# Understanding virulence variability among *Listeria monocytogenes* Clonal Complexes

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## Introduction and Objective

*Listeria monocytogenes* is a foodborne pathogen that causes human listeriosis. This pathogen is characterized by an intra-species heterogeneity so, its strains can be grouped into clonal complexes (CCs) defined either as **hypervirulent** – mainly associated with clinical cases, or **hypovirulent** – associated with food or food processing environments and causing infections mainly in highly susceptible individuals [1, 2]. This pathogen has a complex infection mechanism represented in Figure 1. A crucial step for infection is the internalization of the pathogen into host cells, which is accomplished by the linkage of internalins (A and B) to host receptors. The differences in virulence potential may be explained by the presence of mutations in key virulence genes. The purpose of this study was to explore the virulence capacity based of *L. monocytogenes* CCs through *in vitro* infection assays and to further assess mutations in the *inlA* gene, which is crucial for the invasion into intestinal epithelial cells by the pathogen.

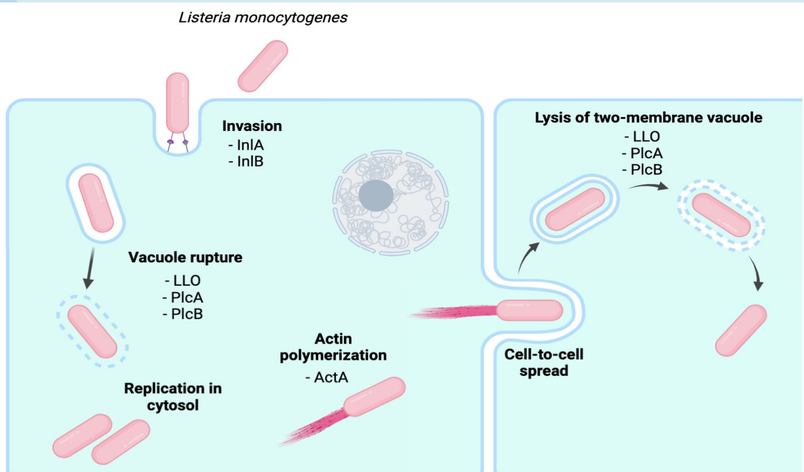
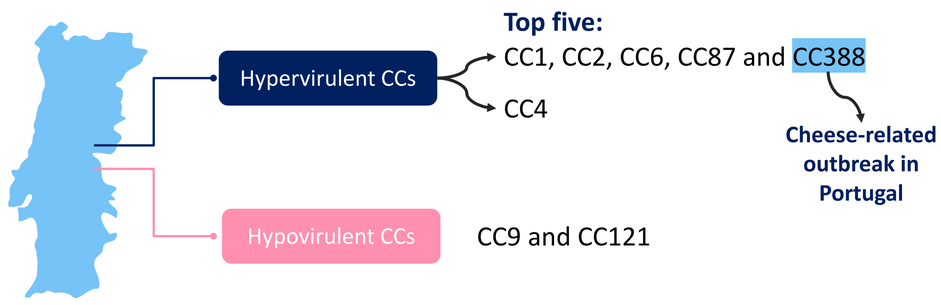


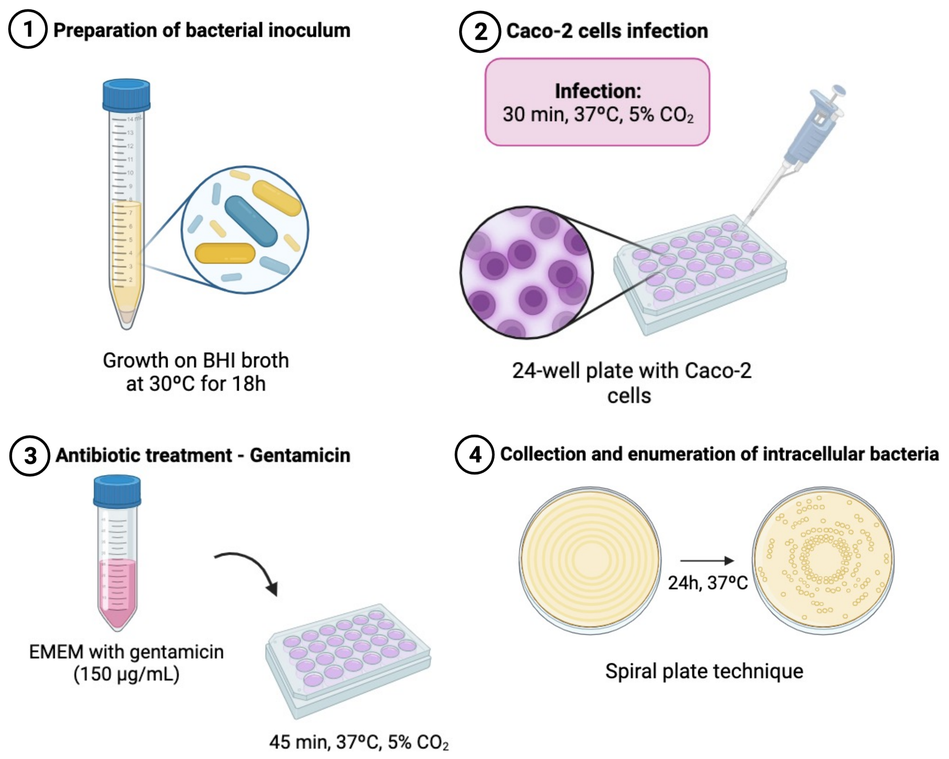

**Figure 1:** Illustration of the invasion and replication of *L. monocytogenes* into eukaryotic cells. Invasion of host cells is achieved through InlA and InlB that binds, respectively, to E-cadherin and C-Met receptors on the host cell. After internalization, the bacteria are engulfed by a vacuole, which is disrupted. Subsequently, the pathogen multiply and form an actin tail, that allows the pathogen to disseminate to proximate cells. After cell-to-cell spread, bacteria are entrapped into a two-membrane vacuole that is lysed. Adapted from Radoshevich and Cossart, 2018.

## Methodology

Eleven isolates were selected based on the top five hypervirulent CCs occurring in Portugal and one strain from CC4 was also included, as this hypervirulent CC is one of the best characterized CCs around Europe. Five isolates from hypovirulent CCs were also used.




Invasion assays were performed in Caco-2 cells and subsequently the presence of PMSC mutations in the *inlA* gene were assessed, using the MEGA software (version 10.1.8)




## Results

Our results show a clear-cut difference on invasion capacity between isolates from hyper- and hypovirulent CCs, with the latter showing significantly reduced efficiencies (Figure 2). Sequence analysis of the *inlA* gene, revealed that only hypovirulent CCs carried PMSC mutations, leading to truncated forms of the InlA protein. A 3-codon deletion was detected in isolates from CC6 (Table 1).

**Table 1.** Mutation types that encode premature stop codons (PMSCs) and the three-codon deletion.

| Mutation type | Isolates (n) | Clonal Complexes |
|---|---|---|
| PMSC mutation type 12 | 2 | CC9 |
| PMSC mutation type 11 | 1 | CC9 |
| PMSC mutation type 6 | 2 | CC121 |
| 3-codon deletion | 2 | CC6 |

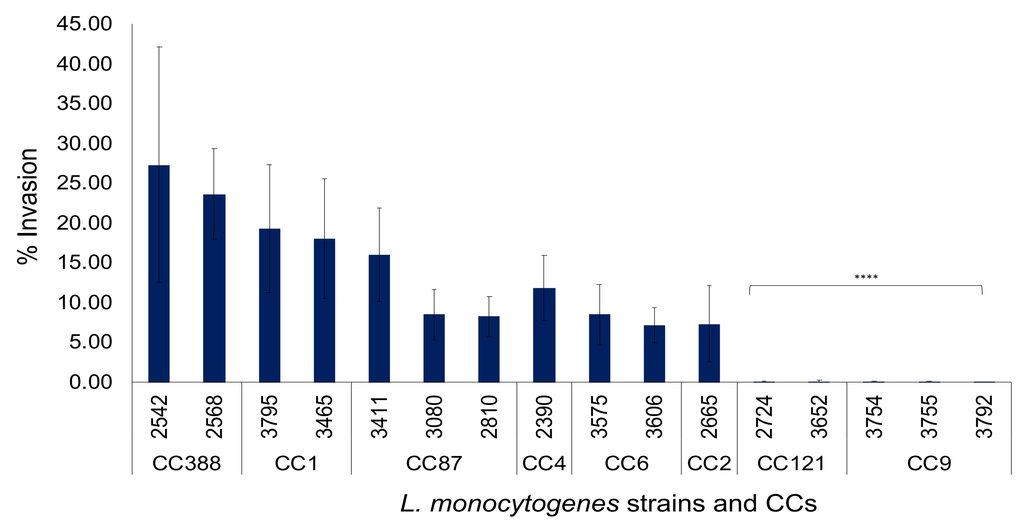



**Figure 2.** The invasion efficiency of *L. monocytogenes* into Caco-2 cells. The bars stand for the mean values of invasion efficiency of at least three independent assays and the error bars represent standard deviations. Hypovirulent CCs were statistically different from hypervirulent CCs (****, p<0.0001).

## Conclusions

- Hypervirulent CCs showed significantly higher invasion efficiencies
- PMSCs reduced the invasion capacity of hypovirulent CCs; the 3-codon deletion did not affect invasion
- *inlA*-dependence to cross intestinal cells may be crucial for infection, reduces the likelihood of host infection

## References

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## Acknowledgements

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