# *L. monocytogenes:* foodborne pathogen and hygiene indicator

**Background:**
Listeriosis is a potentially lethal infection, with the elderly population, pregnant women, and immunocompromised persons at particular risk. Foods, in particular ready-to-eat (RTE) foodstuffs including meat, fish and dairy products, fruit and vegetables, represent the major vehicle for sporadic cases and outbreaks of listeriosis.

## WP1: Strain characteristics

**Phenotypic and genotypic characteristics of Listeria monocytogenes strains isolated during 2011–2014 from different food matrices in Switzerland**
https://www.sciencedirect.com/science/article/abs/pii/S0956713515002571

**Insights:** One hundred and forty two *Listeria monocytogenes* strains isolated from different food matrices in Switzerland between 2011 and 2014 were characterized with respect to their genotypic and phenotypic properties. Analyzed strains originated from various meat, milk, plant-associated food products and production environments as well as from other types of foods including fish, seafood, and ready to eat (RTE) products. The collection included serotype 1/2a (64%), 4b (15%), 1/2c (12%), 1/2b (7%) and 3c (3%). The strains were genetically diverse representing 61 MLST sequence types (ST) including 24 new STs. The most frequent clonal complexes (CC) were CC9 (15%) and CC121 (12%). PCR screening detected presence of the stress survival islet (SSI-1) in 50% of the strains. Phenotypic resistance to benzalkonium chloride (BC) was detected in 18% of the strains. The BC resistance genetic determinants *qacH* and *bcrABC* were detected in 80% and 12% of the strains, respectively. Most (n = 129) of the strains isolated from Swiss food matrices exhibited poor biofilm formation capacity and there were no correlations detected between strain serotypes, genotypes and biofilm production.

**Characterization of Listeria monocytogenes strains isolated during 2011-2013 from human infections in Switzerland**
https://pubmed.ncbi.nlm.nih.gov/25007293/

**Insights:** A total of 93 *L. monocytogenes* strains isolated from different patients in Switzerland from July 2011 to September 2013 were further characterized. Septicemia was reported for 74.2% of the patients, meningitis for 10.8%, and abortion for 3.2%. The majority of the strains belonged to serotype 1/2a (n=58) followed by serotype 4b (n=28), 1/2b (n=5), and 1/2c (n=2). The strains represented 35 multilocus sequence typing sequence types, 8 of which were designated for the first time. Sequence analysis of the inlA gene in the 35 sequence types showed that most of the strains encoded full-length proteins. Screening for Listeriolysin S showed the presence of this virulence factor in 29 of the 33 genetic lineage I strains. By using ApaI and AscI for pulsed-field gel electrophoresis, most strains showed distinguishable patterns.

**Different Shades of *Listeria monocytogenes*: Strain, Serotype, and Lineage-Based Variability in Virulence and Stress Tolerance Profiles**
https://pubmed.ncbi.nlm.nih.gov/35058906/

Insights: The variable distribution of *L. monocytogenes* molecular subtypes with respect to food products and processing environments and among human and animal clinical listeriosis cases is observed. Sixty-two clinical and food-associated *L. monocytogenes* isolates were examined through phenome and genome analysis. Virulence assessed using a zebrafish infection model revealed serotype and genotype-specific differences in pathogenicity. Strains of genetic lineage I serotype 4b and multilocus sequence type clonal complexes CC1, CC2, CC4, and CC6 grew and survived better and were more virulent than serotype 1/2a and 1/2c lineage II, CC8, and CC9 strains. Hemolysis, phospholipase activity, and lysozyme tolerance profiles were associated with the differences observed in virulence. Osmotic stress resistance evaluation revealed serotype 4b lineage I CC2 and CC4 strains as more osmotolerant, whereas serotype 1/2c lineage II CC9 strains were more osmo-sensitive than others. Variable tolerance to the widely used quaternary ammonium compound benzalkonium chloride (BC) was observed. Some outbreak and sporadic clinical case associated strains demonstrated BC tolerance, which might have contributed to their survival and transition in the food-processing environment facilitating food product contamination and ultimately outbreaks or sporadic listeriosis cases. Genome comparison uncovered various moderate

differences in virulence and stress associated genes between the strains indicating that these differences in addition to gene expression regulation variations might largely be responsible for the observed virulence and stress sensitivity phenotypic differences. Overall, our study uncovered strain and genotype-dependent variation in virulence and stress resilience among clinical and food-associated *L. monocytogenes* isolates with potential public health risk implications. The extensive genome and phenotypic data generated provide a basis for developing improved *Listeria* control strategies and policies.

**Lineage-specific evolution and gene flow in Listeria monocytogenes are independent of bacteriophages**
https://pubmed.ncbi.nlm.nih.gov/32483914/

**Insights:** In this work, we used core genome MLST (cgMLST) to identify new outbreaks combined to core genome SNP analysis to characterize the population structure and gene flow between lineages. Whilst analysing differences between the four lineages of L. monocytogenes we have detected differences in the recombination rate, and interestingly also divergence in the SNP differences between sub-lineages. In addition, the exchange of core genome variation between the lineages exhibited a distinct pattern, with lineage III being the best donor for horizontal gene transfer. Whilst attempting to link bacteriophage-mediated transduction to observed gene transfer, we found an inverse correlation between phage presence in a lineage and the extent of recombination. Irrespective of the profound differences in recombination rates observed between sub-lineages and lineages, we found that the previously proposed cut-off of 10 allelic differences in cgMLST can be still considered valid for the definition of a foodborne outbreak cluster of L. monocytogenes.

**Atypical Hemolytic *Listeria innocua* Isolates Are Virulent, albeit Less than *Listeria monocytogenes***
https://pubmed.ncbi.nlm.nih.gov/30670551/

**Insights:** *Listeria innocua* is considered a nonpathogenic *Listeria* species. Natural atypical hemolytic *L. innocua* isolates have been reported but have not been characterized in detail. Here, we report the genomic and functional characterization of representative isolates from the two known natural hemolytic *L. innocua* clades. Whole-genome sequencing confirmed the presence of *Listeria* pathogenicity islands (LIPI) characteristic of *Listeria monocytogenes* species. Functional assays showed that LIPI-1 and *inlA* genes are transcribed, and the corresponding gene products are expressed and functional. Using *in vitro* and *in vivo* assays, we show that atypical hemolytic *L. innocua* is virulent, can actively cross the intestinal epithelium, and spreads systemically to the liver and spleen, albeit to a lesser degree than the reference *L. monocytogenes* EGDe strain. Although human exposure to hemolytic *L. innocua* is likely rare, these findings are important for food safety and public health. The presence of virulence traits in some *L. innocua* clades supports the existence of a common virulent ancestor of *L. monocytogenes* and *L. innocua*.

## WP2: Molecular stress response mechanisms

**Role of cold shock proteins in growth of Listeria monocytogenes under cold and osmotic stress conditions**
https://pubmed.ncbi.nlm.nih.gov/19151183/

**Insights:** We used gene expression analysis and a set of mutants with single, double, and triple deletions of the csp genes to evaluate the roles of CspA, CspB, and CspD in the cold and osmotic (NaCl) stress adaptation responses of L. monocytogenes. All three Csps are dispensable for growth at optimal temperature (37 degrees C). These proteins are, however, required for efficient cold and osmotic stress tolerance of this bacterium. The hierarchies of their functional importance differ, depending on the environmental stress conditions: CspA>CspD>CspB in response to cold stress versus CspD>CspA/CspB in response to NaCl salt osmotic stress. The fact that Csps are promoting L. monocytogenes adaptation against both cold and NaCl stress has significant implications in view of practical food microbial control measures. The combined or sequential exposure of L. monocytogenes cells to these two stresses in food environments might inadvertently induce cross-protection responses.

**The alternative sigma factor sigma(L) of L. monocytogenes promotes growth under diverse environmental stresses**
https://pubmed.ncbi.nlm.nih.gov/19422306/

**Insights:** To assess alternative sigma factor sigma(L) contributions to such stress resistance of L. monocytogenes, quantitative RT-PCR assays and sigL gene deletion mutagenesis were applied in L. monocytogenes EGDe. Transcription of sigL was significantly induced by growth of EGDe under cold, organic acid, and elevated NaCl salt concentration stress conditions. The growth of a DeltasigL strain exposed to these stress conditions was also found to be significantly impaired in comparison to that of its isogenic wild-type strain. The contribution of sigma(L) to transcription control of cold and NaCl

stress adaptation genes, oppA, cspD, and clpP, was also comparatively assessed in DeltasigL and wild-type EGDe cells. Transcription of the oppA gene, which encodes the OppA protein that also promotes L. monocytogenes cold growth, was significantly reduced in cold stress-grown DeltasigL cells compared to levels of the wild-type EGDe strain. These findings therefore suggest important roles of sigma(L) regulatory pathways in facilitating resistance of L. monocytogenes organisms against stress conditions associated with low storage temperatures, exposure to organic acid, and elevated NaCl salt concentrations.

**Evaluation of cold growth and related gene transcription responses associated with Listeria monocytogenes strains of different origins**
https://pubmed.ncbi.nlm.nih.gov/20510784/

**Insights:** The cold growth phenotypes and transcriptional activation of cold stress adaptation genes was evaluated amongst Listeria monocytogenes strains from human listeriosis cases, food products and associated production environments. Significant cold growth phenotypic variation was observed during growth of such strains in rich (BHI) as well as chemically defined minimal (MDM) nutrient conditions. While all twenty analyzed strains grew in BHI at 4 degrees C, only eight of these strains, mostly those recovered from human listeriosis cases, were also able to grow in MDM under similar cold stress. The cold growth phenotypes observed in BHI were used to define two categories of five strains each, which either displayed enhanced and poor cold tolerance relative to the rest of the strain collection. The first group (GP1) consisted of strains characterized by short lag times, whilst the second group (GP2) comprised of strains displaying prolonged lag times before growth resumption during incubation in BHI cultures at 4 degrees C. Transcription level activation of sigB, cspA and pgpH gene expression associated with cold stress exposure in a selection of GP1 and GP2 strains was assessed. Despite similar cold dependent sigB transcript induction between these two strain groups, there were significant differences observed in cold stress dependent induction of cspA and pgpH transcripts. Cold tolerant GP1 strains displayed relatively higher transcriptional activation of cspA and pgpH after cold stress exposure compared to the cold sensitive GP2 strains. This study highlights strain variability in cold stress tolerance phenotypes, as well as in strain capacity to activate specific cold adaptation gene expression responses. In addition the study also shows that enhanced and poor cold growth phenotypes are associated with particular strain capacity to activate important cold stress gene expression responses upon transition of L. monocytogenes into low temperature environments.

**The lmo0501 gene coding for a putative transcription activator protein in Listeria monocytogenes promotes** growth under cold, osmotic and acid stress conditions
https://pubmed.ncbi.nlm.nih.gov/21839374/

**Insights:** In Listeria monocytogenes EGDe, the lmo0501 gene locus encodes a protein similar to the mannitol transcription regulator (MltR) protein in Bacillus subtilis and Bacillus stearothermophilus. In this study we investigated its functional role in L. monocytogenes EGDe cells in view of growth under different stress conditions. Increased lmo0501 gene expression at mRNA level was detected in response to cold, osmotic and organic acid stress exposure. An EGDe Δlmo0501 mutant strain was diminished in growth compared to the wild type strain in minimal defined medium containing either glucose or fructose, as carbon sources. Growth of the lmo0501 null mutant was retarded growth under cold (4 °C), salt (NaCl) and organic acid stress conditions relative to the parental wild type strain. Our results confirm the role of the lmo0501 gene in adaptation of L. monocytogenes cells to food preservation stress conditions as well as to the efficient utilization of glucose and fructose as carbon sources.

**Phenotypic and transcriptomic analyses of Sigma L-dependent characteristics in Listeria monocytogenes EGD-e**
https://pubmed.ncbi.nlm.nih.gov/22850387/

**Insights: I**n this study the phenotypic and transcriptomic traits associated with the alternative sigma factor protein Sigma L in Listeria monocytogenes EGD-e were investigated. It was demonstrated that Sigma L is required for efficient growth in presence of stress associated with food preservative measures such as low temperature and organic acids. Furthermore, besides attenuation of swarming motility, the disruption of Sigma L in this bacterium also reduces resistance to a diverse range of toxic compounds, including some of the antibiotics used in listeriosis treatment. Genes under Sigma L-dependent transcriptional regulation were identified based on comparison of transcriptomes between exponentially growing cells of the EGD-e sigL null mutant and its parental strain cultivated under cold stress (3 °C) and optimized (37 °C) temperature conditions. Four hundred and forty genes under positive Sigma L-dependent transcriptional regulation were identified. The Sigma L regulon as revealed under these conditions comprises genes that code for proteins with diverse cellular functions including protein synthesis, nutrient transport, energy metabolism, cell envelope synthesis, and motility. The diverse range of transcriptome alterations induced by a sigL null mutation is thus consistent with the multiple phenotypic defects observed in the EGD-e ΔsigL mutant. These results demonstrate that Sigma L provides important global transcription

regulatory functions in L. monocytogenes EGD-e. These promote execution of various cellular processes and stress adaptation responses thereby enabling this bacterium to overcome various food preservation measures as well as antibiotics and other toxic chemicals.

**Cold growth behaviour and genetic comparison of Canadian and Swiss Listeria monocytogenes strains associated with the food supply chain and human listeriosis cases**
https://pubmed.ncbi.nlm.nih.gov/24549201/

**Insights:** Sixty-two strains of Listeria monocytogenes isolated in Canada and Switzerland were investigated. Comparison based on molecular genotypes confirmed that strains in these two countries are genetically diverse. Interestingly strains from both countries displayed similar range of cold growth phenotypic profiles. Based on cold growth lag phase duration periods displayed in BHI at 4 °C, the strains were similarly divided into groups of fast, intermediate and slow cold adaptors. Overall Swiss strains had faster exponential cold growth rates compared to Canadian strains. However gene expression analysis revealed no significant differences between fast and slow cold adapting strains in the ability to induce nine cold adaptation genes (lmo0501, cspA, cspD, gbuA, lmo0688, pgpH, sigB, sigH and sigL) in response to cold stress exposure. Neither was the presence of Stress survival islet 1 (SSI-1) analysed by PCR associated with enhanced cold adaptation. Phylogeny based on the sigL gene subdivided strains from these two countries into two major and one minor cluster. Fast cold adaptors were more frequently in one of the major clusters (cluster A), whereas slow cold adaptors were mainly in the other (cluster B). Genetic differences between these two major clusters are associated with various amino acid substitutions in the predicted SigL proteins. Compared to the EGDe type strain and most slow cold adaptors, most fast cold adaptors exhibited five identical amino acid substitutions (M90L, S203A/S203T, S304N, S315N, and I383T) in their SigL proteins. We hypothesize that these amino acid changes might be associated with SigL protein structural and functional changes that may promote differences in cold growth behaviour between L. monocytogenes strains.

**Surviving host - and food relevant stresses: phenotype of L. monocytogenes strains isolated from food and** clinical sources
https://pubmed.ncbi.nlm.nih.gov/30154513/

**Insights:** The aim of this study was to compare the phenotype of 40 strains of L. monocytogenes under food and host relevant stress conditions. The strains were chosen to represent food and clinical isolates and to be equally distributed between the most relevant clonal complexes for clinical and food isolates (CC1 and CC6 vs CC121 and CC9), plus one group of eight strains of rare clonal complexes. Human-associated CC1 had a faster maximal growth rate than the other major complexes, and the lag time of CC1 and CC6 was significantly less affected by the addition of 4% NaCl to the medium. Food-associated CC9 strains were hypohemolytic compared to other clonal complexes, and all strains found to be resistant to increased concentrations of benzalkonium chloride belonged to CC121 and were positive for Tn6188 carrying the qacH gene. Lactic acid affected the survival of L. monocytogenes more than HCl, and there was a distinct, strain specific pattern of acid tolerant and sensitive strains. Strains from CC6 and human clinical isolates are less resilient under acid stress than those from other complexes and from food. One strain isolated from a human patient exhibited significant growth defects across all conditions.

**New Insights on the Role of the pLMST6 Plasmid in *Listeria monocytogenes* Biocide Tolerance and Virulence**
https://pubmed.ncbi.nlm.nih.gov/31338084/

**Insights:** In this study, occurrence and contribution of this plasmid to BC tolerance was examined using PCR, plasmid curing and transformation, RT-qPCR and proteome analysis, respectively. Furthermore, the substrate specificity of the pLMST6 associated EmrC efflux pump and the impact of the plasmid on *L. monocytogenes* virulence were investigated. pLMST6 was detected in 7 (1.6%) of 439 *L. monocytogenes* strains isolated from different sources. A phenotypic role of this plasmid in conferring increased BC tolerance was confirmed by showing that plasmid cure increases BC susceptibility whereas plasmid complementation and transformation increased BC tolerance in different *L. monocytogenes* genetic backgrounds and *L. innocua*. RT-qPCR showed that BC stress exposure strongly induces the expression of mRNAs associated with pLMST6 genes for EmrC and a TetR transcription regulator. A full proteome analysis in a plasmid harboring *L. monocytogenes* strain revealed that the pLMST6 encoded putative TetR family transcription regulator protein is the most upregulated protein in response to BC stress exposure. An investigation into the EmrC efflux pump's substrate spectrum showed that while pLMST6 confers increased tolerance to other quaternary ammonium compounds (QACs) based disinfectants it has no impact on the sensitivity of *L. monocytogenes* to non-QAC disinfectants as well as on antibiotics such as ampicillin, tetracycline and gentamicin. A reduction in the survival of zebrafish embryos infected with pLMST6 plasmid harboring *L. monocytogenes* strains was observed when compared with plasmid cured variants of the same strains suggesting that some pLMST6 harbored genes might contribute to increased virulence capacity. Overall these results confirm the phenotypic contribution of pLMST6 plasmid in promoting and dissemination of BC tolerance in *L.*

*monocytogenes* as well as provide new insights on different molecular levels of pLMST6 associated genes in response to BC stress.

**Transcriptomic and Phenotypic Analyses of the Sigma B-Dependent Characteristics and the Synergism between Sigma B and Sigma L in *Listeria monocytogenes* EGD-e**
https://pubmed.ncbi.nlm.nih.gov/33114171/

**Insights:** Numerous gene expression and stress adaptation responses in *L. monocytogenes* are regulated through alternative sigma factors $\sigma^B$ and $\sigma^L$. Stress response phenotypes and transcriptomes were compared between *L. monocytogenes* EGD-e and its Δ*sigB* and Δ*sigBL* mutants. Targeted growth phenotypic analysis revealed that the *ΔsigB* and *ΔsigBL* mutants are impaired during growth under cold and organic-acid stress conditions. Phenotypic microarrays revealed increased sensitivity in both mutants to various antimicrobial compounds. Genes de-regulated in these two mutants were identified by genome-wide transcriptome analysis during exponential growth in BHI. The Δ*sigB* and Δ*sigBL* strains repressed 198 and 254 genes, respectively, compared to the parent EGD-e strain at 3 °C, whereas 86 and 139 genes, respectively, were repressed in these mutants during growth at 37 °C. Genes repressed in these mutants are involved in various cellular functions including transcription regulation, energy metabolism and nutrient transport functions, and viral-associated processes. Exposure to cold stress induced a significant increase in $\sigma^B$ and $\sigma^L$ co-dependent genes of *L. monocytogenes* EGD-e since most (62%) of the down-regulated genes uncovered at 3 °C were detected in the Δ*sigBL* double-deletion mutant but not in Δ*sigB* or Δ*sigL* single-deletion mutants. Overall, the current study provides an expanded insight into $\sigma^B$ and $\sigma^L$ phenotypic roles and functional interactions in *L. monocytogenes*. Besides previously known $\sigma^B$- and $\sigma^L$–dependent genes, the transcriptomes defined in Δ*sigB* and Δ*sigBL* mutants reveal several new genes that are positively regulated by $\sigma^B$ alone, as well as those co-regulated through $\sigma^B$- and $\sigma^L$–dependent mechanisms during *L. monocytogenes* growth under optimal and cold-stress temperature conditions.

**Strain Variability of *Listeria monocytogenes* under NaCl Stress Elucidated by a High-Throughput Microbial Growth Data Assembly and Analysis Protocol**
https://pubmed.ncbi.nlm.nih.gov/31900307/

**Insights:** Elucidating the intraspecies strain variability of *L. monocytogenes* stress tolerance is crucial for the identification of particularly tolerant strains. To enhance reliable identification of variability in bacterial stress tolerance phenotypes, we compiled a large-scale protocol for the entire data assembly and analysis of microbial growth experiments, providing a systematic approach and checklist for experiments on strain-specific growth ability. Our study illustrated the diversity and strain-specific variation of *L. monocytogenes* stress tolerance with an unprecedented scope and discovered biologically relevant serovar- and lineage-dependent phenotypes of NaCl tolerance.

**The analysis of field strains isolated from food, animal and clinical sources uncovers natural mutations in Listeria monocytogenes Nisin resistance genes**
https://pubmed.ncbi.nlm.nih.gov/33123101/

**Insights:** This study sought to gather more insights into nisin resistance mechanisms in natural *L. monocytogenes* populations by examining a collection of 356 field strains that were isolated from different foods, food production environments, animals and human infections. A growth curve analysis-based approach was used to access nisin inhibition levels and assign the *L. monocytogenes* strains into three nisin response phenotypic categories; resistant (66%), intermediate (26%), and sensitive (8%). Using this categorization isolation source, serotype, genetic lineage, clonal complex (CC) and strain-dependent natural variation in nisin phenotypic resistance among *L. monocytogenes* field strains was revealed. Whole genome sequence analysis and comparison of high nisin resistant and sensitive strains led to the identification of new naturally occurring mutations in nisin response genes associated with increased nisin resistance and sensitivity in this bacterium. Increased nisin resistance was detected in strains harboring $RsbU_{G77S}$ and $PBPB3_{V240F}$ amino acid substitution mutations, which also showed increased detergent stress resistance as well as increased virulence in a zebra fish infection model. On the other hand, increased natural nisin sensitivity was detected among strains with mutations in *sigB*, *vir*, and *dlt* operons that also showed increased lysozyme sensitivity and lower virulence. Overall, our study identified naturally selected mutations involving *pbpB3* (*lm0441*) as well as *sigB*, *vir*, and *dlt* operon genes that are associated with intrinsic nisin resistance in *L. monocytogenes* field strains recovered from various food and human associated sources. Finally, we show that combining growth parameter-based phenotypic analysis and genome sequencing is an effective approach that can be useful for the identification of novel nisin response associated genetic variants among *L. monocytogenes* field strains.

## WP3: Persistence in the production environment

**Pulsed-field gel electrophoresis (PFGE) typing of Listeria strains isolated from a meat processing plant over a 2-year period**
https://pubmed.ncbi.nlm.nih.gov/11139016/

**Insights:** As part of a hygiene monitoring program in a meat processing plant a total of 131 Listeria isolates were detected by sampling different processing areas and meat products within a 2-year period. The isolates were differentiated by means of phenotypic characteristics. Furthermore, the genomic ApaI and SmaI fragment patterns of all isolates were examined by pulsed-field gel electrophoresis (PFGE). PFGE using SmaI and ApaI yielded 15 (Listeria monocytogenes), 20 (Listeria innocua) and six (Listeria welshimeri) pulsotypes. Of the environmental Listeria monocytogenes isolates the predominating PFGE-type B was clearly associated with processing area A whereas PFGE-type E predominated in the meat products. Moreover, the study showed the persistence of closely related Listeria strains over a 2-year period in the environment of this meat processing plant.

**Phenotypic and molecular typing of *Listeria monocytogenes* isolated from the processing environment and products of a sandwich-producing plant**
https://www.sciencedirect.com/science/article/abs/pii/S0956713510001465

**Insights:** The present study investigated the diversity of *L. monocytogenes* in a Swiss sandwich-producing plant over a 12-months period. *L. monocytogenes* were detected by culture after enrichment in 70 (3.5%) of 2028 environmental swabs and 16 (7.4%) of 217 samples from ingredients and sandwiches. Of the 86 *L. monocytogenes* strains, 93% belonged to serotype 1/2a and genetic lineage II. Rep PCR and PFGE analysis yielded each six profiles. Sixty-seven (77.9%) strains belonged to only one genotype, which was repeatedly found on/in slicers, conveyor belts, tables, a bread-feeding machine, spattles, air blow-guns, salmon, and egg sandwiches. Strains of this genotype persisted for more than 9 months in the processing environment, in particular on slicers and conveyor belts, which probably contributed to the contamination of sandwiches. After revision of the cleaning and disinfection procedures, *L. monocytogenes* were no longer found on slicers, conveyor belts, or in products. Besides, these results emphasize the importance of environmental monitoring schemes to identify potential contamination sources and as an early warning system for food business operators.

**Characteristics of Listeria Monocytogenes Strains Persisting in a Meat Processing Facility over a 4-Year Period**
https://pubmed.ncbi.nlm.nih.gov/30866522/

**Insights:** We subtyped 124 strains of *L. monocytogenes* isolated from a meat processing facility in Switzerland by serotyping, multi locus sequence typing (MLST) typing and whole genome sequencing. We then analyzed their ability to form biofilms and their resistance to the disinfectants benzalkonium chloride (BC) and peracetic acid (PAA). The genotyping results of the strains showed that several clonal populations of *L. monocytogenes* belonging to CC9, CC204 and CC121 had persisted in this meat processing facility for at least four years. All of the strains showed biofilm forming capacity comparable to a known high biofilm forming strain. Known efflux pumps for BC were present in CC204, CC9 (*brcABC*) and CC121 (*qacH*) strains, while strains from other CC showed very low minimal inhibitory concentrations (MICs) for BC. For PAA, minimal bactericidal concentrations of 1.2¯1.6% for 20 min and minimal inhibitory concentrations between 0.1 and 0.2% were observed. These values were close to or above the recommended concentration for use (0.5¯1%), suggesting that PAA might be ineffective at controlling *L. monocytogenes* in this and potentially other meat processing facilities.

**Whole-Genome Sequencing-Based Characterization of 100 Listeria monocytogenes Isolates Collected from Food Processing Environments over a Four-Year Period**
https://pubmed.ncbi.nlm.nih.gov/31391275/

**Insights:** In this study, whole-genome sequencing (WGS) was applied as a tool to characterize and track 100 *L. monocytogenes* isolates collected from three food processing environments. These WGS data from environmental and food isolates were analyzed to (i) assess the genomic diversity of *L. monocytogenes*, (ii) identify possible source(s) of contamination, cross-contamination routes, and persistence, (iii) detect absence/presence of antimicrobial resistance-encoding genes, (iv) assess virulence genotypes, and (v) explore *in vivo* pathogenicity of selected *L. monocytogenes* isolates carrying different virulence genotypes. The predominant *L. monocytogenes* sublineages (SLs) identified were SL101 (21%), SL9 (17%), SL121 (12%), and SL5 (12%). Benzalkonium chloride (BC) tolerance-encoding genes were found in 62% of these isolates, a value that increased to 73% among putative persistent subgroups. The most prevalent gene was *emrC* followed by *bcrABC*, *qacH*-Tn*6188*, and *qacC.* The *L. monocytogenes* major virulence factor *inlA* was truncated in 31% of the isolates, and

only one environmental isolate (*L. monocytogenes* CFS086) harbored all major virulence factors, including *Listeria* pathogenicity island 4 (LIPI-4), which has been shown to confer hypervirulence. A zebrafish embryo infection model showed a low (3%) embryo survival rate for all putatively hypervirulent *L. monocytogenes* isolates assayed. Higher embryo survival rates were observed following infection with unknown virulence potential (20%) and putatively hypovirulent (53 to 83%) *L. monocytogenes* isolates showing predicted pathogenic phenotypes inferred from virulence genotypes.

## WP4: Outbreak investigations

**Outbreak of listerosis due to imported cooked ham, Switzerland 2011**
https://pubmed.ncbi.nlm.nih.gov/23725774/

**Foodborne transmission of *Listeria monocytogenes* via ready-to-eat salad: A nationwide outbreak in Switzerland, 2013–2014**
https://www.sciencedirect.com/science/article/abs/pii/S0956713515001978

**How can patients and their physicians contribute to an outbreak investigation? Experiences from a nationwide listeriosis outbreak in Switzerland**
https://pubmed.ncbi.nlm.nih.gov/27878798/

**Local Outbreak of Listeria monocytogenes Serotype 4b Sequence Type 6 Due to Contaminated Meat Pâté**
https://pubmed.ncbi.nlm.nih.gov/28379731/

**Listeriosis caused by persistence of *Listeria monocytogenes* serotype 4b Sequence Type 6 in cheese production environment**
https://pubmed.ncbi.nlm.nih.gov/33350924/