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Source attribution of *Listeria monocytogenes* genotypes in meat processing facilities

Diplomarbeit
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ABSTRACT

Listeria (L.) monocytogenes is a zoonotic pathogen with a high mortality rate (20-30%) and therefore requires special attention. The cause of transmission lies in the fact that the pathogen forms reservoirs in food and the food environment, which is why measures must be taken. This will be achieved through the cooperation of stakeholders, i.e. the food producing companies and the evaluating institutes.

The companies should learn from the data they have to obtain from environmental monitoring as part of their self-monitoring. This means deriving measures to reduce *L. monocytogenes* in the environment and to eliminate hypervirulent *L. monocytogenes* on the premises. We know that there is hypo- and hypervirulent *L. monocytogenes* that are present in the environment as well as in facilities. Some of them are highly infectious, have adapted to their environment and persist.

The purpose of this study is to describe this situation using typing data from two companies and to make the data as simple as possible so that it can be quickly assessed and used retrospectively by a quality management team. For this purpose, 297 isolates from two companies were used, which were characterized by MLST. A rapid screening focusing on the *abcZ*, *bglA* and *Idh* alleles is used as a predictor of the clonal complex (CC), which can be used to determine whether the CCs are hypo- or hypervirulent. In a second step, the data set is plotted over space, niche, and time to determine whether persistent clones are present. Hypervirulent clones are eliminated first, followed by virulent ones. And in the case of persistent clones, special cleaning concepts may need to be considered. Testing the effectiveness of the biocides used against persistent strains helps indirectly.

ZUSAMMENFASSUNG

L. monocytogenes ist ein Zoonoseerreger mit einer hohen Sterblichkeitsrate (20-30%) und erfordert daher besondere Aufmerksamkeit. Die Ursache der Übertragung liegt in der Reservoirbildung des Erregers in Lebensmitteln und in der Lebensmittelumgebung, weshalb Maßnahmen ergriffen werden müssen. Dies wird durch die Zusammenarbeit der Beteiligten, d.h. der lebensmittelproduzierenden Unternehmen und der bewertenden Institute, erreicht.

Die Unternehmen sollen aus den Daten lernen, die sie im Rahmen ihrer Eigenkontrolle aus dem Umweltmonitoring gewinnen müssen. Das bedeutet, dass sie Maßnahmen ableiten müssen, um *L. monocytogenes* in der Umwelt zu reduzieren und hypervirulente *L. monocytogenes* auf dem Betriebsgelände zu eliminieren. Wir wissen, dass hypo- und hypervirulente *L. monocytogenes* sowohl in der Umwelt als auch in Anlagen vorkommen. Einige von ihnen sind hochinfektiös, haben sich an ihre Umgebung angepasst und sind persistent.

Ziel dieser Studie ist es, diese Situation anhand von Typisierungsdaten aus zwei Betrieben zu beschreiben und die Daten so einfach wie möglich zu gestalten, damit sie von einem Qualitätsmanagementteam schnell ausgewertet und retrospektiv genutzt werden können. Zu diesem Zweck wurden 297 Isolate aus zwei Unternehmen verwendet, die mittels MLST charakterisiert wurden. Ein schnelles Screening mit Fokus auf die *abcZ*-, *bglA*- und *Idh*-Allele wird als Prädiktor für den klonalen Komplex (CC) verwendet, um zu bestimmen, ob die CCs hypo- oder hypervirulent sind. In einem zweiten Schritt wird der Datensatz über Raum, Nische und Zeit aufgetragen, um festzustellen, ob persistente Klone vorhanden sind. Hypervirulente Klone werden zuerst eliminiert, gefolgt von virulenten Klonen. Für persistente Klone können spezielle Reinigungskonzepte erforderlich sein. Die Prüfung der Wirksamkeit der eingesetzten Biozide gegen persistente Stämme ist indirekt hilfreich.

ABBREVIATIONS

%	Percent
°C	Degree Celcius
µl	Microliter
µM	Micromole
BIGSdb	Bacterial Isolate Genome Sequence Database
bp	Base pair
CC	Clonal complex
CDC	Centers for Disease Control and Prevention
cgMLST	Core Genome MLST
DEPC	Diethyl pyrocarbonate
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
E.	*Escherichia*
EDTA	Ethylenediaminetetracetic acid
EU	European Union
FCS	Food contact surface
FPE	Food processing environment
g	Gram / Relative centrifugal force
HCl	Hydrochloric acid
kb	Kilobase
L.	*Listeria*
LMO	*Listeria monocytogenes*
M	Mole
Mg	Magnesium
$MgCl_2$	Magnesium chloride
min	Miunte
ml	Milliliter
MLST	Multilocus sequence typing
mM	Millimole
NFCS	Non-food contact surface
nM	Nanomole
NTC	Non-template control
PA	Product-associated
PCR	Polymerase chain reaction
PE	Personnel-associated
RASFF	Rapid Alert System for Food and Feed
RM	Raw material
RTE	Ready-to-eat
s	Second
ST	Sequence type

TAE	Tris-Acetate-EDTA
TIFF	Tagged Image File Format
Tris	Tris(hydroxymethyl)aminomethane
U	Unit
UV	Ultraviolette
V	Volt
µM	Micromole

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1. INTRODUCTION

L. monocytogenes is a pathogenic species of the gram-positive bacterial genus *Listeria* and the cause of listeriosis [1]. Foodborne listeriosis has become a public health problem with a major economic impact on the food industry. *L. monocytogenes* is generally considered to be ubiquitously distributed in the natural environment [2]. It has been specifically isolated from many different environments including soil, water, vegetation, sewage, animal feed, farm environments and food-processing environments [3]. Unlike other foodborne pathogens, *L. monocytogenes* can tolerate high salt concentrations (up to 20%), can multiply over a wide range of temperatures (1 to 45°C), and can adapt to and survive acid stress [4]. There are two main forms of listeriosis: non-invasive and invasive. The non-invasive form occurs in immunocompetent individuals, whereas the invasive form occurs in pregnant women and newborns, the elderly and immunocompromised individuals. In pregnant women, the disease may result in late miscarriage or severe infection of the newborn [5].

1.1. *L. MONOCYTOGENES* OUTBREAKS AND RECALLS

Among zoonotic agents, *L. monocytogenes* was responsible for 35 foodborne outbreaks in the EU in 2022. In comparison, most outbreaks were caused by *Salmonella* (1014), unspecified bacterial toxins (636), Norovirus (and other calicivirus) (332) and *Bacillus cereus* toxins (306). However, the mortality of *L. monocytogenes* was very high compared to other zoonotic agents (9.46%). Other pathogens with high mortality were Shiga toxin-producing *E. coli* (0.25%), toxin-producing *Staphylococcus aureus* (0.18%) and *Salmonella* (0.12%); In 2022, *L. monocytogenes* caused 43.8% (n=28) of all deaths in foodborne outbreaks in the EU, highlighting the impact of this pathogen on public health.

The official *L. monocytogenes* sampling carried out by competent authorities in the manufacture of ready-to-eat foods in 2022 showed that the highest proportion of positive single samples was in the categories fish and fishery products (2.5-2.6%), followed by meat products other than fermented sausages (2.5%) and dairy products (0.14-1.5%) [1].

The Centers of Disease Control and Prevention (CDC) investigates and issues outbreak notifications for foodborne outbreaks in the United States. From 2006 to 2024, 16% of multistate foodborne outbreaks were attributable to *L. monocytogenes,* with *Salmonella (50%)* and pathogenic *E. coli (25%)* being the only causative agents with a higher proportion of outbreaks (Figure 1).

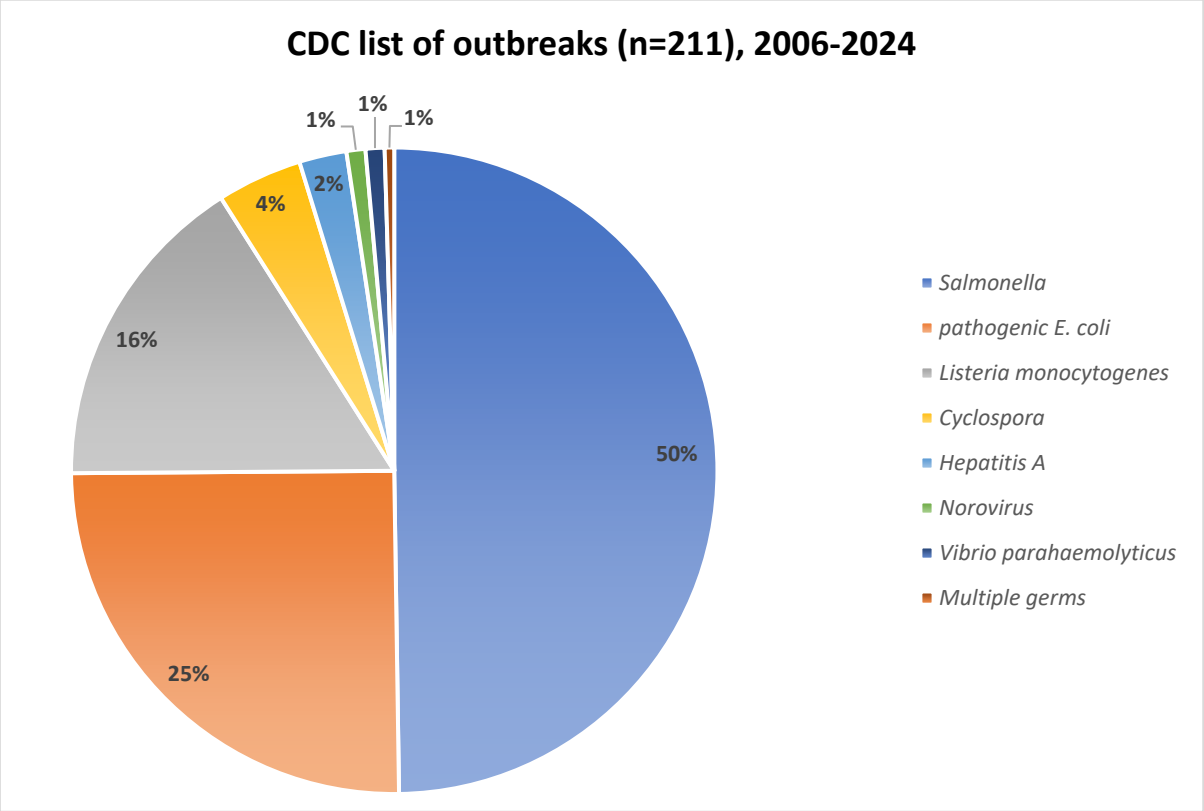


Figure 1: Distribution of foodborne outbreak pathogens in the U.S. as reported by the CDC.

Source: https://www.cdc.gov/foodborne-outbreaks/outbreaks/index.html accessed on: 11-02-2026.
Abbreviations: CDC = Centers of Disease Control and Prevention, *E. Coli* = *Escherichia Coli*.

The 34 outbreaks associated with *L. monocytogenes* were most frequently detected in the following food categories: dairy products (13 or 38.2%), fruits and vegetables (11 or 32.5%), and meat products (5 or 14.7%) (Figure 2). The most prevalent food items in the fruits and vegetables category were Enoki mushrooms, salads, and leafy greens. The dairy products most frequently included in the study were cheese and ice cream. Meat products included cooked chicken, deli meats, deli ham and pork products. The egg product category contained hard-boiled eggs (https://www.cdc.gov/foodborne-outbreaks/outbreaks/index.html accessed on: 11-02-2026).

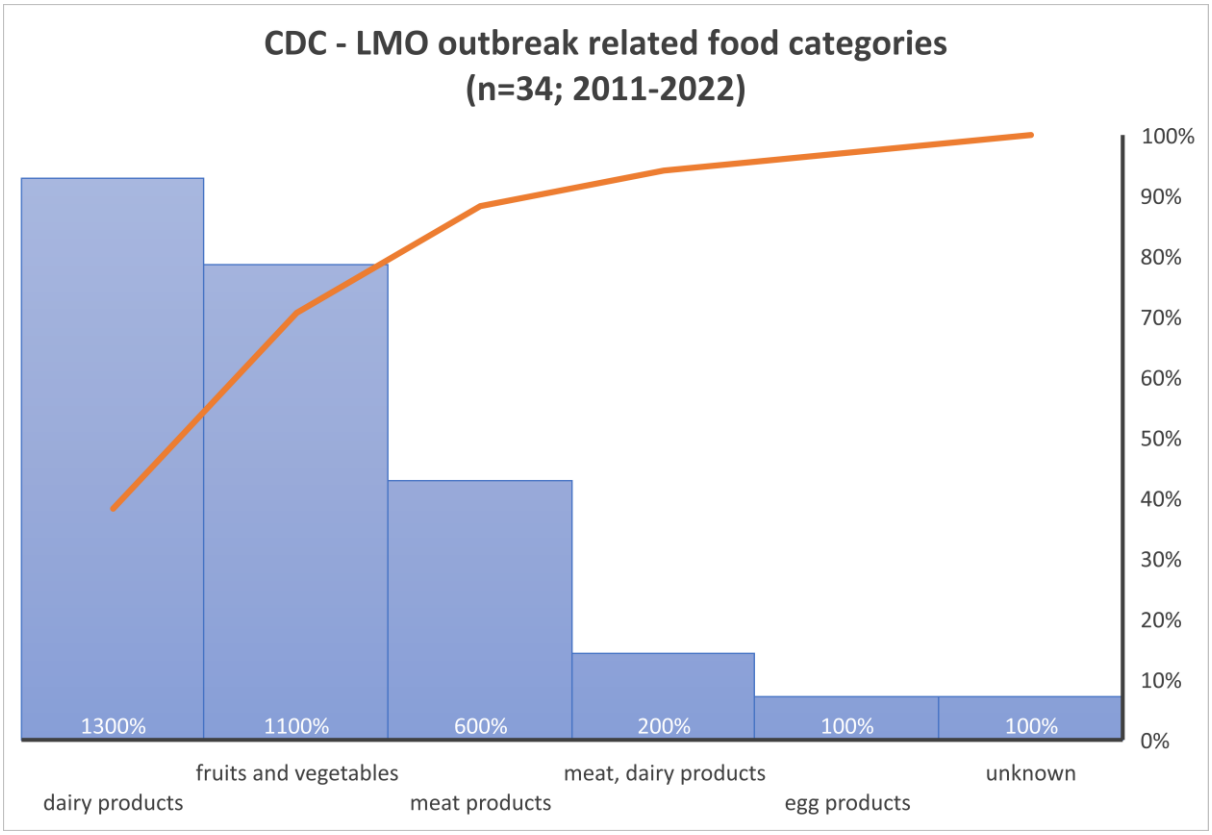


Figure 2: *L. monocytogenes* outbreaks in the U.S. attributed to food categories.

Source: https://www.cdc.gov/foodborne-outbreaks/outbreaks/index.html accessed on: 11-02-2026.
Abbreviations: CDC = Centers of Disease Control and Prevention, LMO = *Listeria monocytogenes*.

The EU Rapid Alert System for Food and Feed (RASFF) registered 740 recalls or foodborne outbreaks due to *L. monocytogenes* between 2020 and 2024. The most frequently affected food categories were milk and milk products (n=200), fish and products thereof (n=181), and meat and meat products (except poultry) (n=169). The distribution of the 740 notifications across different food categories is shown in Figure 3.

In the context of the meat categories and their respective products (n = 99 defined categories out of 235 notifications), *L. monocytogenes* was identified most frequently in chicken (42%), beef (19%), and pork (14%). (Figure 4A). The most commonly affected ready-to-eat meat RTE products in the data set with specification (n=114) were sausage (30%), ham (17%), and bacon (11%), (Figure 4B) (https://webgate.ec.europa.eu/rasff-window/screen/search?searchQueries= accessed on: 11-02-2026).

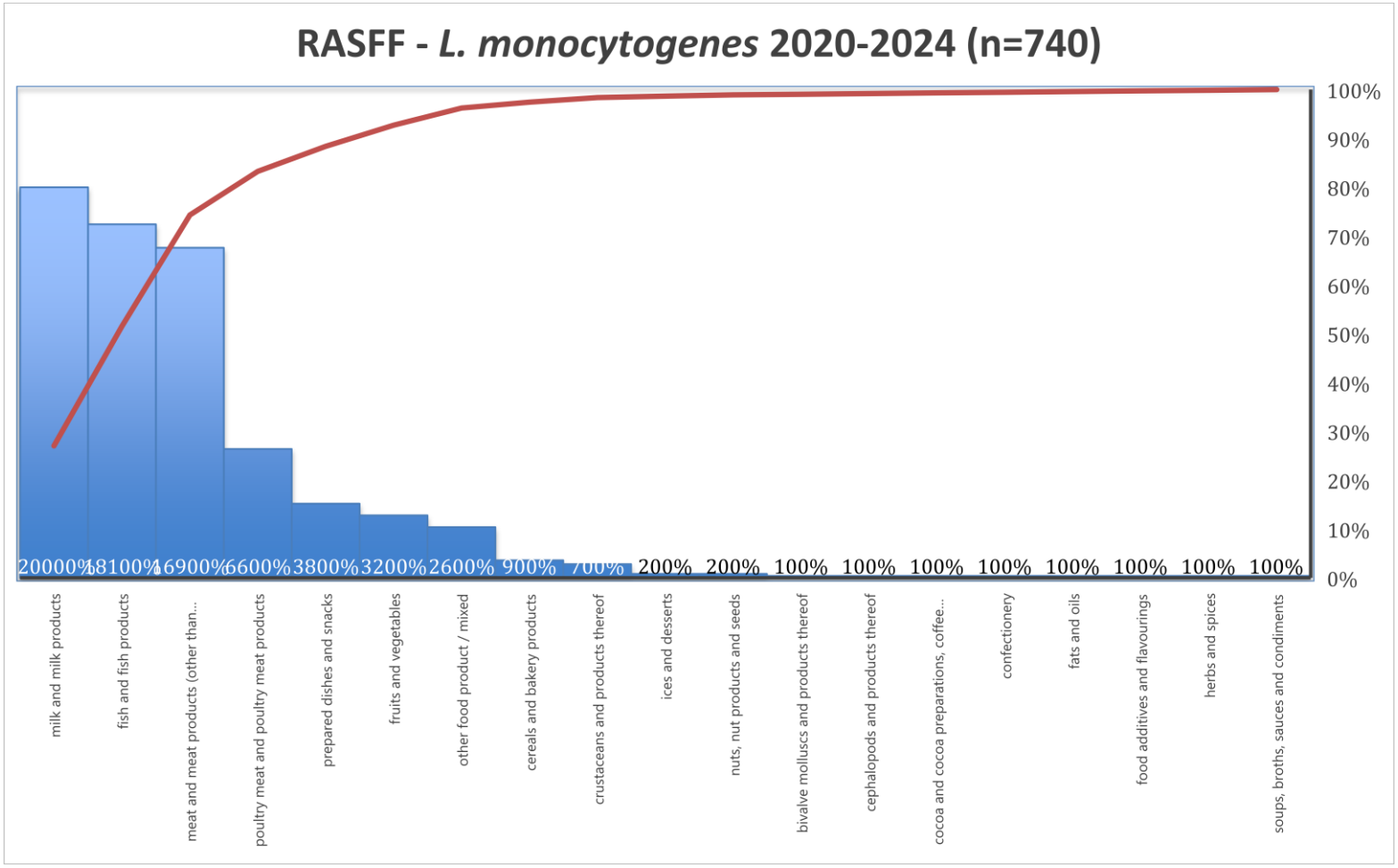


Figure 3*: L. monocytogenes* recalls and outbreaks in the EU notified by the RASFF, by food category.

Source: https://webgate.ec.europa.eu/rasff-window/screen/search?searchQueries= accessed on: 11-02-2026.
Abbreviations: RASFF = Rapid Alert System for Food and Feed, *L. = Listeria*.

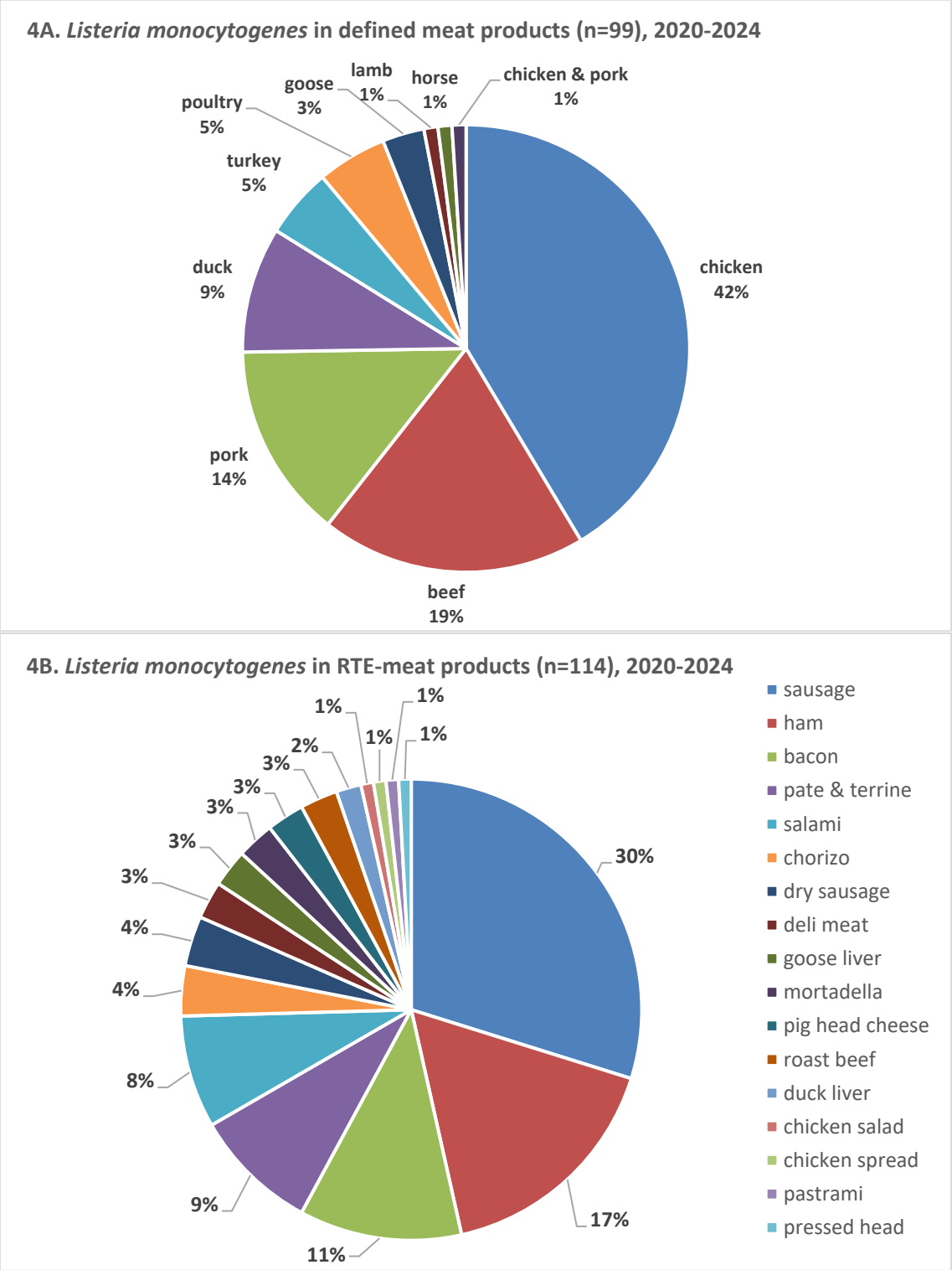


Figure 4*:* Proportion of meat and meat products resp. ready-to-eat meat products affected by *L. monocytogenes* recalls and outbreaks in the EU notified by the RASFF (2020-2024).

Source: https://webgate.ec.europa.eu/rasff-window/screen/search?searchQueries= accessed on: 11-02-2026.
Abbreviations: RASFF = Rapid Alert System for Food and Feed, RTE = ready-to-eat, *L. = Listeria*.

1.2. *L. MONOCYTOGENES* PREVALENCE

L. monocytogenes is divided into four genetic lineages (I-IV), with strains of genetic lineages I and II being the primary cause of human disease. Each of these lineages contains numerous clonal complexes (CCs), subtypes of *L. monocytogenes*, with varying prevalence.

The relative prevalence of lineage II isolates is higher in food (especially CC121 and CC9), and that of lineage I isolates is higher in humans (especially CC1 and CC4) [6]. While clonal complexes such as CC5, CC8, CC121 and CC204 appear to be distributed across all food categories, CC9 is mainly found in meat and meat products [7].

1.3. *L. MONOCYTOGENES* GLOBAL DISTRIBUTION

L. monocytogenes is a bacterium that is widely distributed in nature, found in soil, water and the digestive tract of animals. The bacterium is transmitted to humans primarily through the consumption of contaminated food, including vegetables through soil and ready-to-eat foods during processing (https://www.who.int/news-room/fact-sheets/detail/listeriosis; accessed on: 11-02-2026). *L. monocytogenes* has the capacity to survive and even multiply at low temperatures during various stages of food production, the cold chain, and in the consumer's household. This renders it a significant food safety concern, particularly in refrigerated environments [8, 9].

The global distribution of *L. monocytogenes* includes some widespread and globally distributed clones (i.e., clonal complexes). These clones have been identified in various sources across five continents, indicating their wide distribution [10]. The bacterium's ability to survive in various environments, including meat processing facilities and slaughterhouses, underscores the need for comprehensive hygiene and control measures to prevent contamination and subsequent outbreaks [11, 12].

Listeriosis, a zoonosis caused by *L. monocytogenes*, manifests itself in two main forms: non-invasive and invasive. The non-invasive form generally manifests in healthy individuals and results in mild symptoms, including febrile gastroenteritis. The invasive form is more severe and can lead to serious health problems, including sepsis, meningoencephalitis, and loss of the unborn child, particularly in at-risk groups such as pregnant women, the elderly, and persons with weakened immune systems. [13].

The implementation of effective hygiene practices, environmental monitoring, and food safety management systems constitutes a multifaceted approach to the control and prevention of *L. monocytogenes*. Education and training of food handlers and consumers, especially those belonging

to at-risk groups, are crucial for the prevention of listeriosis. (https://www.cdc.gov/listeria/about/index.html; accessed on: 11-02-2026).

It is imperative that the global distribution and virulence of individual *L. monocytogenes* be taken into account in training. (https://www.ecdc.europa.eu/en/listeriosis/threats-and-outbreaks; accessed on: 11-02-2026) [14].

The clonal complexes of *L. monocytogenes* exhibit divergent virulence potential. In particular, hypervirulent CCs (e.g., CC1, CC2, CC4, CC6) are responsible for the most frequent epidemic outbreaks worldwide, while hypovirulent CCs (e.g., CC9, CC121) occur in every food production chain and have adapted well to the production environment. [3, 15].

1.4. *L. MONOCYTOGENES* HYPOVIRULENCE, HYPERVIRULENCE, PERSISTENCE

L. monocytogenes lineage I subtypes, such as CC1, CC4, and CC6, are considered hypervirulent because they are more common in human isolates, show high virulence in in vitro studies, and possess specific virulence factors. Subtypes of lineage II, such as CC121, CC9, and CC31, are considered hypovirulent because they are more common in food isolates but can still cause infections in immunocompromised individuals [16, 17].

Due to its persistence, *L. monocytogenes* demands great attention in food-processing environments (FPEs) [18]. Persistence can be defined as “the ability of an organism to be established in niches within the FPE for a long term, despite the frequent application of cleaning and disinfection”. This ability to persist may lead to recurrent food contamination, which can be detected by repeated isolation of strains over several months in the same premises or equipment. However, if the same germ is repeatedly introduced into the facility, it is not considered to be persistent [7]. The clonal complexes more commonly associated with persistence in FPEs are CC5, CC6, and CC2 of genetic lineage I as well as CC121, CC8, and CC9 of lineage II [6]. Sequence types found to be persistent are ST121 and ST9 [19]. The reasons for the persistence in FPEs are not yet fully understood. On the one hand, improper hygiene measures or conditions, and on the other hand, strains with specific genetic determinants, such as the ability to form biofilm and adapt to physical and chemical stress, could be the cause [20].

The most common risk factors for FPEs are equipment cleanability, food residue, and moisture accumulation [21]. For food contact surfaces (FCS), persistent *L. monocytogenes* are most commonly isolated from equipment and conveyors, and for non-food contact surfaces (NFCS), from drains and floors. Repeated isolation from drains and floors may indicate that persistence is located elsewhere, as these sites are collecting points for *L. monocytogenes* [7].

1.5. AIM OF THE STUDY

The objective of the present study is to ascertain the *L. monocytogenes* genotypes from a set of isolates derived from two meat-processing facilities, with a view to tracing them back to the site of entry or niche, and to perform a source attribution. The results of this study will provide the manufacturer with the necessary information to develop a set of measures to prevent the spread and establishment of these genotypes in the FPE.

The categories distinguish between transient *L. monocytogenes* contamination (sporadic), repeated but irregular occurrences (recurrent), and long-term environmental survival (persistent), based on isolation frequency and source. This helps target control measures effectively (Table 1).

Table 1: Categorization of *L. monocytogenes* isolates by source and frequency.

Category	Definition	Example Sources
Sporadic CCs	Clonal complexes (CCs) isolated **only once** during the study period, with no evidence of recurrence. These likely represent **transient contamination** events with limited risk of persistence or spread in the facility.	Raw material (RM), personnel (PE), product associated (PA), food contact surfaces (FCS), non-food contact surfaces (NFCS)
Recurrent CCs	CCs isolated **more than once**, but not consistently from the same niche or time point. These may indicate **repeated introduction** (e.g., via raw material) or **intermittent survival** in the environment.	Raw material (RM), personnel (PE), food contact surfaces (FCS), non-food contact surfaces (NFCS)
Persistent CCs	CCs isolated **repeatedly over multiple sampling events**, particularly from **hard-to-clean niches** (e.g., drains, floors, or equipment crevices). These suggest **established environmental reservoirs** or **ongoing contamination**.	Non-food contact surfaces (NFCS) such as drains, floors, or equipment with poor accessibility.

Abbreviations: FCS = food contact surface, **NFCS** = non-food contact surface, **PA** = product-associated, **PE** = personnel-associated, **RM** = raw material.

These scenarios illustrate how *L. monocytogenes* isolation patterns inform source attribution. CC-X suggests reintroduction from raw material, CC-Y indicates persistence in drains, and CC-Z reflects transient contamination from personnel, guiding targeted interventions (Table 2).

Table 2 *:* Practical example: Persistence vs. Reintroduction.

Scenario	Interpretation	Actionable Insight
CC-X found in RM (Month 1) and FCS (Month 2)	Likely reintroduction via RM	Strengthen RM supplier controls
CC-Y found in drains (Months 1, 3, 5)	Likely persistent in niche	Deep clean drains, review sanitation
CC-Z found in PE (Month 2) only	Sporadic, likely transient	Reinforce personnel hygiene training

2. MATERIAL AND METHODS

2.1. ISOLATE DESCRIPTION

The list of devices and consumables used in this study are listed in Appendix Table 1.

A *L. monocytogenes* isolate set (n=297) was provided by Dr. Beatrix Stessl (Center for Food Science and Public Veterinary Medicine) for closer examination.

The strain set contained *L. monocytogenes* isolates from two food-producing companies (Company A n=232 isolates and Company B n=65). The isolates were obtained from four sample categories: raw material (RM), food contact surfaces (FCS; conveyors, belts, tables, slicers, molds, and knives), non-food contact surfaces (NFCS; floors, walls, and drains), personnel-associated (PE) samples and product-associated (PA) samples. Details can be found in the following illustrations (Figure 5).

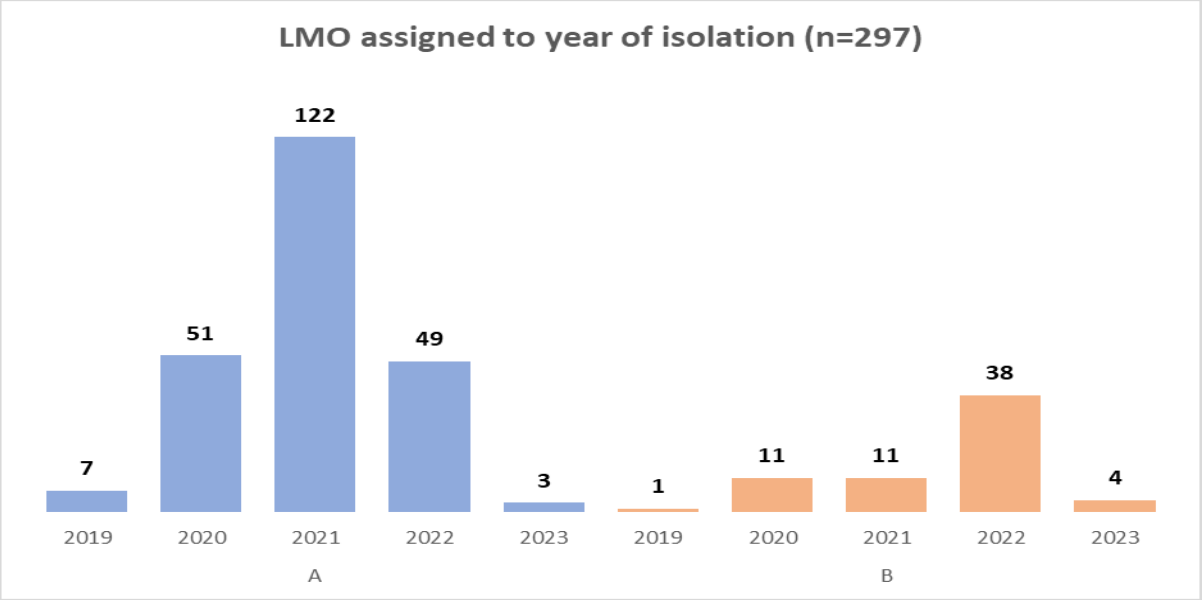


Figure 5*:* Overview of *L. monocytogenes* isolates included in this study from Company A and B.

Abbreviations: LMO = *Listeria monocytogenes, L. = Listeria*.

In total 297 *L. monocytogenes* isolates were collected between 2019-2012; Thereof 232 isolates were assigned to Company A and 65 isolates to Company B. The number of isolates per year varies greatly between the two companies, with most isolates from Company A in 2021 (122) and from Company B in 2022 (38).

The variation of the number of isolates between the companies are due to the different in-house monitoring systems in place and the size of the food-processing facilities. Company A has a self-monitoring system with a large building and extension, Company B a self-monitoring system with only one building. Company A produces a wide range of meat products, most of which are heated, while company B concentrates on convenience meat products (convenience levels IV and V) (Figure 5).

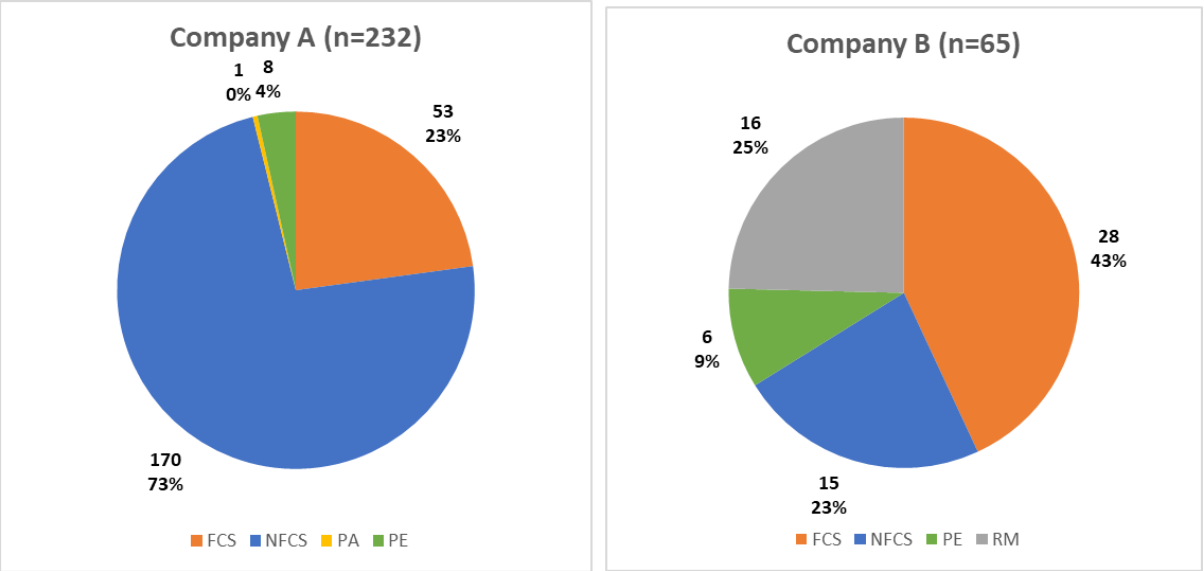


Figure 6*: L. monocytogenes* positive sampling sites assigned to Company A and B.

Abbreviations: FCS = food contact surface, **NFCS** = non-food contact surface, **PA** = product-associated, **PE** = personnel-associated, **RM** = raw material, *L. = Listeria*.

The isolates were assigned to one of the 5 specified sampling sites associated with the sample type of the specific product or non-product. However, there were no isolates from "raw material" from Company A. The distribution of isolates differs between the two companies. In Company A, most of the 232 isolates came from "non-food contact surfaces" (170), followed by "food contact surfaces" (53). Most of the 65 isolates from Company B came from "food contact surfaces" (28), "raw material" (16) and "non-food contact surfaces" (15) (Figure 6).

In the practical part of this work, the following steps were carried out and the methods described below were applied to obtain the final data of the experiment for an analysis of the occurrence, frequency, and genetic diversity of *L. monocytogenes*. This includes the steps from DNA extraction to processing up to the final data analysis. The sequencing of the PCR (polymerase chain reaction) products was performed by LGC Genomics (https://shop.lgcgenomics.com/).

Quality analysis and raw data analysis were carried out in the laboratory of the Department of Food Microbiology (Vetmeduni Vienna). The bioinformatics tool of the Institute Pasteur (*Listeria* Pasteur MLST sequence definition database) was used to compare the sequences obtained with a database and to determine their clonal complexes (CCs).

2.2. DNA EXTRACTION AND PCR MASTER MIX

The *L. monocytogenes* isolates used in this study were obtained directly from the collection of the Center for Food Science and Public Veterinary Medicine (Vetmeduni Vienna, Team Dr. Beatrix Stessl). The *L. monocytogenes* strain set was previously validated using the Rapid L'Mono agar and PCR method [22] and stored in a cryoculture collection at -80°C.

To obtain the allelic variants of the three relevant housekeeping genes (*abcZ*, *bglA* and *ldh*), Chelex DNA extraction was used to separate cellular material from bacterial DNA [23]. Housekeeping genes are genes that are “essential for basic cellular functions, regardless of their specific function in the organism, and are expressed uniformly with low variance under control and experimental conditions” [24].

In a first step, *L. monocytogenes* isolates were transferred with a 1 µl loop to a tube and mixed with 0.01 M Tris-HCl buffer. Next, 400 µl of Chelex Resin was added, which purifies the solution and keeps the DNA stable for further processing. It binds metal ions such as Mg2+, which are essential for DNA function. This ensures that any active enzymes that might degrade the DNA are rendered inactive. In addition, the Chelex Resin binds cell detritus while the DNA remains in solution (Bio-Rad, 2023). The cell suspensions were then heated to 100°C for 10 minutes. To separate the DNA from fragments and other cellular components, the tubes were centrifuged at 10,000g for 5 seconds. The free DNA (100 µl) was carefully transferred to new tubes suitable for PCR (maximum recovery tubes). The Chelex Resin component at the bottom was not shaken as it contained all unwanted cell debris. The *L. monocytogenes* DNA was not further purified and could be used directly for target-specific PCR or stored at -20 °C until further use.

The purified DNA was then amplified by simple MLST (multilocus sequence typing)-specific PCR reactions for the housekeeping genes *abcZ*, *bglA* and *ldh* (Tables 3 and 4).

A Taq DNA polymerase master mix (recombinant) and a dNTP mix (Invitrogen, Thermo Fisher) were used and target-specific primers for *abcZ*, *bglA* and *ldh* (synthesized by https://www.microsynth.com/home-ch.html) were added to the PCR reaction volume of 45 µl.

Finally, the *L. monocytogenes*-specific DNA templates (5 µl) were added to each reaction volume (50 µl total). In addition, a negative control for DNA extraction, a positive control (*L. monocytogenes* ATCC BAA-679™) and a non-template control (NTC) for the master mix reaction were added to the master mix aliquots (45 µl master mix and 5 µl control each).

Table 3: Overview of targeted housekeeping genes.

Locus	Amplicon length	Housekeeping gene
***abcZ* (*lmo*2752)**	537 bp	ABC transporter
***bglA* (*lmo*0319)**	399 bp	Beta glucosidase
***ldh* (*lmo*0210)**	453 bp	L-lactate dehydrogenase

Table 4*:* PCR master mix components and their concentrations and volumes.

PCR master mix (housekeeping gene *abcZ* & *bglA*)			
Component	**Final concentration**	**Stock concentration**	**Volume for one sample (µl)**
DEPC water			31.3
10x PCR buffer	1x		5
$MgCl_2$	2.5 mM	50 mM	2.5
Primer F	200 nM	5000 nM	2
Primer R	200 nM	5000 nM	2
dNTPs	200 µM	5000 µM	2
Taq polymerase	1 U	5U/µl	0.2
DNA template			5
PCR master mix (housekeeping gene *ldh*)			
Component	**Final concentration**	**Stock concentration**	**Volume for one sample (µl)**
DEPC water			15.2
10x PCR buffer	1x		5
$MgCl_2$	2.5 mM	50 nM	2.5
Primer F	1000 nM	5000 nM	10
Primer R	1000 nM	5000 nM	10
dNTPs	200 µM	5000 µM	2
Taq Polymerase	1.5 U	5U/µl	0.3
DNA-template			5

Abbreviations: PCR = polymerase chain reaction.

After preparing the master mix, 5 µl template DNA was added to 45 µl master mix in 0.5 ml DNA-free PCR tubes (Table 4). Throughout the process, the filled tubes and PCR chemicals had to be stored in pre-cooled racks to avoid loss of polymerase efficiency and premature degradation of the components. In addition, all pipetting was performed under a laminar flow workbench to avoid contamination with unwanted DNA.

The DNA master mix was now ready for amplification in a PCR thermal cycler (VWR Doppio PCR thermal cycler). Again, different programs had to be used depending on the allele targeted to ensure optimal

PCR amplification. Table 5 lists the thermal cycler settings including run times, repeats and temperatures.

Table 5*:* PCR thermal cycler settings.

PCR conditions	Temperature	Duration	Cycles
Initial denaturation	94°C	72°C	
Denaturation	94°C	30 s	30
Annealing	52°C (*abcZ*, *ldh*) 45°C (*bglA*)	30 s	
Elongation	72°C	2 min	
Final elongation	72°C	10 min	

Abbreviations: PCR = polymerase chain reaction.

2.3. PCR GEL ELECTROPHORESIS

In this method, macromolecules such as DNA are run through an agarose gel to separate them by size and charge. In this thesis, gel electrophoresis was used to confirm the success and accuracy of all previous steps and to ensure that the PCR product was suitable for sequencing. Since the three target DNA fragments (*abcZ*, *bglA*, and *ldh*) were of different lengths, their migration distance in the agarose gel was used to determine whether they were the PCR-targeted amplicons.

To prepare a 1.5% agarose gel, 1.5 g of agarose powder (Peqgold, VWR International) and 1x Tris-Acetate-EDTA (TAE) buffer (100 ml) were mixed in an Erlenmeyer flask. After initial cooling, a DNA dye (peqGREEN, fluorescent dye, VWR International) was added and dispersed in the solution. The melted agarose was then poured into a gel casting system (Bio-Rad) and a comb was inserted into the freshly cast gel to form chambers. After cooling (30 min), the gel was placed in the electrophoresis chamber with 1x TAE buffer. The comb side had to be oriented to allow the samples to migrate from the cathode to the anode, as the DNA is negatively charged and follows the positive charge. 5 µl of PCR product and loading dye solution (2-3 µl) were mixed and pipetted into the comb pockets. In addition, a DNA ladder (GeneRuler 1 kb DNA Ladder, Thermo Fisher) was added for comparison, as well as a positive and negative control for each PCR product. Each well corresponded to a sample that was mapped for later data processing. The gel was then ready for electrophoresis. After the tube was sealed with a lid, a power supply connected to the tube generated a voltage of 120 V (Bio-Rad). The DNA fragments began to migrate through the agarose according to their size and were kept in motion for approximately 20-30 minutes.

The DNA amplicons were visualized using a GelDoc Imaging System (Bio-Rad). This device uses UV light to visualize the PCR products, as the dye reacts to the UV radiation and makes them glow. An image in TIFF format was generated for further quality control and interpretation of the results. This provided immediate evidence of the success of both the PCR and the gel electrophoresis. The results could then be compared to the original samples for allelic variation. While *abcZ* and *ldh* were approximately the same length, *bglA* was a shorter fragment, which should correspond to shorter DNA migration in the gel. The 1-kb DNA ladder was used to verify that the DNA fragments were in the correct regions and to check the validity of the positive and negative controls.

Sequencing was performed externally on a Sanger sequencer (Premium Run, Sanger Sequencing, LGC Genomics (https://shop.lgcgenomics.com/). After evaluation and confirmation that the correct PCR amplicons were present and purified, the PCR product was sent in a skirted PCR microplate with barcodes.

The FASTA sequences obtained were then compared with the Bacterial Isolate Genome Sequence Database (BIGSdb) of the Institute Pasteur (https://bigsdb.pasteur.fr/listeria/), operated by software developed at the University of Oxford.

This database contains genotypic data of *L. monocytogenes* isolates collected using 7-allele MLST and Core Genome MLST (cgMLST). Individual raw sequences or batches can be entered into the software, which uses the bioinformatics algorithms BURST and BLAST to match the entry to the database. Either an existing perfect match or the closest match is displayed, and mutations were highlighted to identify allelic differences. An allelic profile was generated for each allele (*abcZ, bglA* and *ldh*), which in turn could be entered to determine the specific sequence type (ST) of the *L. monocytogenes* isolate. In addition, information on the clonal complex (CC) and lineage was provided [25].

By comparing which STs are found in which frequency and at which location, conclusions can be drawn about the hygiene measures, persistent *L. monocytogenes* and *L. monocytogenes* recurrently introduced by raw materials.

3. RESULTS

3.1. RESULTS OF MLST ASSIGNED TO GENETIC LINEAGE I AND II

Out of a total of 297 isolates from Company A and Company B, 30 isolates (10.1%) were identified as genetic lineage I, while 267 isolates (89.9%) were classified genetic lineage II.

Within genetic lineage I, the most prevalent clonal complexes (CCs) were CC59/CC191, comprising 26.67% (n = 8) of the isolates, followed by CC6 at 23.33% (n = 7), and CC1 at 20% (n = 6), as depicted in Figure 7.

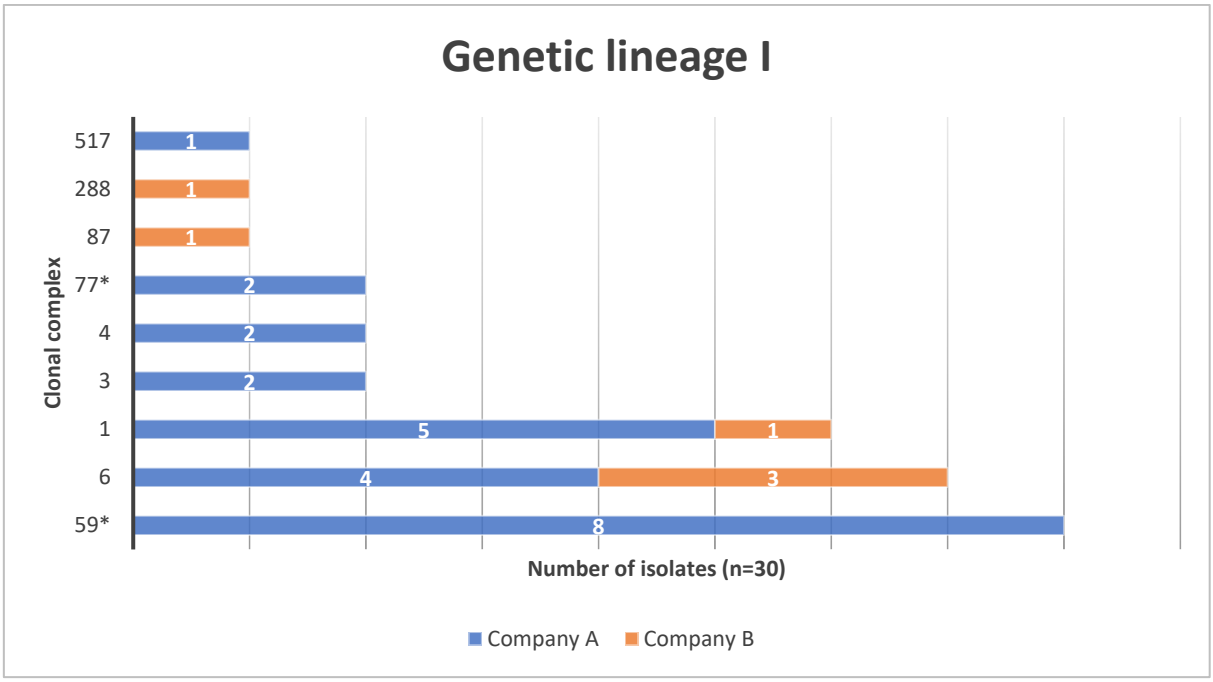


Figure 7*:* Distribution of *L. monocytogenes* clonal complexes of genetic lineage I.

Abbreviations: ST = sequence type. 59*, corresponding to CC59/191; 77*, corresponding to CC77/489.

For genetic lineage II, the predominant clonal complexes were CC37, accounting for 34.83% (n = 93) of the isolates, followed by CC9 at 19.1% (n = 51), and CC8 at 11.61% (n = 31), as illustrated in Figure 8. Additionally, Company A exhibited greater diversity in the distribution of clonal complexes compared to Company B.

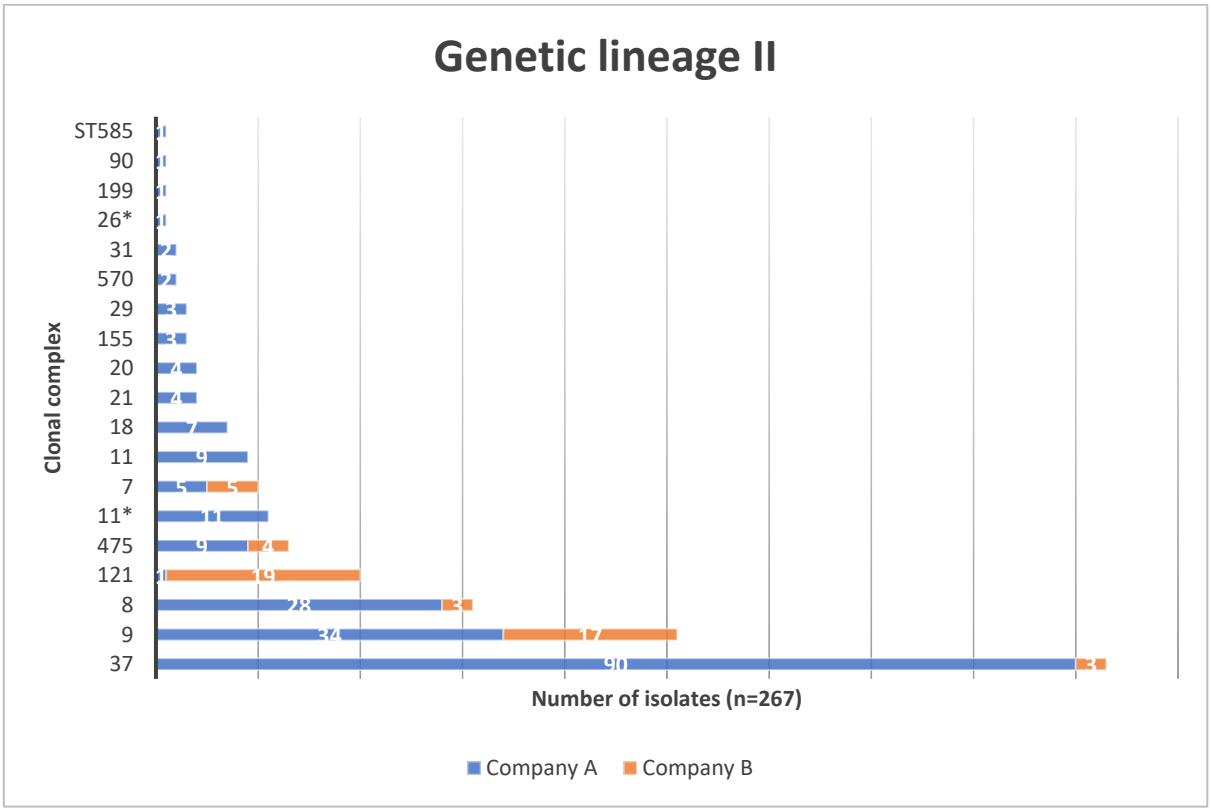


Figure 8*:* Distribution of *L. monocytogenes* clonal complexes of genetic lineage II.

Abbreviations: ST = sequence type. 11*, corresponding to CC11/89; 26*, corresponding to CC26/901/1503/1515/2454.

Certain clonal complexes (CC) were exclusive to one of the two companies. As an example, Company A frequently harbored clonal complexes such as CC59/ CC191, CC3, CC4, and CC77/CC489 from lineage I, as well as CC11/CC89 from lineage II. Conversely, clonal complexes CC87 and CC288 from lineage I were identified solely in Company B.

When examining the proportions of clonal complexes (CCs) across both genetic lineages, CC37, CC9, CC8, and CC121 of genetic lineage II emerged as the most prevalent overall, with frequencies of 31.31%, 17.17%, 10.44%, and 6.73%, respectively. Table 6 provides a comprehensive overview of the frequencies and distribution of these clonal complexes.

Table 6: Frequencies and distribution of clonal complexes from a total of 297 isolates.

CC	*abcZ*	*bglA*	*ldh*	number	percentage
Genetic lineage I					
59 & 191	11	1	1	8	2.69
6	3	9	1	7	2.36
1	3	1	1	6	2.02
4	1	2	5	2	0.67
3	4	4	1	2	0.67
77 & 489	11	9	1	2	0.67
195 & 517	11	3	1	1	0.34
87	12	1	39	1	0.34
288	34	39	1	1	0.34
CC	*abcZ*	*bglA*	*ldh*	number	percentage
Genetic lineage II					
37	5	7	8	93	31.31
9	6	5	4	51	17.17
8	5	6	3	31	10.44
121	7	6	37	20	6.73
475	7	438	37	13	4.38
11& 89	7	6	2	11	3.70
7 & 1211	5	8	2	10	3.37
11	7	5	4	9	3.03
18	7	6	6	7	2.36
21	7	7	6	4	1.35
20	17	13	7	4	1.35
155	7	10	2	3	1.01
29	15	10	3	3	1.01
570	5	5	7	2	0.67
31	7	14	8	2	0.67
26&901&1503&1515&2454	5	10	2	1	0.34
199	9	10	26	1	0.34
90	21	6	2	1	0.34
ST585	30	6	26	1	0.34

Abbreviations: CC = clonal complex, **ST** = sequence type.

Certain clonal complexes of genetic lineage I and II occurred significantly more often than others in Company A and Company B in the different sampling years from 2019 to 2023. The following two graphs illustrate the assignment of clonal complexes of lineage I (Figure 9) and II (Figure 10) to the two companies and to each year.

3.2. RESULTS OF MLST ASSIGNED TO COMPANY A AND B DURING THE YEARS OF SAMPLING

In Company A, the most prevalent CC of genetic lineage I was CC59/CC191, identified during eight sampling events throughout the year 2020. The second most common was CC1, which occurred during five sampling events, with three instances in 2020 and two in 2021. The third most frequent clonal complex of lineage I in Company A was CC6, recorded four times in 2021. In contrast, Company B did not have any clonal complexes from lineage I that appeared more than once in any given year. However, CC6 was detected once in 2020 and reappeared in both 2022 and 2023.

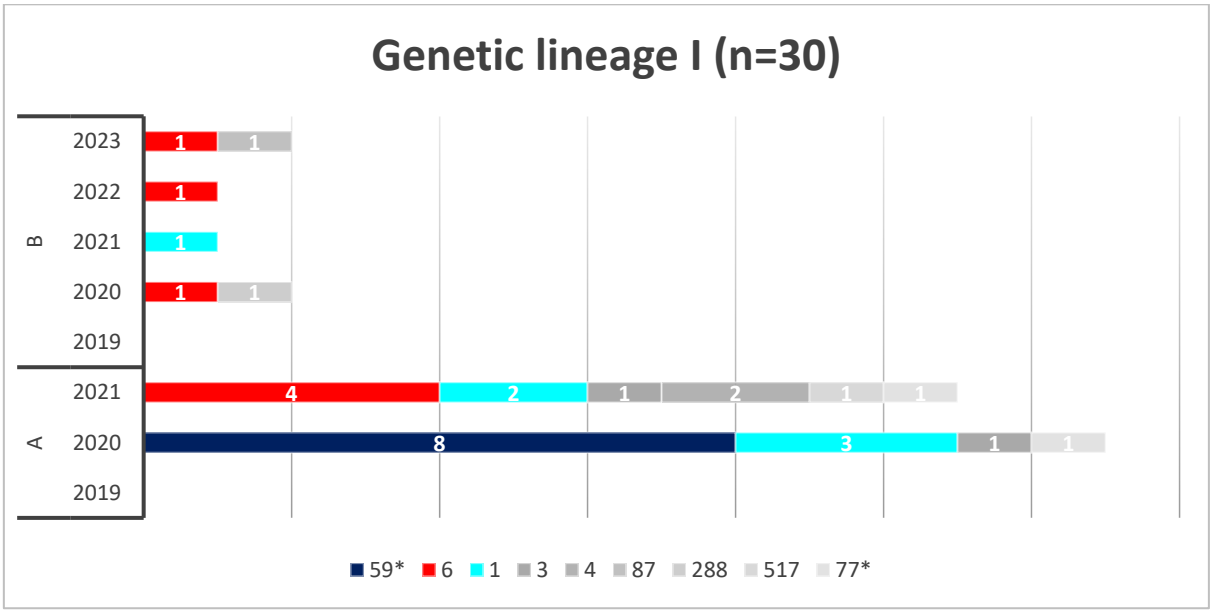


Figure 9*: L. monocytogenes* clonal complexes of genetic lineage I assigned to Company A and B as well as year of sampling.

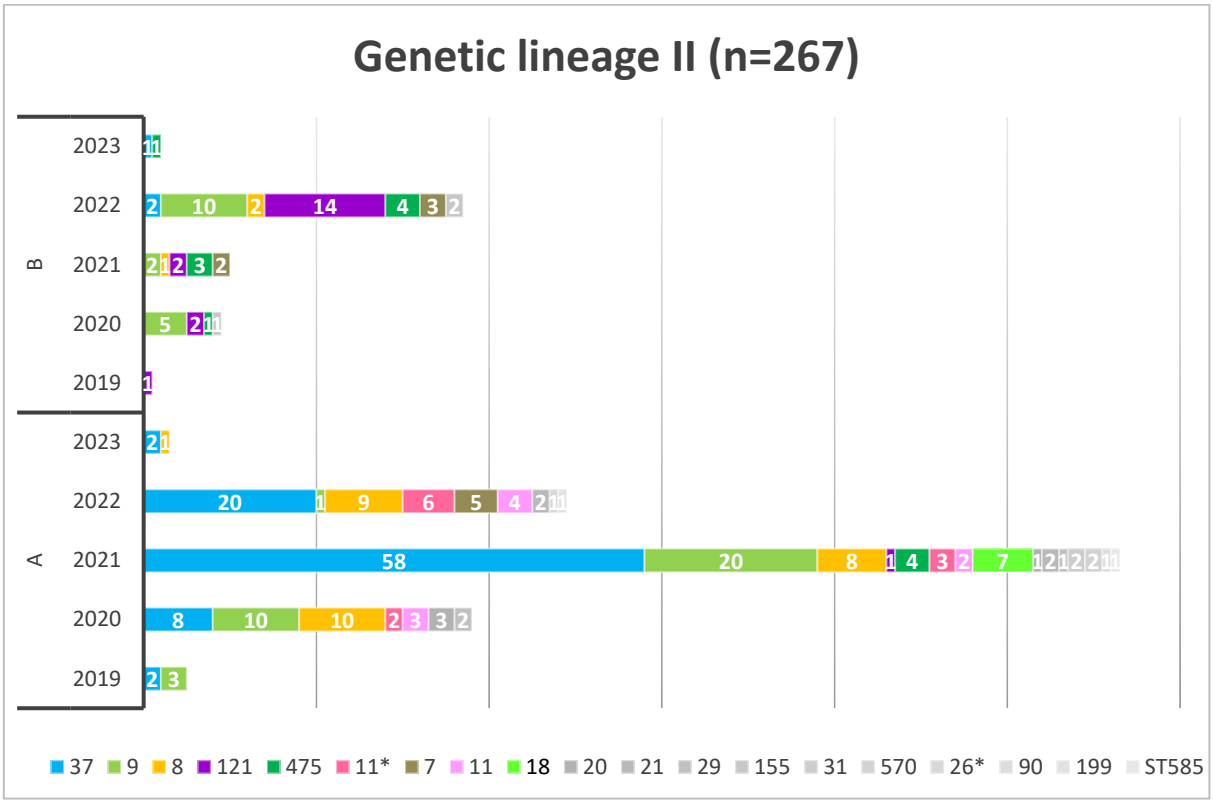


Figure 10*: L. monocytogenes* clonal complexes of genetic lineage II assigned to Company A and B as well as year of sampling.

Abbreviations: ST = sequence type. 26*, corresponding to CC26/901/1503/1515/2454.

The most prevalent clonal complex of genetic lineage II in Company A was CC37, which was identified in a total of 90 isolates. Although a significant proportion of these occurrences was recorded in 2021 (n=58) and 2022 (n=20) - years with more positive sampling events - CC37 was detected in each of the five sampling years from 2019 to 2023 in Company A. This continuous presence may indicate an underlying persistence. The second and third most common clonal complexes of lineage II in Company A were CC9 (n=34) and CC8 (n=28), respectively. The continuous presence of both CC9 (2019–2022) and CC8 (2020–2023) in Company A strongly suggests environmental persistence. Their temporary absence during years with fewer isolates and improved hygiene further supports this, as reduced contamination likely suppressed detection rather than eradication. This emphasizes the importance of maintaining sanitation efforts in areas that are difficult to clean, even during periods of lower overall *Listeria* prevalence. In Company B, the most frequently identified clonal complex from Lineage II was CC121, which was detected during 19 sampling events. This complex was most prevalent in 2022 (n=14) and was consistently found in samples from 2019 to 2022 over a four-year period. Additionally,

CC475 was also detected consistently for four consecutive years, from 2020 to 2023. Overall, CC475 was the third most common clonal complex of Lineage II in Company B, with detection during nine sampling times, while CC9 ranked second, being identified 17 times consistently over the three-year span from 2020 to 2021. The persistence of these three clonal complexes may contribute to their continued presence.

3.3. TRANSMISSION MAPPING

The maps of the operational spaces in Company A and Company B, depicted in Figures 11 and 12, illustrate the distribution of clonal complexes within each company over the specified time period. The rooms outlined in black adhere to a one-way system within the clean zone, progressing sequentially from left to right in row 1 and then from left to right in row 2. It is important to note that certain products, such as schnitzel, sausages or cooked ham were produced in specific rooms. Additionally, the variously numbered packaging rooms (I, II, and III) are designated for the packaging of distinct products and are not interconnected.

Distribution of subtypes in Company A across facility areas

During 2019/2020, *L. monocytogenes* was detected in four rooms of Company A's processing rooms: raw ham processing, packaging I, II, and III, with raw ham processing showing the highest diversity of nine clonal complexes. CC37 was the most prevalent, present in all positive rooms. In 2021, CC37 was found in five areas (cooking room, raw ham processing, selch room, packaging II, and slicing room), while CC18 was identified in three areas (cooking room, raw ham processing, and slicing room).

During 2022/2023, CC8 was detected in five rooms (cooking room, raw ham processing, selch room, packaging II, and slicing room), and CC37 in three rooms (cooking room, raw ham processing, and selch room). Some clonal complexes persisted in the same areas over multiple years. For example, CC9 and CC37 were consistently found in the raw ham processing room from 2019 to 2023. Other complexes, such as CC20 and CC29 in raw ham processing, CC11/CC89 and CC37 in the cooling room, and CC8 and CC37 in the smoking room, were present for at least two consecutive periods (Figure 11).

Distribution of subtypes in Company B across facility areas

In 2019/2020, *L. monocytogenes* was detected in four rooms of Company B, with CC9 present in all locations: the poultry cutting room, cutting room, tumbler, and convenience production area (Figure 12). In 2021, detections occurred in nearly the same areas, with no clonal complex found in more than

two locations. During 2022/2023, *L. monocytogenes* was detected in 7/8 rooms, excluding the tumbler room (Figure 12). Four clonal complexes were identified across multiple rooms: CC9, CC37, CC475, and CC7/CC1211, each present in three different areas.

Certain clonal complexes persisted in specific rooms over time. CC9 was consistently detected in the poultry cutting room from 2019 to 2023, and CC7/CC1211 in the cutting room from 2021 to 2023. This persistence suggests potential environmental establishment, while intermittent absences may indicate reintroduction of the pathogen.

Figures 11 and 12 illustrate the detection frequency of clonal complexes in various rooms of Company A and Company B, categorized by sampling site type (FCS, NFCS, PA, PE), offering insights into their prevalence.

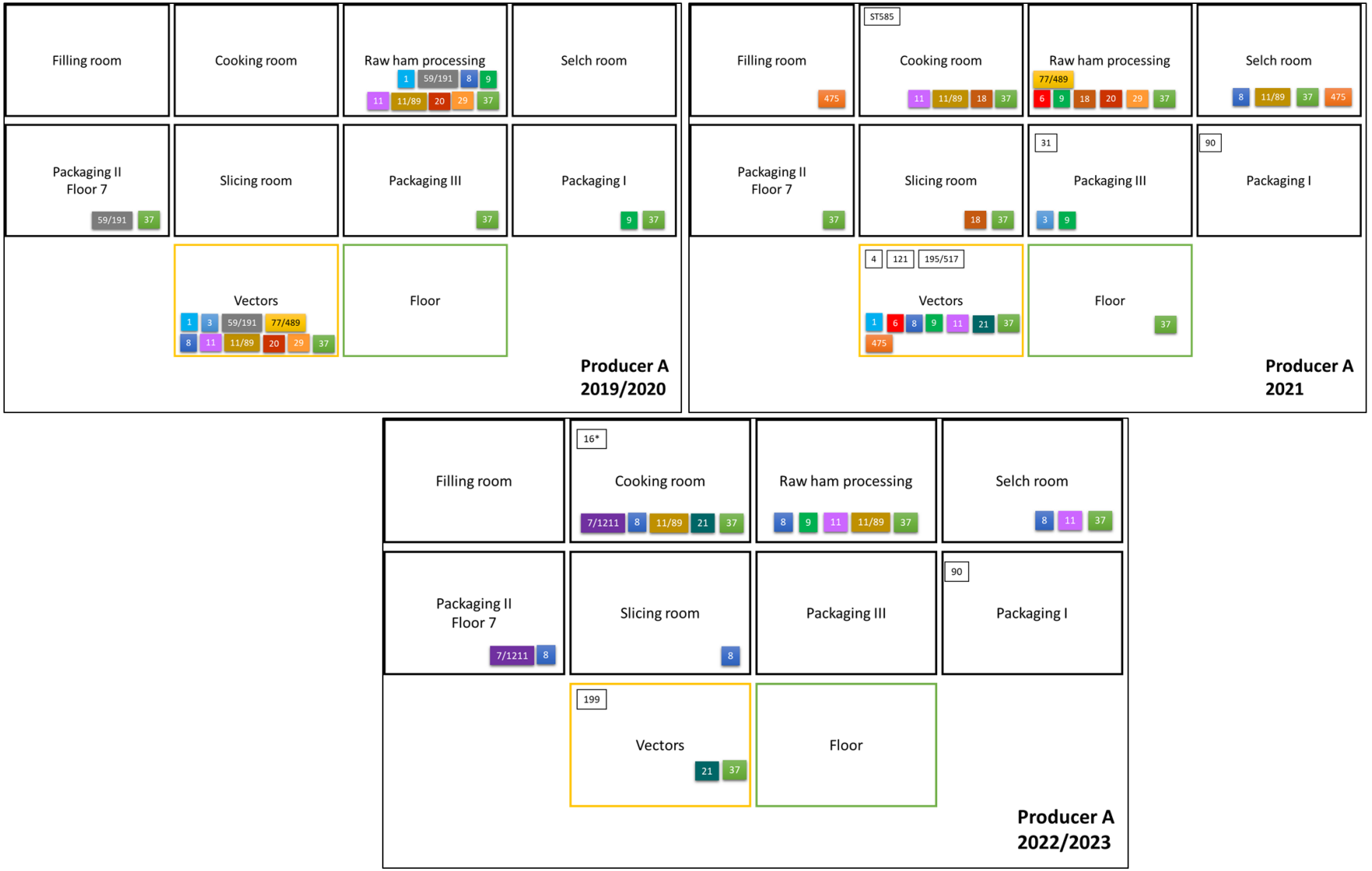


Figure 11: Allocation of *L. monocytogenes* clonal complexes to Company A rooms from 2019-2023.



Figure 12: Allocation of *L. monocytogenes* clonal complexes to Company B rooms from 2019-2023.

3.4. DETAILED *L. MONOCYTOGENES* DISTRIBUTION

In 2019/2020, CC9 (genetic lineage II) was the most frequently detected clonal complex in Company A. It was identified among 12 isolates on non-food contact surfaces (NFCS) in the raw ham processing room (see Figure 13), which suggests that it is likely to persist in this area. The same room and surfaces also harbored five isolates of CC59/CC191 (lineage I) and four isolates of CC8 (lineage II), highlighting the presence of multiple clonal complexes in this environment. Furthermore, CC8 and CC37 (both lineage II) were detected five and four isolates among vectors, respectively, suggesting potential routes for cross-contamination or spread within the facility. These findings emphasize the importance of targeted sanitation and monitoring in the raw ham processing room to mitigate the risk of *L. monocytogenes* persistence and transmission.

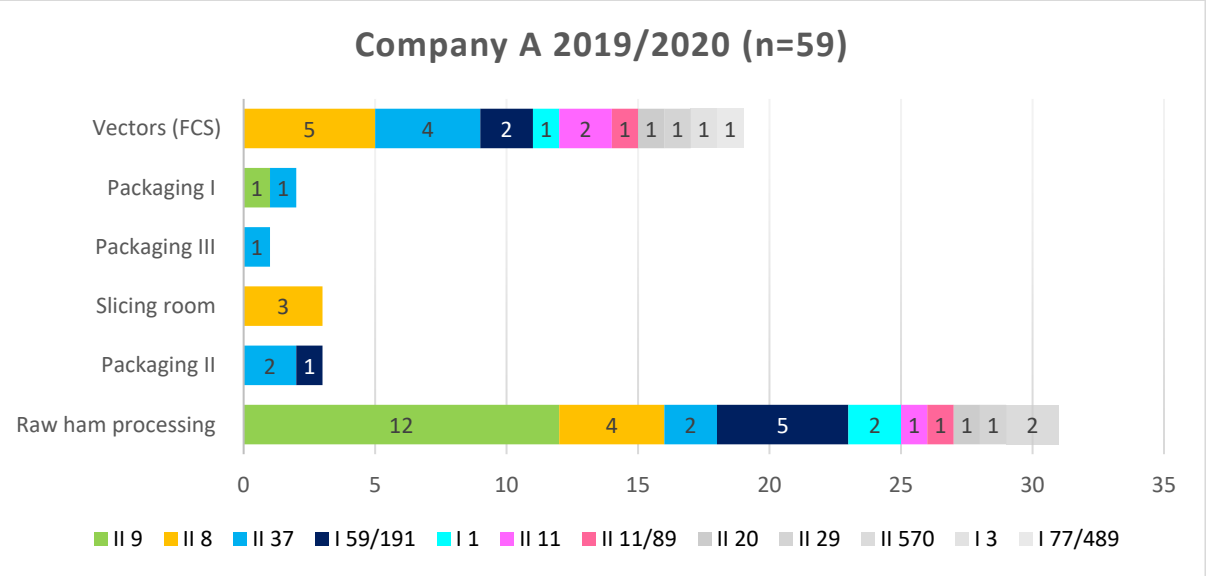


Figure 13*: L. monocytogenes* clonal complexes assigned to rooms and sampling sites of Company A in 2019/2020.

Abbreviations: FCS = food contact surface, **NFCS** = non-food contact surface, **PA** = product-associated, **PE** = personnel-associated.

During the same period (2019-2020), Company B's distribution of clonal complexes across rooms and sampling sites was relatively balanced, with only single detections recorded (see Figure 14). Notably, CC9 was found twice on food-contact surfaces (FCS) in the poultry cutting room.

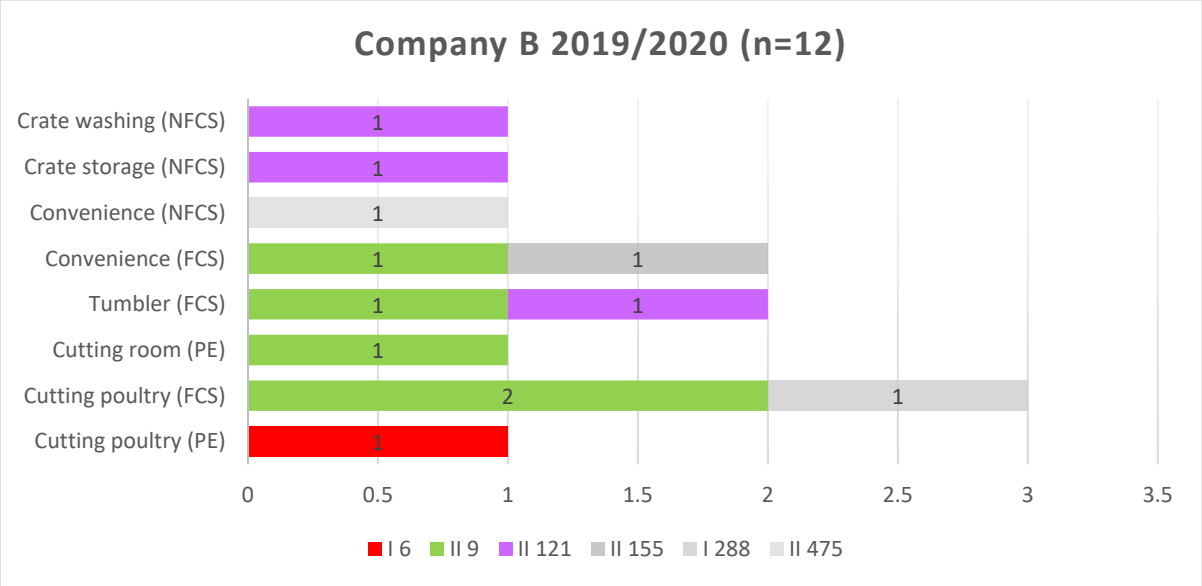


Figure 14*: L. monocytogenes* clonal complexes assigned to rooms and sampling sites of Company B in 2019/2020.

Abbreviations: FCS = food contact surface, **NFCS** = non-food contact surface, **PA** = product-associated, **PE** = personnel-associated.

In Company A, CC37 (lineage II) emerged as the dominant clonal complex. It was detected in 25 isolates on non-food contact surfaces (NFCS) in the cooking room (Figure 19), which indicates a significant, persistent reservoir in this area. It was also found among 11 isolates in the Packaging Room II and seven isolates in the Selch Room, exclusively on NFCS, suggesting widespread environmental contamination in multiple processing areas. Meanwhile, CC9 (Lineage II) remained prevalent, with 13 isolation events on NFCS in the raw ham processing room, further establishing its presence as a persistent strain in this facility.

In contrast, Company B exhibited a more sporadic distribution of clonal complexes in 2021, consistent with the previous sampling period. CC9 (Lineage II) was detected only twice on food-contact surfaces in the poultry cutting room (Figure 15), with no evidence of clustering or persistence. These differences highlight the variations in contamination dynamics between the two companies and indicate targeted areas for intervention in Company A, as well as a lower baseline level of contamination in Company B.

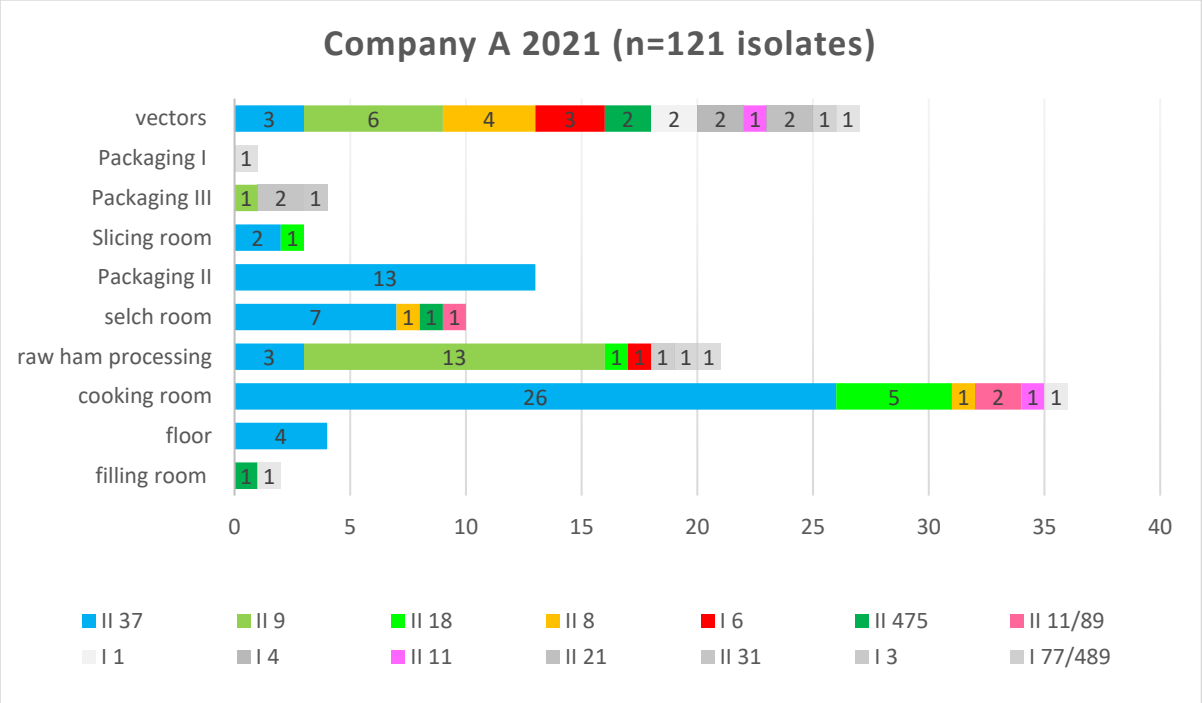


Figure 15*: L. monocytogenes* clonal complexes assigned to rooms and sampling sites of Company A in 2021.

Abbreviations: FCS = food contact surface, **NFCS** = non-food contact surface, **PA** = product-associated, **PE** = personnel-associated.

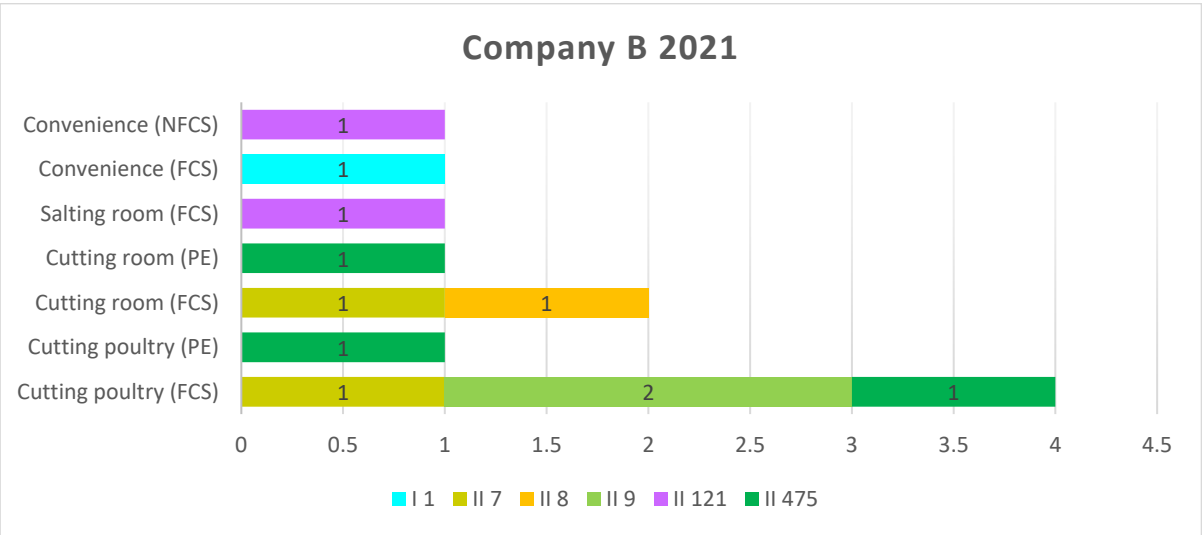


Figure 16*: L. monocytogenes* clonal complexes assigned to rooms and sampling sites of Company B in 2021.

Abbreviations: FCS = food contact surface, **NFCS** = non-food contact surface, **PA** = product-associated, **PE** = personnel-associated.

In 2022/23, CC37 (lineage II) was the most frequently identified clonal complex in a particular area of Company A, predominantly on NFCS in the cooking room (n = 14) (see Figure 16). This clonal complex was also detected among four isolates in the smoking room on NFCS. Additionally, there were each five isolates assigned to CC8 (lineage II) in the cooking room (NFCS), and to CC11/CC89 (lineage II) in the raw ham processing room (NFCS).

During the same period, the most common clonal complexes found in Company B were CC121 (lineage II), detected in the convenience room on FCS (n = 7) and in the cooling room on raw materials (n = 4); and CC9 (lineage II), identified in the cooling room on raw materials (n = 4) and in the crate washing area on non-food contact surfaces (n = 4) (see Figure 17).

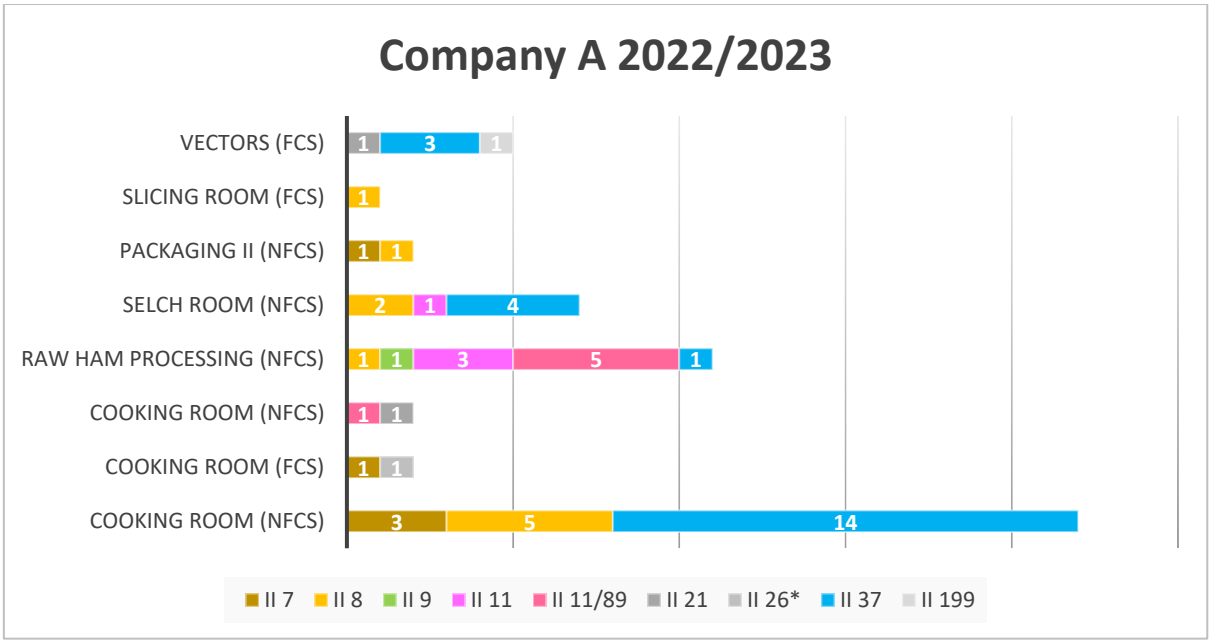


Figure 17*: L. monocytogenes* clonal complexes assigned to rooms and sampling sites of Company A in 2022/2023.

Abbreviations: ***FCS*** *= food contact surface,* ***NFCS*** *= non-food contact surface,* ***PA*** *= product-associated,* ***PE*** *= personnel-associate*

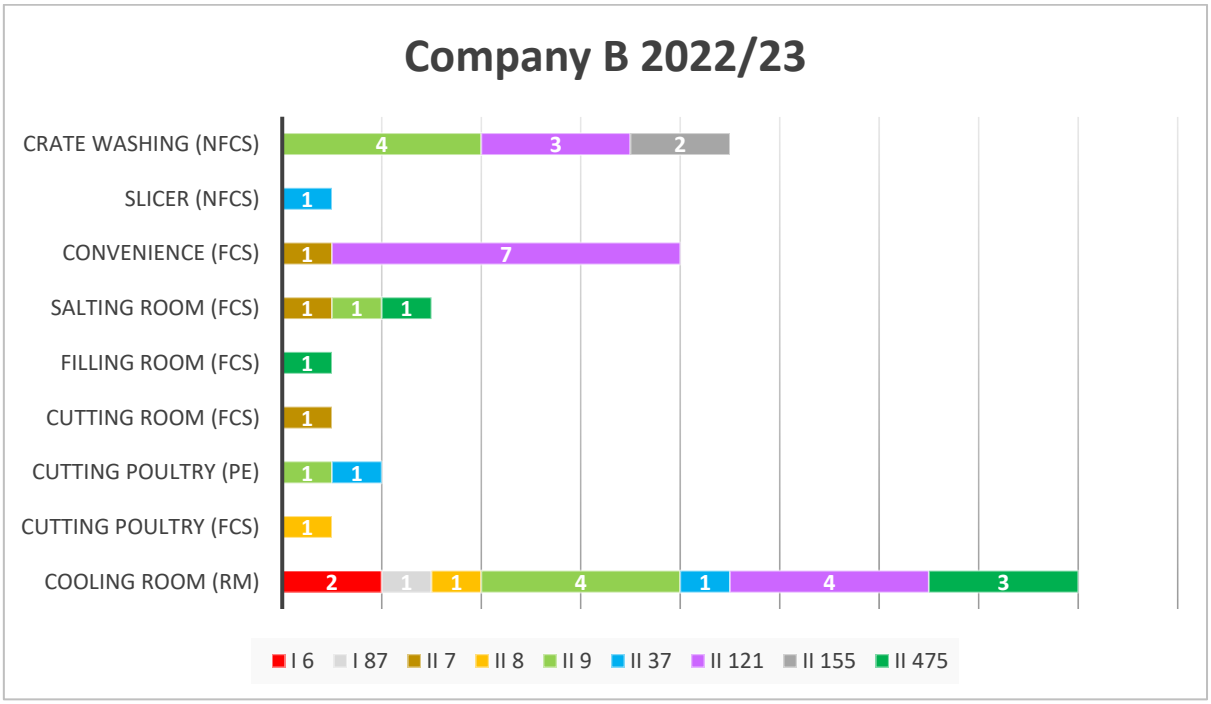


Figure 18: *L. monocytogenes* clonal complexes assigned to rooms and sampling sites of Company B in 2022/2023.

Abbreviations: FCS = food contact surface, **NFCS** = non-food contact surface, **PA** = product-associated, **PE** = personnel-associated.

3.5. *L. MONOCYTOGENES* PERSISTENCE

In company A, *L. monocytogenes* clonal complexes CC59, CC6, and CC1 (genetic lineage I) were strongly associated with persistence in NFCS niches and contamination of FCS. Other clonal complexes were identified either recurrently or sporadically and were primarily linked to raw materials, FCS or personnel as vector (Table 7).

Within genetic lineage II, a greater number of persistent clonal complexes (CCs) were observed — up to 13 in total, with 11 identified in Company A (Table 8). Among these, CC7, CC9, and CC475 led to persistence in both facilities, enabling survival in protected niches.

Table 7 *:* Genetic lineage I: Assignment to persistence, recurrence, and sporadic detection of *L. monocytogenes.*

	A					B					
CC	**2019/2020**	**2021**	**2022/2023**	**Source**	**Category**	**2019/2020**	**2021**	**2022/2023**	**Source**	**Category**	n
59*	8			FCS, NFCS	persistent						8
6		4		FCS, NFCS	persistent	1		2	RM, PE	recurrent	7
1	3	2		FCS, NFCS	persistent	1			FCS	sporadic	6
3	1	1		FCS, NFCS	recurrent						2
4	2			FCS	recurrent						2
77*	1	1		FCS, NFCS	recurrent						2
87				RM		1			FCS	sporadic	1
288				FCS		1			FCS	sporadic	1
517	1			FCS	sporadic						1

Table 8: Genetic lineage II: Assignment to persistence, recurrence, and sporadic detection of *L. monocytogenes.*

	A					B					
CC	**2019/2020**	**2021**	**2022/2023**	**Source**	**Category**	**2019/2020**	**2021**	**2022/2023**	**Source**	**Category**	n
37	10	58	22	PE, PA, FCS, NFCS	persistent			3	RM, PE, NFCS	recurrent	93
9	13	20	1	PE, FCS, NFCS	persistent	5	2	10	RM, PE, FCS, NFCS	persistent	51
8	12	6	10	FCS, NFCS	persistent	1		2	RM, FCS	recurrent	31
121	1			RM, FCS, NFCS	sporadic	3	2	14	RM, FCS, NFCS	persistent	20
475	4			RM, PE, FCS, NFCS	persistent	1	3	5	RM, PE, FCS, NFCS	persistent	13
11*	2	3	6	FCS, NFCS	persistent						11
7			5	FCS, NFCS	persistent	2		3	FCS, NFCS	persistent	10
11	3	2	4	PE, FCS, NFCS	persistent						9
18	7			FCS, NFCS	persistent						7
21	2		2	FCS, NFCS	persistent						4
20	3	1		FCS, NFCS	persistent						4
29	2	1		FCS, NFCS	persistent						3
155				FCS, NFCS		1		2		persistent	3
31	2			NFCS	recurrent						2
199			1	FCS	sporadic						1
570	2			NFCS	recurrent						2
90	1			PE	sporadic						1
26*			1	NFCS	sporadic						1
ST585	1			NFCS	sporadic						1

4. DISCUSSION

While the usual concepts for monitoring *Listeria* in meat and convenience product processing generally focus on sampling after heat treatment and during packaging, our current study shows that it is also necessary to sample upstream facilities and raw materials in order to effectively assess contamination risks [26]. When setting up a *Listeria* monitoring program, it is crucial for food business operators to determine the baseline level of contamination throughout the entire operation. It is therefore recommended that the initial sampling be accompanied by a comprehensive assessment of the contamination status—whether sporadic, localized (hotspots), or widespread [27].

In our local food industry, meat processing and convenience processing companies often grow from small businesses into medium-sized enterprises, with their buildings being expanded organically around the original structure. With these extensions, the original construction—especially the drain system—remains unchanged and has been a source of *L. monocytogenes* contamination in the past [28]. Without adequate hygienic separation between the facility and the external environment, even newly constructed adjacent buildings can quickly become colonized by the same persistent *L. monocytogenes* genotypes found in the older structures [29]. *L. monocytogenes* is capable of colonizing and surviving in food processing environments (FPEs) for years, even decades as also observed in this study. This persistence is largely facilitated by the formation of biofilms, which protect the bacteria from environmental stressors and disinfection measures. Biofilms are often formed by hygiene indicators that are actually associated with resistance to some cleaning chemicals (pseudomonads, soil bacteria such as *Janthinobacter, Psychrobacter, Acinetobacter*, or spore formers). These form a physical barrier and enable the survival of otherwise sensitive *L. monocytogenes* strains under harsh conditions, including exposure to biocides and heavy metals. Studies have shown that persistent strains can adapt to and thrive in niches such as drains, floors, and equipment, making them difficult to eradicate [30]. CC1 and CC4 are considered hypervirulent, are often associated with severe infections in humans, and are found in fresh, minimally processed products. CC1 is particularly common in cattle and food products made thereof and is the most common clonal group associated with listeriosis in humans worldwide [32, 33]. CC6 are often involved in epidemic, multi-country outbreaks involving meat and vegetables and are the dominant genotype in the few raw material studies, such as poultry meat in an Italian study (https://www.ecdc.europa.eu/en/listeriosis/threats-and-outbreaks; accessed on: 11-02-2026) [34]. Although CC59 is not as strongly linked to large-scale outbreaks as CC1, CC4 or CC6, its presence in food processing environments and products highlights

the importance of ongoing monitoring and risk assessment, especially in areas where it has been identified (https://bigsdb.pasteur.fr/listeria/; accessed on: 11-02-2026).

In this study, CC1 and CC6 were also identified as persistent genotypes in Company A. This requires an analysis of the measures used to eliminate *L. monocytogenes* and the derivation of measures to improve environmental hygiene. The question is whether these types were introduced into the facility via contaminated raw materials, whether they were able to adapt to routine cleaning, or whether there were niches that were overlooked in the hygiene concept. It is also possible that not enough hygiene barriers, such as profile gates to storage rooms or hygiene locks to clean areas, were established to provide a clear boundary between delivery and raw material preparation.

In the initial risk assessment, we assume that the raw materials used are contaminated with a high variability of *L. monocytogenes* genotypes and that this must be considered in the manufacturing processes and hygiene concepts. This was also discussed with the two companies.

In *L. monocytogenes* genetic line II, there were many more genotypes that led to persistence at companies A and B between 2019 and 2023. These genotypes, such as CC121 and CC9, are the most successful genotypes worldwide that have adapted to different production facilities [35]. CC121 isolates tend to cluster closely in phylogenetic analyses, suggesting limited genetic divergence within the clonal complex. This uniformity is consistent with its adaptation to specific environmental niches, such as meat processing plants and ready-to-eat food production [36]. *L. monocytogenes* CC9 has a fairly homogeneous core genome globally, with recent diversification occurring in the area of plasmids and prophages. It has three main ST9 clusters, indicating niche adaptation in meat processing [37]. Premature Stop Codons (PMSCs) in *inlA* are present in CC121 and CC9 indicating hypovirulence [38]. Some clonal complexes of genetic lineage II (Table 8) can develop unique genetic traits depending on local environmental stresses such as disinfectant use, soil conditions, or host interactions, and are still undergoing genetic diversification, which is characterized by a heterogeneous genome [39].

For the food business operators (FBOs) of companies A and B, the entries for *L. monocytogenes* genotypes are particularly important. In company A, 7 and 18 different genotypes were present in genetic lines I and II, respectively, in the period 2019-2023. In company B, there were far fewer genotypes (4 and 7 different CCs in genetic lines I and II) that either occurred sporadically and were associated with the raw material or led to temporary persistence in the environment.

Manufacturers can now use rapid three-allele MLST prediction to identify the most important CCs, learn which CCs are particularly frequently associated with outbreaks, or survive very stubbornly as

persisters in biofilms. This learning effect makes it easy to derive rapid response measures and intensive cleaning protocols to gradually eliminate contamination in the food environment.

Intensive cleaning protocols pay particular attention to gully remediation, as these are often clogged with food soil, leading to water accumulation in the manufacturing environment, which promotes the presence of *Listeria.* In production, the new knowledge and awareness that raw materials are contaminated with *L. monocytogenes* to a small degree enables responsible and logistical planning and thus safe food production [40]. Furthermore, follow-up studies should examine the adaptability of certain CCs to biocides [41].

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6. DECLARATION OF INDEPENDENCE

I hereby declare that I have written this diploma thesis independently and that I have not used any resources or literature other than those cited in the text and listed in the references page. All persons who contributed to the preparation of this thesis are named in full. This thesis has not been submitted to any other examination authority and has not been published in any form. Generative artificial intelligence tools were used in a supporting manner for drafting and editing parts of the sections Introduction and Methods. All content generated with their assistance was critically reviewed, verified and where necessary revised by Dr. med. vet. Beatrix Stessl and myself.

Vienna, 4 February 2026.

David Petz.

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8. APPENDIX

Appendix Table 1: Equipment and consumables.

Devices	
Bunsen burner	Schuett-biotec GmbH, Göttingen, Germany
Centrifuge 5424	Eppendorf AG, Hamburg, Germany
E count colony counter	Heathrow Scientific LLC., Illinois, USA
Freezer -20°C	Liebherr-International AG, Bulle, Switzerland
Freezer -80°C	Sanyo Electric Co., Ltd., Osaka, Japan
Gel Doc	Bio-Rad Laboratories Inc., Hercules, California
Heating block	Kleinfeld Labortechnik GmbH., Gehrden, Germany
Incubator 30°C	Ehret GmbH, Mahlberg, Germany
Incubator 37°C	3 M, Minnesota, USA
Incubator 42°C	Sanyo Electric Co., Ltd., Osaka, Japan
Merck Millipore Milli-Q™ Reference Ultrapure Water Purification System	Merck Millipore, Merck KGaA, Darmstadt, Germany
Microwave	Silva Schneider GmbH, Salzburg, Salzburg
Pipetus	Hirschmann Laborgeräte GmbH & Co. KG, Eberstadt, Germany
Power pac 1000 (gel electrophoresis)	Bio-Rad Laboratories Inc., Hercules, California
Scale	Tanita Corporation, Tokyo, Japan
Scale (for gel electrophoresis)	Santorius AG, Göttingen, Germany
Thermal cycler T100	Bio-Rad Laboratories Inc., Hercules, California
Consumables	
Agarose	VWR International GmbH, Darmstadt, Germany
Chelex Resin®	Bio-Rad Laboratories Inc., Hercules, California
Cryogenic vials 2ml	Biologix Group Ltd., Shandong, China
Deoxynucleoside triphosphate (dnTP) mix	Thermo Fisher Scientific Inc., Massachusetts, USA
Diethyl pyrocarbonate (DEPC)-treated water	Sigma-Aldrich Co., St. Louis, USA
Finnipipettes 1-1000µl	Thermo Fisher Scientific Inc., Massachusetts, USA
Glas pasteur pipettes	Brand GmbH & Co. KG, Wertheim, Germany
Inoculation loops 1,10µl	Sarstedt AG & Co. KG, Nürnbrecht, Germany
Latex gloves	Semperit Technische Produkte GmbH, Wien, Austria
Maxymum recovery tubes	Axygen Inc., California, USA
Nitrile gloves	Paul Hartmann GmbH, Wiener Neudorf, Österreich
PCR tubes (0.2ml)	NeoLab Migge GmbH, Heidelberg, Germany
PEQ Green	VWR International GmbH, Darmstadt, Germany

Platinum taq polymerase	Thermo Fisher Scientific Inc., Massachusetts, USA
Primer: *abcZ* (ABC transporter), *bglA* (beta glucosidase), *ldh* (L-lactate dehydrogenase)	Microsynth AG, Balgach, Switzerland
Safe-lock tubes 1,5ml, 2ml, 5ml	Eppendorf AG, Hamburg, Germany
Serological pipette 10ml, 25ml	Sarstedt AG & Co. KG, Nürnbrecht, Germany
Sterile filter tips 10µl, 100µl, 1250µl	Greiner-Bio One, Kremsmünster, Austria
Thermo Scientific™ GeneRuler™ 1kb DNA-ladder	Thermo Fisher Scientific Inc., Massachusetts, USA
Trisaminomethane (Tris) HCL	Merck KGaA,Darmstadt, Germany
Trisaminomethane-acetate-Ethylenediaminetetraacetic acid (TAE) buffer	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Culture media	
Trypto-Casein-Soy Agar (TSA)	Biokar Diagnostics, Pantin Cedex, France
RAPID'L.mono Agar	Bio-Rad Laboratories Inc., Hercules, California
Brain Heart Broth (BHI)	Biokar Diagnostics, Pantin Cedex, France
Glycerol	Merck KGaA, Darmstadt, Germany