



Persistence of *Listeria monocytogenes* in food processing environments

Lauren Alteio
Senior Researcher
FFoQSI GmbH



ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
AGRICULTURAL UNIVERSITY OF ATHENS



UNIVERSIDAD
DE BURGOS



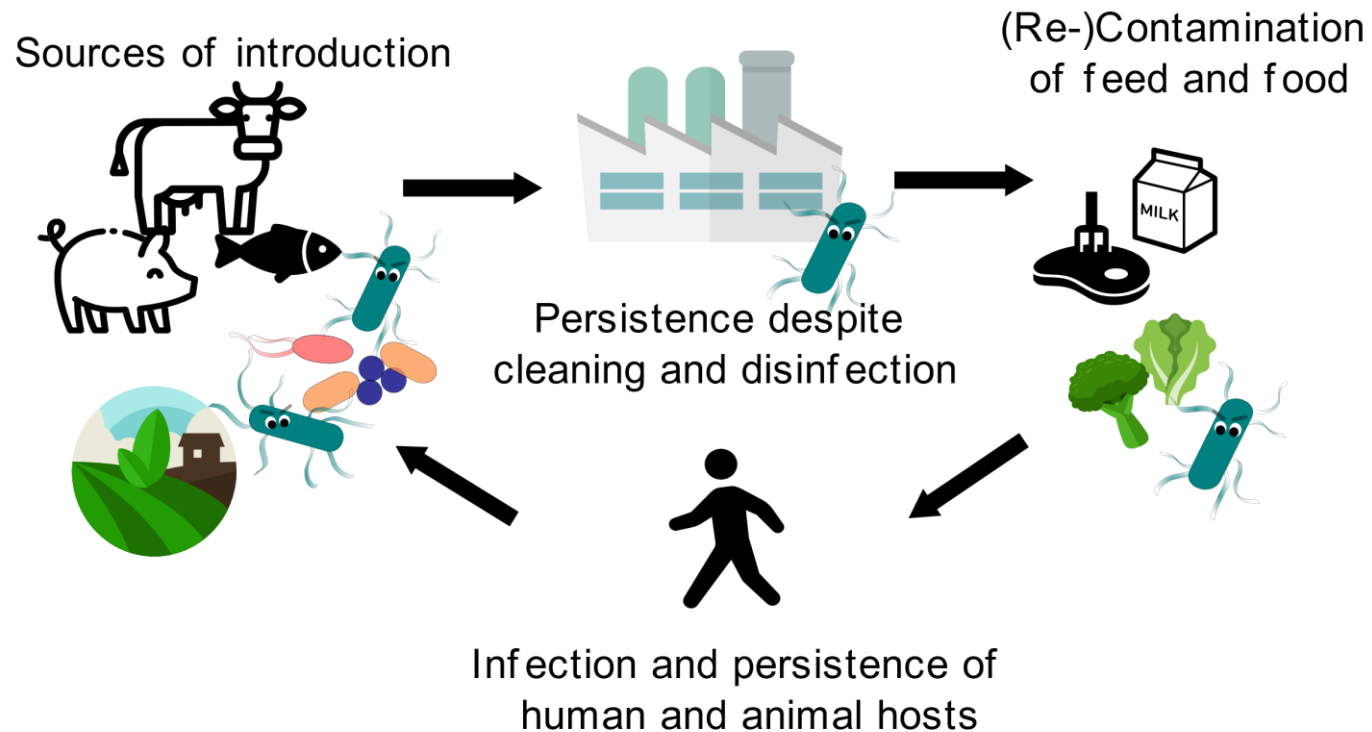
This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 101060698

7/6/2026

Persistence of *L. monocytogenes* in food processing environments



Persistence → long-term survival of identical *Listeria monocytogenes* isolates in the same facility

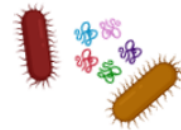


Challenges in disentangling *L. monocytogenes* persistence



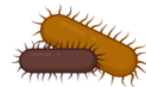
Biofilm formation (single and multispecies).

Yuan et al., 2020 ; Fagerlund et al., 2021.



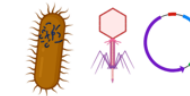
Inter- and intra-species interaction.

Madsen et al., 2012; Yuan et al., 2018, 2020.



Co-occurring microbiome.

Rolon, Chandross-Cohen, et al., 2023; Rolon, Tan, et al., 2023; Tan et al., 2019.



Mobile genetic elements.

Madsen et al., 2012; Kwon et al., 2020; Palma et al., 2020.




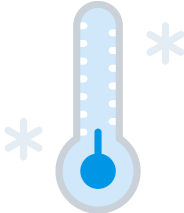

Genomic traits.

(Anast et al., 2022; Chen et al., 2020; Hingston et al., 2019, 2017; Schmitz-Esser et al., 2021)

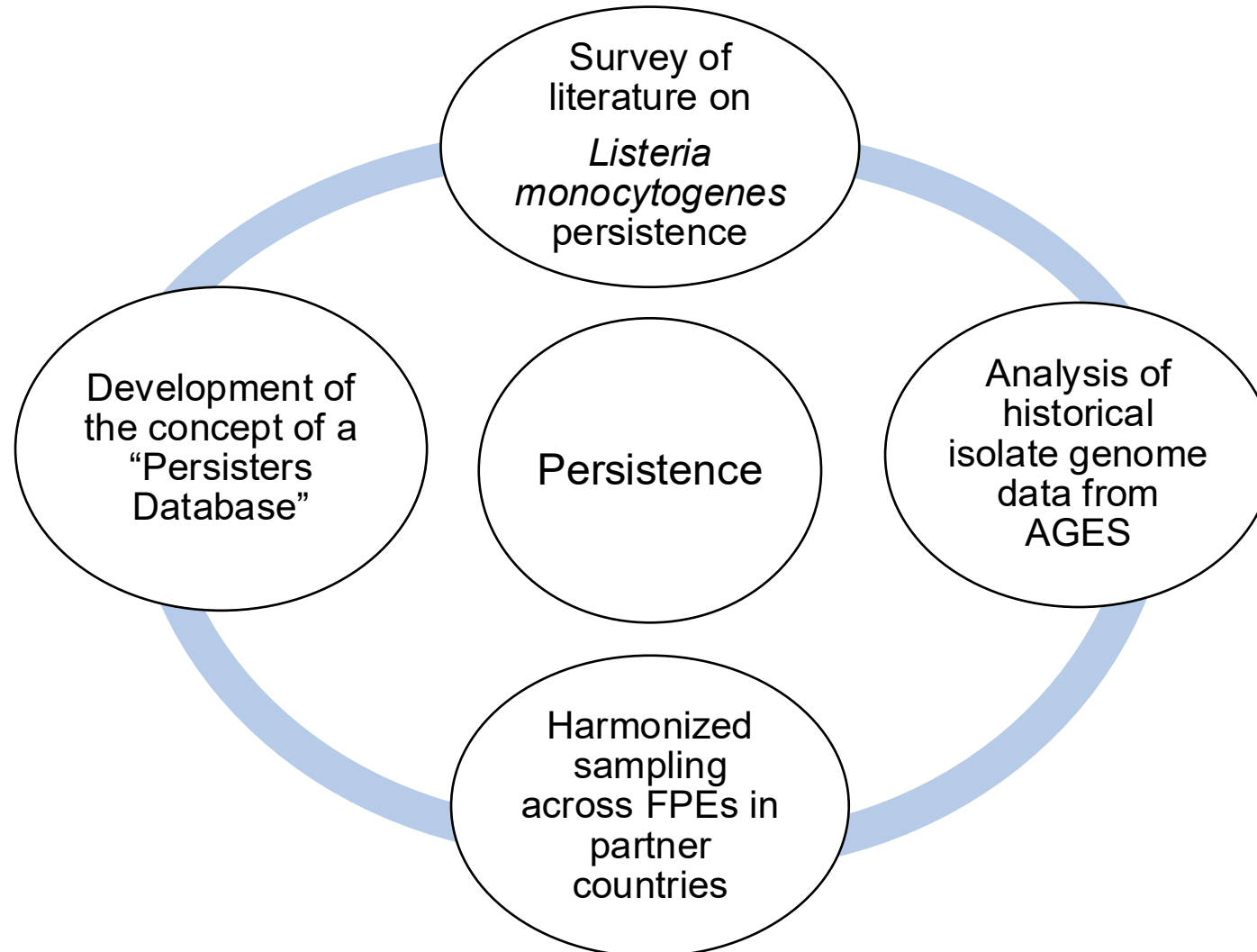
Various factors investigated in association with persistence of *L. monocytogenes* – no clear picture!

Goals in FoodSafeR

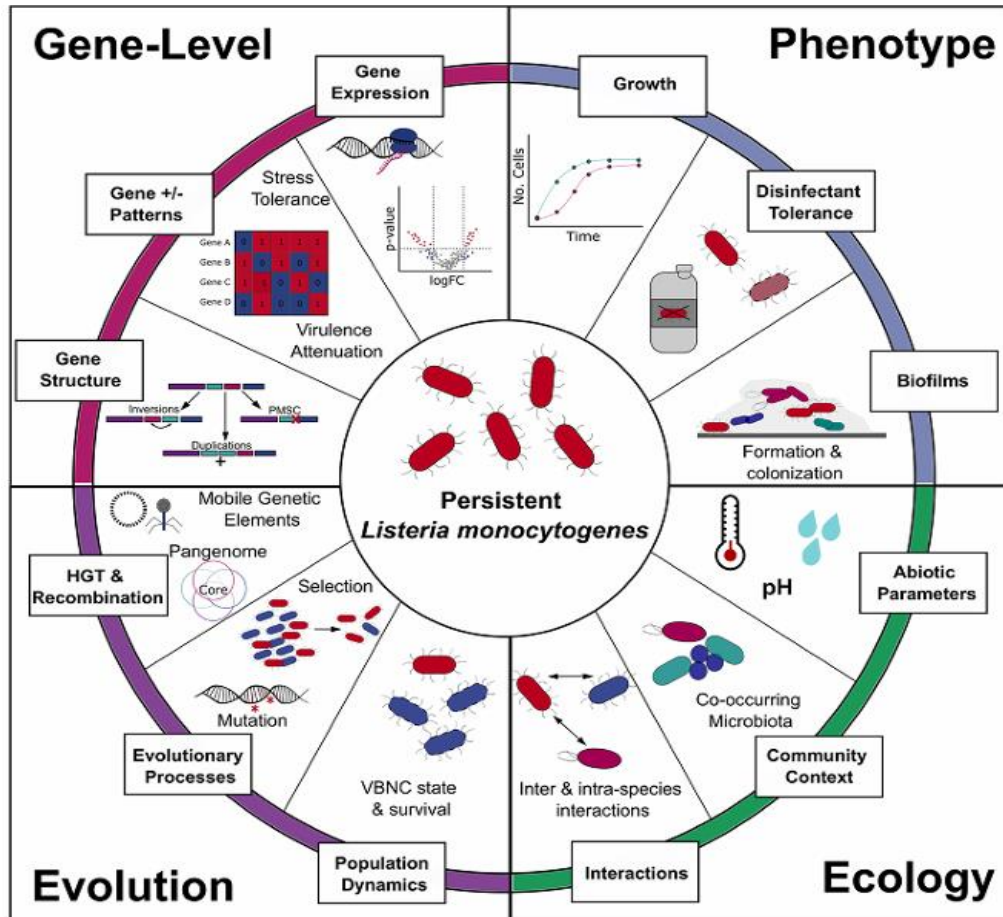


- Identify **genetic traits** linked to *L. monocytogenes* persistence in food processing environments 
- Apply a more **holistic view** of persistence by integrating **expanded metadata** into studies of persistence 
- Generate a concept for the “**Persisters Database**” containing four data facets:
 1. Sample metadata
 2. Isolate sequence typing & persistence classification
 3. Phenotypic & genetic traits
 4. Microbial co-occurrence data

Multi-faceted approach in FoodSafeR



Historical perspective from literature and AGES culture collection



- Leveraged large database generated by the Austrian Agency for Health and Food Safety (AGES) with >28,000 *L. monocytogenes* genomes sequences
- Analyzed 1,699 whole genome sequences from *L. monocytogenes* isolates from 23 sequence types across 18 Austrian FPEs

Detection and characterization of persistent isolates across partner countries



Partner	Country	Facility Type
FFoQSI	Austria	1 Dairy, 1 Fish
AUA	Greece	2 Meat
UBU	Spain	5 Dairy, 2 Meat



Felix Spiegel

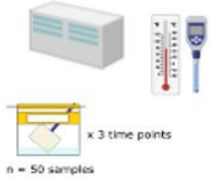
- **Three** repeated samplings, **50 samples** per event
- Sampled food contact/non-contact, before/after cleaning and disinfection
- **Eleven facilities** sampled – 6 Dairy, 4 Meat, 1 Fish
- Total of **1,538 swab samples** collected



Isolation, sequencing, and typing of *L. monocytogenes*



1. Facility Sampling



Swabs and Metadata

Hotspots: food contact surfaces, drains, floors, pallets, conveyor belts, slicers/cutting machines, smear robots, etc.

2. Preparation of environmental samples



+ 1x PBS



Rapid L. mono

3. Enrichment and Isolation of Listeria



+ HF
48h @ 30°C



+ VF
48h @ 37°C



Rapid L. mono



PCR Confirmation

Microbiome Sample DNA



qPCR (16S, prfA)



Cryo Culture

Sequencing
AGES and Nestle



- Hosted 6-month research stay
- Whole genome sequencing and 16S rRNA gene sequencing

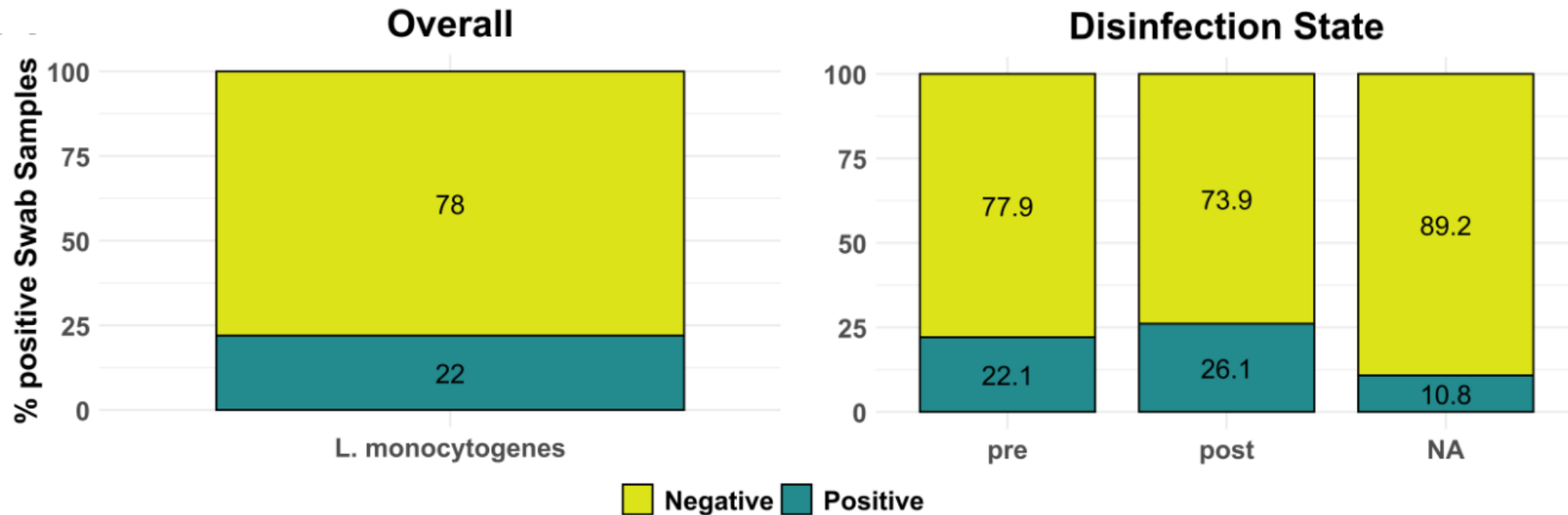


- Multi-locus sequence typing

Following modified ISO 11290-1:2017

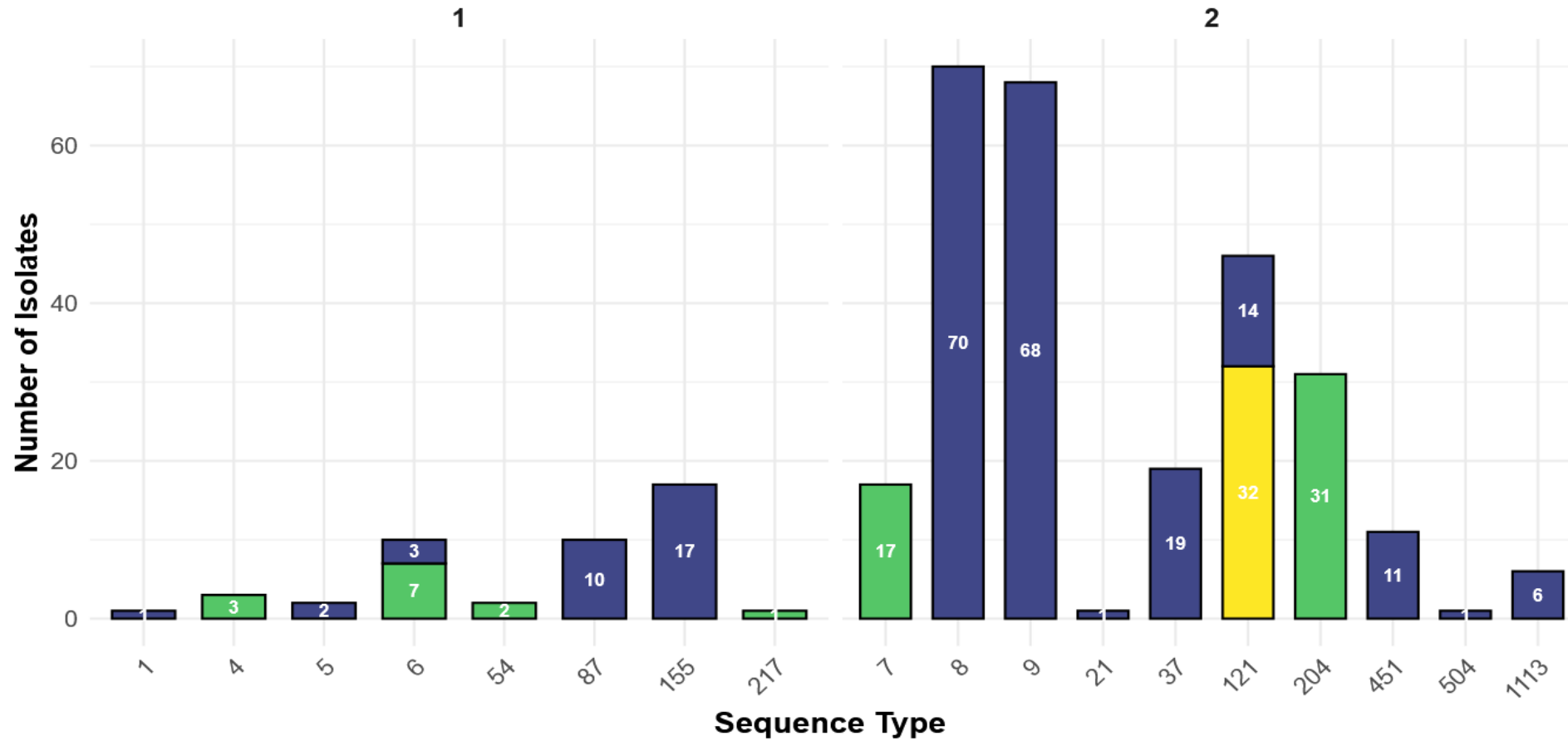


L. monocytogenes detection rate across FPEs



- Of 1,538 swab samples, 338 were positive for *L. monocytogenes* = **22% positive**
- Detection rate remained **similar before and after cleaning and disinfection**

Sequence typing of 316 genomes revealed 18 STs across FPEs



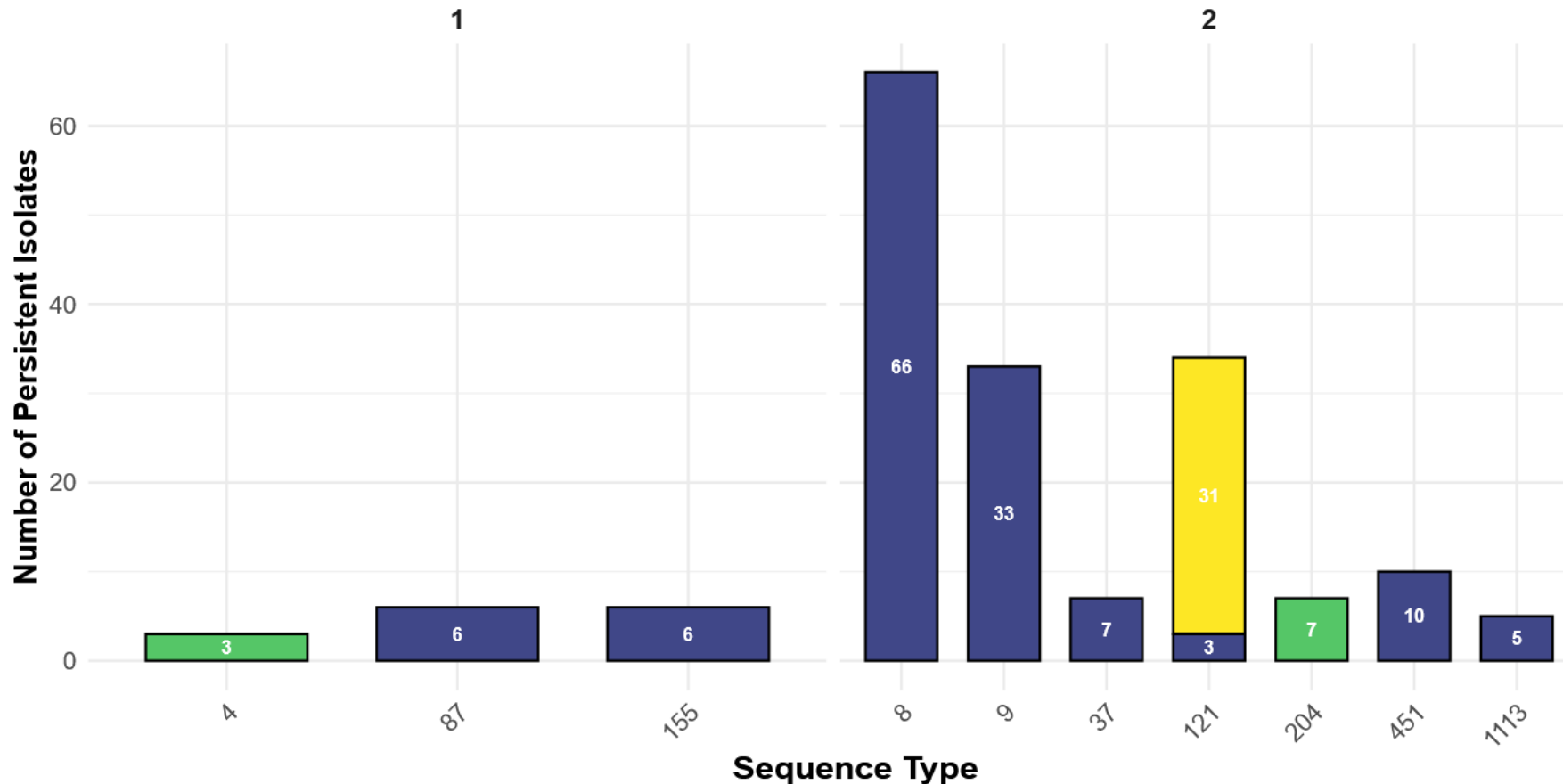
Operational definition of persistence at the Single Nucleotide Polymorphism (SNP) level



Working definition: the repeated isolation (3x) of **identical isolates** from the same facility location over a period of at least two calendar years.

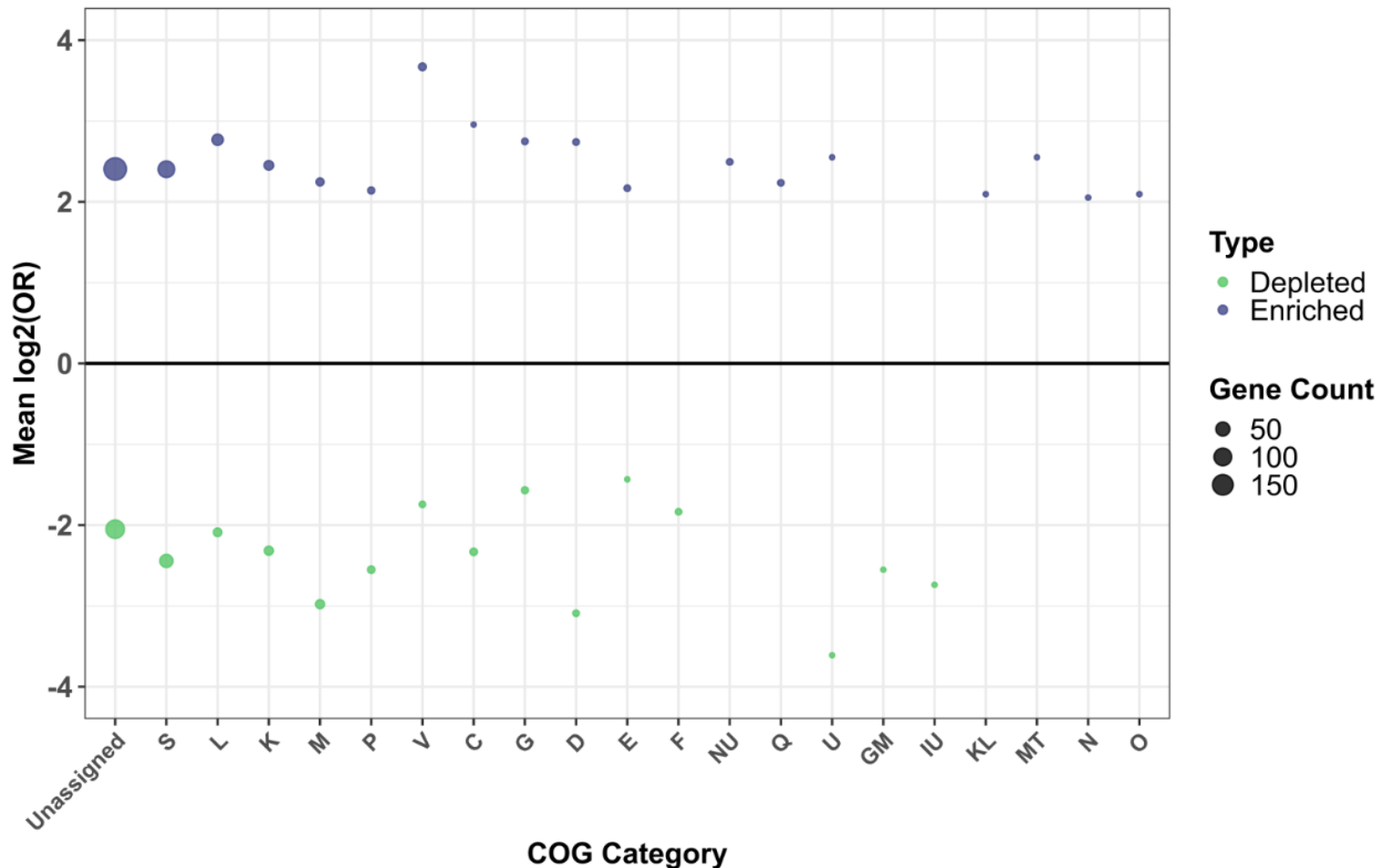
Identical isolates: ≤ 10 SNP differences based on whole genome SNP analysis.

56% of *L. monocytogenes* isolates classified as persistent (177 isolates)



- 10 STs and 15 cgMLSTs have isolates classified as persistent
- Persistence identified as a widespread phenomenon

Protein families associated with persistence



- 364 genes associated with persistence
- Persistent isolates enriched in genes involved in **transcriptional regulation, DNA repair, and phage elements.**
- No differences in virulence gene profiles between persistent and non-persistent isolates
- Stress tolerance gene profiles largely ST-type specific



Concept for the „Persisters Database“

Sample Metadata	Isolate Sequence Typing & Persistence Classification	Genetic & Phenotypic Traits	Microbial Co-occurrence
<ul style="list-style-type: none">• Sample location• Surface type• Temperature and humidity• Disinfection status & disinfectant used	<ul style="list-style-type: none">• 316 <i>L. monocytogenes</i> isolates and whole genome sequence data• 18 STs and 70 CTs• 177 persistent, 139 non-persistent	<ul style="list-style-type: none">• Accessory genome, virulence profile & stress tolerance genes (Spiegel <i>et al.</i>, <i>in Review</i>)• Biofilm formation capacity of selected isolates	<ul style="list-style-type: none">• Co-occurrence between <i>L. monocytogenes</i> and other <i>Listeria</i> species• Correlation with other microbiota using full-length 16S rRNA sequencing

Concept for the „Persisters Database“ available on FoodSafeR DigitalHUB

Can be implemented and further analyzed in Multivariate Food Predictor Tool (by Panos Skandamis)

Summary & Outlook



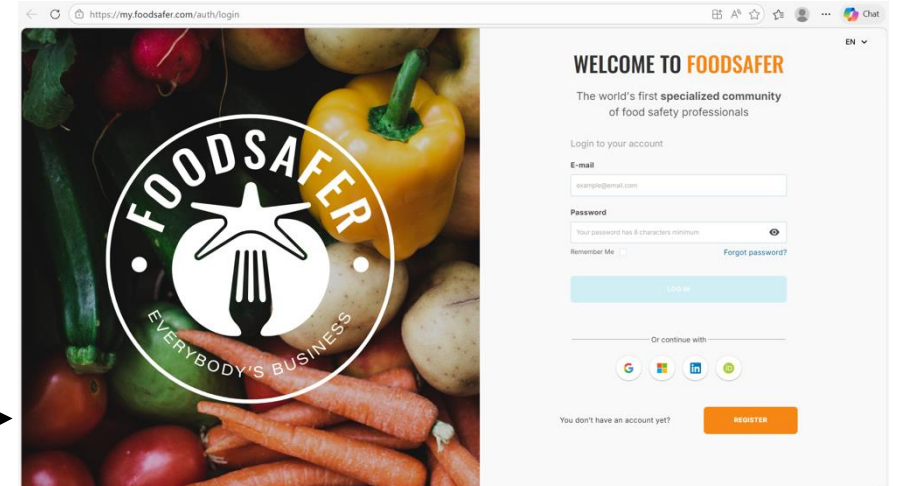
- **Persistence is a widespread phenomenon** -> many STs capable of persistence
- **No single genetic marker for persistence** -> persistent isolates enriched in transcriptional regulators, DNA repair mechanisms, acquisition of mobile genetic elements and phage
- **No significant differences in biofilm formation capacity observed between persistent and non-persistent isolates** -> may be a factor, but not the most important factor in explaining persistence
- ***L. monocytogenes* changes microbial community structure, and could be supported by other bacteria** -> further investigation needed
- **Encourage future studies to apply a holistic view toward an improved understanding of persistence!**



Thank You!



Join us on the FoodSafeR DigitalHub!



7/6/2026