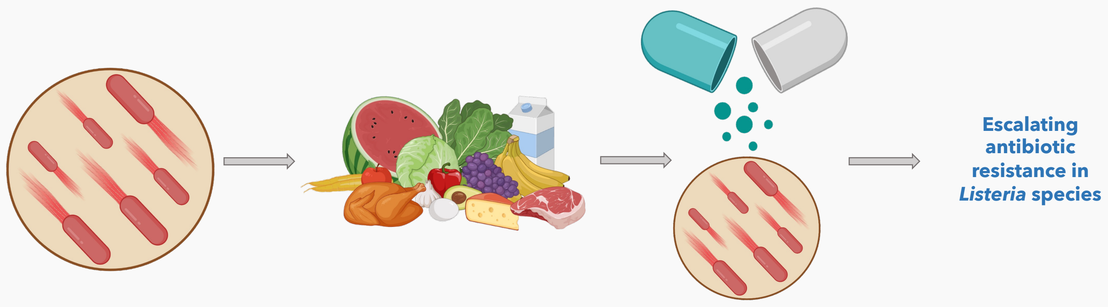


Figure 2. Core-genome clustering analysis of *L. monocytogenes* (thirteen isolates). The Minimum Spanning Tree (MST) was constructed based on the cgMLST 1748-loci Pasteur schema. Each circle (node) contains the sample code and represents a unique allelic profile, with numbers on the connecting lines representing allelic distances (AD) between nodes. Cluster analysis was conducted with ReporTree and data visualization was adapted from GrapeTree dashboard. Straight and dotted lines reflect nodes linked with allelic distances (ADs) below and above a threshold of seven ADs, which can provide a proxy to the identification of genetic clusters with potential epidemiological concordance. The surrounding orange shadow highlights a cluster supported by ≤7ADs.

Conclusions: Food safety and public health

We used Whole Genome Sequencing (WGS) alongside epidemiological data to link strains to human illnesses and potential food sources. Through cgMLST analysis, we identified genetic clusters of closely related isolates, all linked to the same producers.

This approach helped us pinpoint common sources of contamination and gain insights into the transmission dynamics of *L. monocytogenes* in the context of food safety and public health. The escalating antibiotic resistance in *Listeria* species, particularly in *L. monocytogenes*, emphasizes the need for heightened surveillance and improved hygiene practices in the food industry to curb the spread of antibiotic resistance and ensure food safety.



Acknowledgments: This work was supported by the projects UIDP/ CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). This work was supported by the Associate Laboratory for Green Chemistry–LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020). Adriana Silva is grateful to FCT (Fundação para a Ciência e a Tecnologia) for financial support through the PhD grant SFRH/ BD/04576/2020.