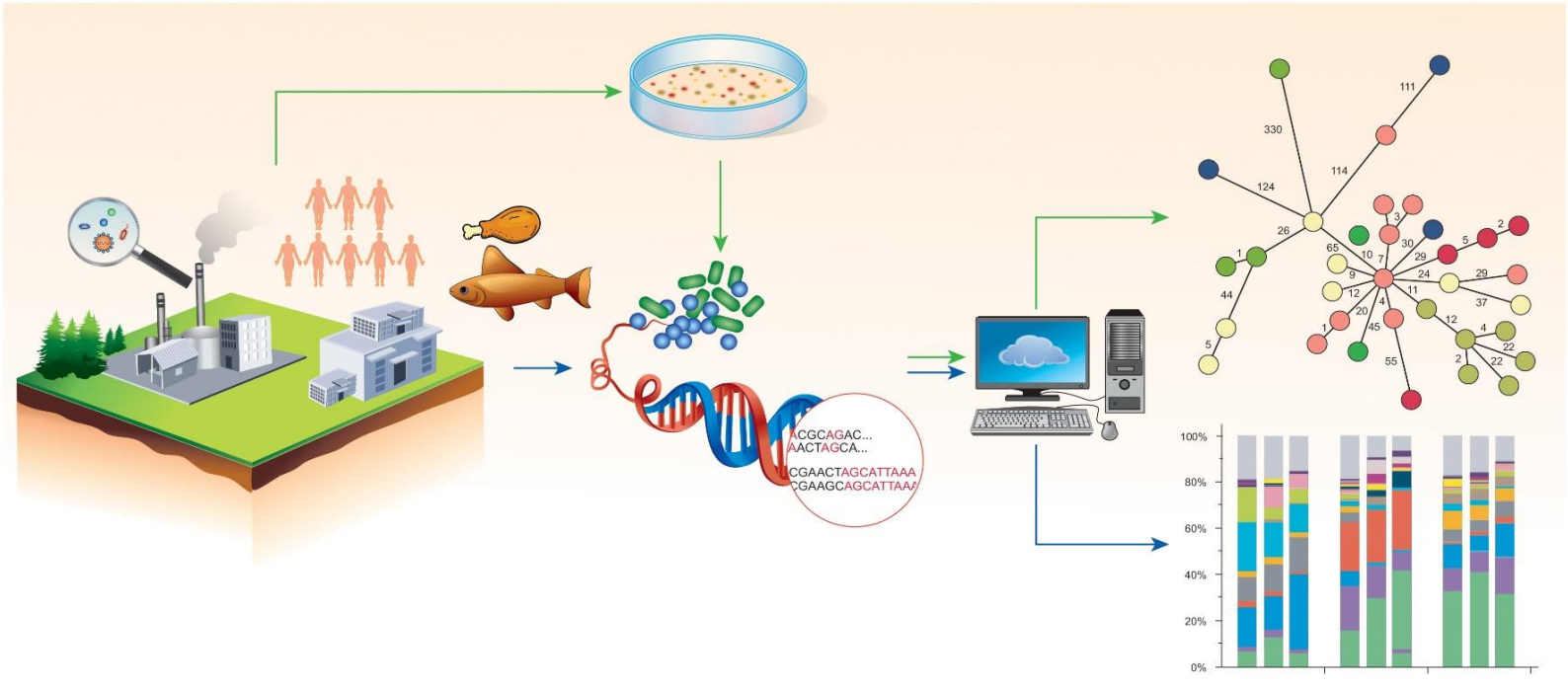
Guideline:
Use of whole genome sequencing for prevention and control of *Listeria monocytogenes* in the food industry



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PREFACE

Food producers are responsible for ensuring that food is safe, together with downstream actors in the food chain such as retailers, restaurants and consumers. Important parts of a control program for *Listeria monocytogenes* in food factories are source tracking, risk analysis, and implementation of targeted corrective actions. Whole genome sequencing is a technology that could aid the food safety management in the food industry, but it is unfortunately still primarily used in research projects and by authorities during outbreak investigations.

The purpose of this guideline is to facilitate the implementation of whole genome sequencing by food companies. Originally, the guideline was prepared for the Norwegian food industry and in Norwegian. Although examples are from Norway, we believe it could also benefit food industries and actors supporting the food industries worldwide. We would stress the need for a better mapping of problematic *Listeria monocytogenes* strains in more countries, as we recognize variation in this regard.

This guide was written in connection with the project PathoSeq, led by Dr. Annette Fagerlund and is a deliverable from a work package led by Dr. Solveig Langsrud. PathoSeq brought together an interdisciplinary group of actors from academia, industry, and non-governmental organizations. We would like to thank our project partners from the University of Oslo, University of Veterinary Medicine (Vienna, Austria), Nortura, Grilstad, Norsk Kylling, Orkla Foods Norway, the Norwegian Meat and Poultry Industry Association (KLF), SinkabergHansen, SalMar, Slakteriet, The Norwegian Seafood Federation, Eurofins Genomics, and Aquatiq Sense for a fruitful cooperation.

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CONTENT

SUMMARY

Listeria monocytogenes (listeria) is a foodborne pathogenic bacterium primarily associated with ready-to-eat, cold-stored food products. The bacterium is a persistent problem in food production, which can be explained by the fact that 1) It is widespread in the environment and can enter the factory from raw materials, equipment and passenger traffic. 2) It thrives well in the production environment and can establish itself on equipment and contaminate the products. 3) It can grow even at refrigeration temperatures and in vacuum-packed products, and it is not possible for consumers to know whether the product has listeria based on taste or smell.

Although most food companies occasionally find listeria in the production environment or on products, there are wide variations. While some rarely or never find the bacterium, others struggle with daily or weekly findings, uncertainty about sources of contamination and contamination routes, that they are unable to remove listeria from the processing environment, or that they regularly receive raw materials containing listeria. When listeria becomes a persistent problem or listeria is found on products, it is crucial to find the cause to be able to implement effective corrective actions.

Sequencing of the entire genome of listeria can be used as a tool both to find out more about the characteristics of the problem bacterium and its genetic relationship to other listeria, which can be used to find reservoirs and contamination routes. Among the methods that exist to characterize bacteria, whole genome sequencing has the greatest resolution and can also provide standardized information across laboratories. Whole genome sequencing is still rarely used by food producers, but there are good examples of companies that use the technology in their hazard control, source tracking and for training purposes[i]. However, there are several barriers that must be reduced to exploit the full potential of the method among food producers, both in terms of knowledge about listeria, technology, costs, ownership and time before test results.

The purpose of this guide is to make it easier for food manufacturers to assess the usefulness of whole genome sequencing in their business. It will also be a tool for using whole genome sequencing effectively in monitoring, problem solving and training, as well as for communication with suppliers, customers and authorities.

WHAT IS SEQUENCING?

Whole genome sequencing (WGS) is a technology to determine the order of the nucleotides in the genome (DNA or genome), and for listeria this means a sequence of letters of about 3 million nucleotides. This sequence, or parts of it, can be used to determine whether a bacterial isolate belongs to the species *Listeria monocytogenes* (listeria). All bacteria within this species have common characteristics, such as the ability to cause disease in humans and grow at low temperatures. The species *Listeria monocytogenes* can be further grouped into different sequence types (ST) or clonal groups (clonal complex, CC) that have more in common. For example, some sequence types more frequently cause disease and some more often establish themselves as house strains in food companies. The sequence type is determined from the sequence of seven of the 3,000 genes of listeria. In practice, however, it is most cost-effective to sequence the entire genome and then extract information about only the seven relevant genes to determine sequence type[ii].

There may also be variation within the same sequence type. To obtain more information one can use larger parts of the sequence or all of the sequence information. If you have a collection of listeria that has been sequenced, you can create evolutionary trees (phylogenetic trees, or "family trees") that show how similar the isolates are genetically. Here one must be aware that the tree is calculated based on statistical methods that give a probable relationship, and not exact differences[iii]. The evolutionary trees are often based on an extended sequence typing where instead of looking at seven genes, 1700 genes (core genome MLST; cgMLST) or all 3000 genes (whole genome MLST, wgMLST) are used. It is also possible to compare the entire genome sequence with a reference (SNP analysis). When comparing closely related bacterial isolates, with few differences that distinguish them from each other, whole genome MLST and SNP analysis will provide approximately equally good answers about genetic relationships.

How can the genome say anything about relationships between isolates?

Bacteria reproduce by cell division. In principle two daughter cells have exactly the same genome as their mother. Exactly the same bacteria are called clones and they are perceived as the same "individual". Over time, however, mutations will arise in the genetic material that will be inherited to the daughter cells and further for several generations. How quickly this happens (the mutation rate) will depend on growth conditions. A single mutation is referred to in the scientific literature as a "single nucleotide polymorphism" ("single nucleotide polymorphism" or SNP, pronounced *snip*). If you have two listeria isolates that differ from each other with only one or a few snips, you have reason to believe that they have a common origin not so far back in time.

Authorities use whole genome sequencing in connection with outbreak investigations. The sequence of outbreak isolates is compared with listeria from foods that are a suspected source of infection (for example, something all patients have eaten), and if they find the same or similar clone, they are quite certain that the source of infection has been found. The fact that listeria of the same subgroup of listeria is found (in an analysis based on 7 or 1700 genes) in both the food product and a patient is not enough to conclude about a connection. Whole genome sequencing is also widely used in research, for example to map variation within a species or to find out which genes are responsible for which traits. The method has been used in many research projects for and with the food industry to gather the background information necessary to interpret and use sequence data.

Whole genome sequencing is used by some food producers, among other things, to reveal sources of contamination and contamination routes and to obtain information about the properties of the problem bacterium. Whole genome sequencing can, for example, be used in hazard analysis and in communication with customers and suppliers. However, there are still many barriers to broad use of the method, such as price, analysis time and complexity, lack of analysis standards and uncertainty about ownership of results, requirements for sharing data, and risk of misinterpretation of test results by customers and authorities[iv].

WHAT DOES GENOME SEQUENCE TELL US ABOUT RISK?

In principle, all *Listeria monocytogenes* may cause illness and should be eliminated from the raw materials and production environment of ready-to-eat products. In a risk-based food safety management system, it will nevertheless be common practice that findings on product contact surfaces or repeated findings in the same sampling point trigger stronger measures than, for example, a sporadic finding in a drain.[v] Whole genome sequencing enables a more advanced approach, where the bacteria's properties, such as their ability to cause disease or establish itself in the production environment is included in the evaluation of risk.

Most often, disease is caused by foods where listeria can grow, and thus reach high numbers: 92% of listeriosis cases is caused by food with at least 2,000 listeria cells per gram[vi]. For some product categories where listeria has not previously been considered a hazard, it may still be relevant to include highly virulent variants of listeria (variants with high disease capacity) in the evaluation of risk. Highly virulent variants of listeria can cause disease at lower numbers and pose a risk even in products that do not allow the growth of listeria (for example, frozen food).

ABILITY TO CAUSE DISEASE

In a risk-based food safety system, the severity of a microbial contaminant must be evaluated, which includes both the severity of the disease, the number of bacteria ingested to become ill, the possible growth of the product, and the expected use of the product. There is a growing recognition that there are large differences in the ability to cause disease for different *Listeria monocytogenes* : The probability of getting sick from a highly virulent variant is hundred times as likely as from a low-virulent variant[vii]. Note that this difference is at the same magnitude as the difference in risk between the part of the population that is over and under 65 years of age.

The sequence type can give an indication of whether an isolate is highly virulent or low-virulent, despite that there are differences within sequence types. An important part of the infection mechanism of listeria is to invade host cells, and some sequence types more often have changes in the genes needed for invasion. As an example, a study of listeria from Norwegian food companies showed that the vast majority of listeria belonging to CC9 (ST9), CC121 (ST121) and CC5 (ST5) had mutations in the gene that codes for the protein internalin A (also called *inlA*). Internalin A is the most important virulence factor in listeria we know today, and the mutations leads to a non-functional protein. These isolates therefore had low ability to invade epithelial cells. The World Health Organization (WHO) has identified CC8, CC9 and CC121 as typically low-virulent listeria. In Norway there is a situation where some isolates of CC121 have an intact internalin A gene and a larger proportion of people with listeriosis are infected with CC121 than in other countries, so it is somewhat uncertain whether CC121 should be considered low or medium virulent. All thirteen CC8 isolates in the Norwegian study had intact internalin A, which shows that it is not necessarily possible to extrapolate from international findings to national ones. Table 1 shows a virulence ranking according to the report from WHO[vii] and according to the Norwegian study[viii].

Table 1. Ranking of listeria in terms of ability to cause disease using the proposed classification from WHO. The examples of listeria types that typically belong to the different levels are given[Vii viii]

	Description	Examples WHO (CC)	Examples, Norway (CC)
Highly virulent	Lineage I & LIPI1/3/4 &full-length InlA	1, 3, 4, 5, 6, 54, 87	1, 2, 3, 4, 88, 220
Medium virulent	LIPI1 & full-length InlA (excluding highly virulent)	37, 54, 155, 204	7, 8, 11, 14, 18, 19, 20, 21, 37, 91, 101, 121, 177, 200, 403, 415
Low virulent	Non-functional InlA	8, 9, 121	5, 9, 121

PROBABILITY OF CONTAMINATION

A risk-based listeria control program should include an evaluation of the likely occurrence of listeria in the product, including the potential contamination from the specific process and production environment. Listeria on a contact surface is much more likely to contaminate the product than listeria that is on the floor or in a drain. Furthermore, listeria found in the production environment for only a single day (sporadically) will be much less of a problem than listeria which has established itself in the production environment (house strain) and which can be a source of contamination every single day.

It is well known that some listeria variants are more often established as house strains. Table 2 shows listeria clones (as proved by close genetic relationship) isolated repeatedly over at least two calendar years in more than one Norwegian food processing plant [ix]. These pervasive listeria clones belonged to CC7 (ST7), CC8, CC9, CC14, CC19, CC121, CC415, and more commonly harbored genes making them fit to survive cleaning and disinfection. In addition to these pervasive types, clones belonging to CC1, CC5, CC7 (ST732), CC37, CC91, CC177, CC199, CC315 and CC403 were re-occurring for at least two years in single factories. If conditions are right, any listeria can probably establish itself in the production environment. Still, one could argue that if a listeria that belong to a typically pervasive CC or ST (and especially of they find a specific clone persisting in several factories) is detected in the monitoring program, it should be considered a greater risk than a typically sporadic listeria type (e.g one only previously found in the nature).

Table 2. Examples of house strains: Number of Norwegian food factories with listeria house strains and typical areas where listeria had established itself.

CC	ST	Number of factories (total 15)	Floor	Drain	Conveyor belt	Gutting machine
CC7	ST7	**6**	3	2	1	
CC8	ST8	**4**	2	2	1	1
CC9	ST9	**3**	2	3		
CC14	ST14	**2**	1	1	2	1
CC19	ST1416	**2**	1	1		
CC121	ST121	**2**	2	1	1	1
CC415	ST394	**2**	2	1		

LISTERIA THAT CAUSES OUTBREAKS

If we look at which listeria types have most frequently caused disease in Norway in recent years, we will observe that it reflects both the virulence properties of listeria and which listeria more frequently establish themselves in the production environment (Table 3). This supports that a risk based approach should consider both incidence and ability to cause disease.

Similarly, for international outbreaks, both listeria with high and low disease capacity are implicated (Table 4 and Appendix). There is a strikingly greater problem in Norway than other countries with the low-virulent, pervasive type CC121 (ST121). It is not known whether this is due to specific eating habits or whether the ST121 variants in Norway are more virulent. This underlines the necessity of mapping listeria variants locally or nationally to enable efficient use of sequence type information in food factories

Ideally, sequence information from isolates associated with ongoing outbreaks should be published continuously to allow the food industry and others to compare with listeria found in food production environments, raw materials and products. Unfortunately, most public health authorities are very reluctant to share this information.

Table 3. The most common listeria clusters linked to disease in Norway from 2010-2022 (black), with indication of whether they have been detected as pervasive (red) or not (green). Whether the types are considered highly virulent (red), intermediate (orange) or low virulent (green) is also indicated.

	CC7	CC8	CC121	CC1	CC20	CC87	CC9	CC6	CC14
2010									
2022									
Pervasive									
Virulence*									

* Two colour codes for the same CC are given in case of discrepancy between WHO and Norwegian data or variation within CC.

Table 4. Examples of listeria clusters (CC) and sequence types (ST) that have produced more than one outbreak.

CC	ST	Number of outbreaks	Continent (number of countries)	Food (number of outbreaks)	Sick	Died
CC6	6	14	Europe (10), Americas (2), Africa (1), Australia (1)	Smoked salmon (4), vegetables, cheese (2), meat (7)	1568	276
CC8	8, 1247, 120, 292	10	Europe (4) Europe (5) America (1)	Meat (2), Salmon (5) Salmon/Trout (2) Meat	195	37
CC1	1	7	Europe (2) America (3)	Coleslaw, caramel apples, meat, cheese (4)	715	215
CC5	5	6	America (1)	Cheese (4), melon, ice cream	37	4
CC7	7	5	Europe (3), America (2)	Fish cake, melon, meat (2), cheese	>67	>7
CC155	155, 372	4	Europe (3) America (1)	Meat/fish, fish, butter Egg	82	11
CC2	2, 290	3	Europe (1), America (1) America (1)	Pate, cheese Milk	423	15
CC19	802, 378, 398	3	Europe (4) America (1)	Rakfisk Celery Cheese	33	8
CC11	11	2	America (1)	Meat, cheese	38	5
CC388	388, 558	2	Europe (1) America (1)	Meat Cheese	235	8
CC554	554	2	America (1)	Cheese, sprouts	20	3
CC4	4	2	Europe (1), America (1)	Cheese, meat	16	5
CC9	9	2	Europe (1), America (1)	Sausages salmon	84	17
CC403	403	2	Europe (3)	Cheese, salmon	33	7
CC101	101	2	America (2)	Cheese	43	5
CC121	121	2	Europe (2)	Salmon	7	0

WHAT DOES THE GENOME SEQUENCE TELL US ABOUT SOURCES AND CONTAMINATION ROUTES?

Sequencing is valuable because it can be used to say something about the properties of listeria, but another strength is the possibility of comparing isolates, such as listeria found over years in the same company, across and along different value chains from primary production to consumer, and between countries. Whole genome sequencing is not currently suitable for daily monitoring because it takes too long for test results, but it is nevertheless useful to plan for listeria sequencing as a routine part of the monitoring program or to use it during periods of problems. Some examples are:

- Find the reservoir or route of contamination: Investigate whether a sequence type on the production line or product has previously been found in a raw material, at the raw material supplier or from an unclean zone in the company.
- Uncovering house strains (listeria that have attached, survived and reproduce in this location): Investigate whether listeria with a similar sequence has been found several times in the same point. This may indicate a reservoir for the clone at the point or near/upstream on the processing line.
- Detect dispersal: Investigate whether the listeria clone has spread resulting in additional reservoirs.

Below are some real-life examples of how sequencing can be used to uncover reservoirs or routes of contamination.

RAW MATERIAL SUPPLIER

Sometimes companies suspect that a listeria house strain originates from a specific supplier. To be able to make this claim, whole genome sequencing can be helpful. Figure 1 shows an example of an evolutionary tree for listeria from a company that believed that listeria problems occurred after a particular delivery of salmon. In this example, all the isolates (circles) in the evolutionary tree are of the same sequence type. The colors in the tree represent different Norwegian factories, each factory with its own color. Listeria from the factory in question is colored turquoise and it was evident that a house strain had established itself, since very closely related listeria was found in hygiene samples from the same area over a long period of time. The sequence of a listeria found on the well-boat delivering salmon at the start of the listeria problems was very similar to the house strain. It was therefore very likely that the suspected well-boat was the source of the house strain. The boat delivered fish to several companies and may therefore have caused problems at several slaughterhouses and processing plants.

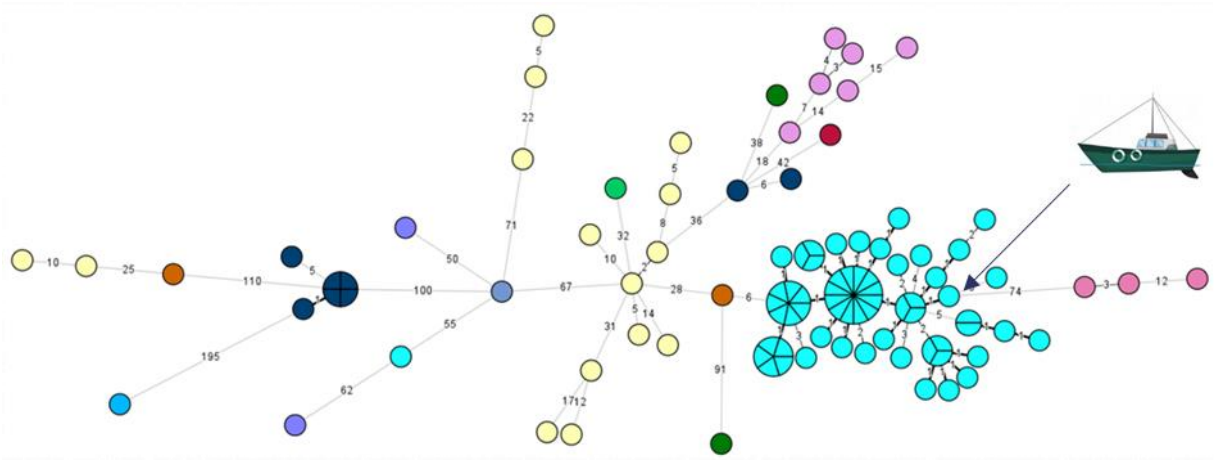

Figure 1. Evolutionary tree for listeria from a company that ran into problems with a house strain after a delivery of salmon for slaughter. The colors of the tree represent different factories, each factory with a different color

DETECTING HOUSE STRAINS

If you find listeria in the same sample point over time, it may either be that you have a house strain or that listeria comes in frequently (for example, from raw materials). If it is a house strain, you have to introduce corrective actions such as maintenance, replacement of parts and/or cleaning of the niche where the house strain thrives.

Figure 2 is an example of an evolutionary tree for evaluating the establishment of house strains in a meat company. Each colored circle represents one listeria isolate from the company (pink tones for department with boiled product) and the numbers on the lines between the circles show the number of genes with differences in sequence between the isolates. The white circles represent isolates from other Norwegian companies.

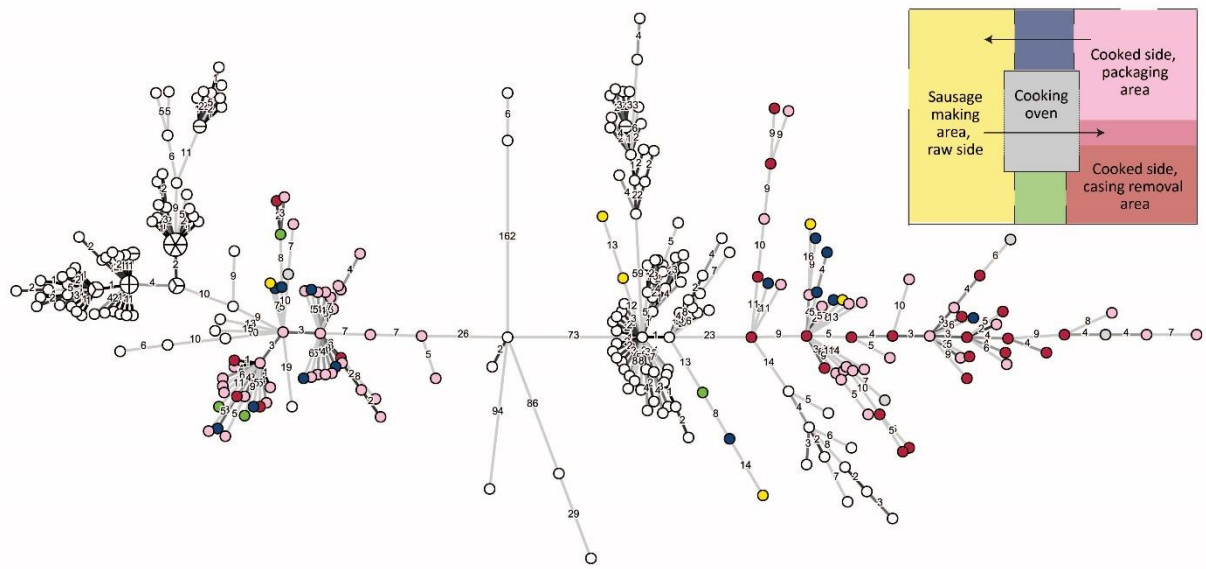

Figure 2. Evolutionary tree for listeria isolates from several meat processing companies. The color codes indicate where each listeria isolate was found in one specific company. White symbols are listeria isolates from other companies. [x]

Of 99 listeria isolates detected over six years of sampling at the cooked products department, 97 were of sequence type 9 (ST9/CC9). In the raw materials department, on the other hand, several different types of listeria were found in the same period. Virtually all of the isolates from the cooked products department came from drains and floors, and listeria was never found on contact surfaces. This indicated that listeria had established itself in drains and/or floors. It was evident from examining the evolutionary relationships between isolates that there were two variants of ST9 in the department, each dominating in separate areas of the room. Some of the ST9 from the raw materials department (yellow) were different from those in the cooked products department. This, together with the fact that there were some differences between the isolates, indicated that it has been a while since breaches of the hygienic barriers had been made. Thus, the ST9 bacterium that originally entered into the cooked zone had now been present there for many generations (and thus managed to mutate several times). An alternative explanation – that there were constant breaches of zone separation so that listeria keeps coming into the department – seemed unlikely, judging from the spread of ST9 variants in different meat companies and the variation in listeria types found in the raw materials department.

RECENT BREACHES OF HYGIENE BARRIERS

Sporadic detections of listeria in a high-risk zone may be due to a breach of hygiene barriers from the raw zone or sporadic spread of a house strain from an area in the high-risk zone that is not sampled. Figure 3 illustrates an example of the former. Circles of red colour are from the cooked products department, while the yellow and green are from two different raw material departments that were next door to the high-risk zone. We see that there are two groups of listeria (B1, B3) from the high-risk zone that have very close relatives in one of the raw material departments. Here it is difficult to say which way the contamination is going, it can be from cooked to raw department or *vice versa*. The last group (B2) dominates in the second raw materials department and there are three sporadic detections of very close relatives in the high-risk zone, i.e. it appears that there are recent barrier violations. Upgrading the division between the departments, with a change of footwear and clothing as well as hand washing would be a good measure in this case.

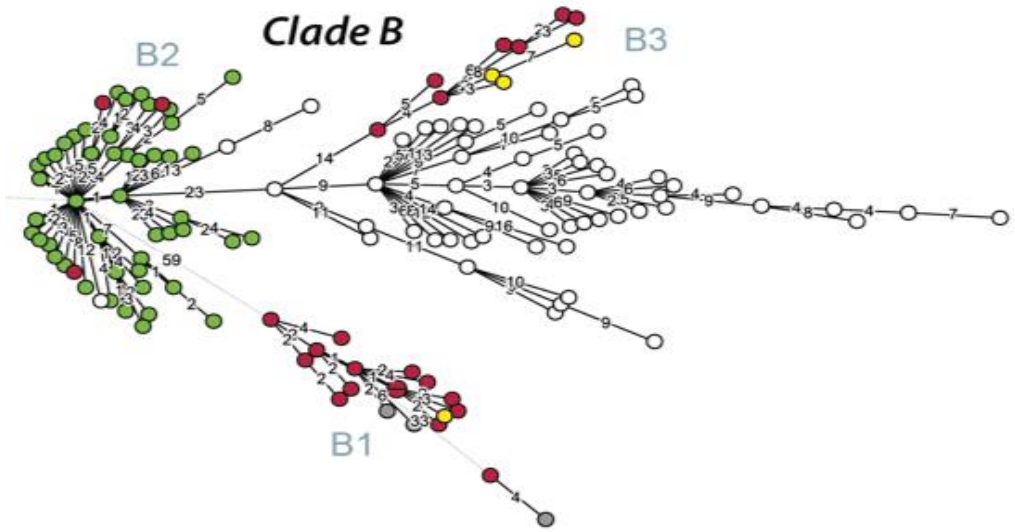

Figure 3. Evolutionary tree showing breach of barriers between raw material departments (yellow, green) and a department with cooked products (red).[xi]

USED EQUIPMENT

There are many examples of companies experiencing listeria problems in connection with changes in the production lines. In some cases, it is suspected that used equipment originating from another factory or department has brought in a new house strain. Figure 4 shows an example of how it could be demonstrated that a new house strain originated from used equipment. In this case, the factory (Company A) had other listeria variants in their drains over time, but not on the production line. When installing a "second hand" conveyor belt, listeria-positive hygiene samples from that processing line started to appear. Whole genome sequencing showed that the new variant was very similar to an isolate found on the same machine a few years earlier, when it was installed in the factory where it was originally located (Company B). In other words, it was not listeria from drains in Company A that had spread to the line. The machine was replaced and the problem solved.

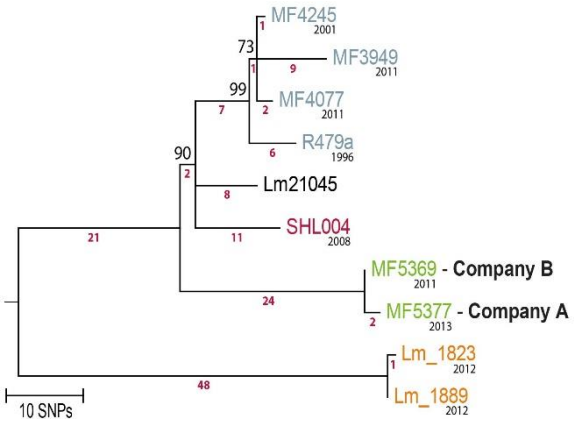

Figure 4 Evolutionary tree illustrating house strains on used equipment (green)[Xi]

USE OF WHOLE GENOME SEQUENCING IN PRACTICE

This chapter deals with how whole genome sequencing can be used in food safety management in practice. For general guidance on monitoring and handling of listeria, we refer to other sources[xii,xiii,xiv,xv,xvi,xvii,xviii,xix,xx].

As described in the chapters above, whole genome sequencing of listeria offers many opportunities. When assessing the scope of sequencing, there is much to take into account, where analysis costs, resources for interpreting sequence data (time and expertise) and risk must balance short- and long-term benefits.

Table 5 provides an example of how a simple sampling program for the preservation of isolates for sequencing can be set up.

Table 5. Example of program for collection of isolates for whole genome sequencing.

	Keep	Sequencing	Keep after sequencing
Product	Always	All	One isolate per sequence variant per year
Raw materials*	Always	One per supplier per year and 2-3 for recurring detections	One isolate per sequence variant per year
Product contact surface	Monthly and in case of recurring detections	Annually or in case of recurring detections from the same site or product	Five per year and two isolates from each reservoir or sampling point in case of recurring findings (first and last)
Environmental test	Monthly and in case of recurring detections	Annual selection or in case of a suspected house strain	Five per year and two isolates from each reservoir or sampling point in case of recurring findings (first and last)

* Applicable for ready-to-eat products without a listeria elimination step, such as raw salmon for sushi

ISOLATES AND SEQUENCE INFORMATION

KEEPING ISOLATES FOR SEQUENCING

There is no definitive answer to how many listeria samples should be taken in the monitoring program and which listeria should be kept. This will depend on production quantity, product (growth potential for listeria, type of consumer), intended use (ready to eat or not), process (steps that kill listeria, contamination after heat treatment), production hygiene (zone separators, flow of goods, temperatures, hygienic design, cleaning), customer requirements and, not least, the situation (detection in product or contact surfaces, suspected house strain or spread, connection to outbreaks, customer complaints).

It may be appropriate to preserve all (for companies with few findings) or a small selection of listeria isolates (covering from different points and over time) from routine monitoring to build up a strain collection of historical isolates and perform hazard analysis. If you suspect that the cleaning does not remove listeria, or that you have a house strain, you should for a period of time keep isolates from the same sample point over time. At the same time, listeria from other sampling points should also be collected to find out whether the listeria variant has spread and established itself in more locations. Isolates from products and raw materials should also be included.

WHICH ISOLATES SHOULD BE SEQUENCED?

For use in risk- based food safety system , a representative set of listeria isolates from routine monitoring should be sequenced annually. In case of problems, for example recurrent detections, 3-5 isolates from the same sampling point/raw material/product should be sequenced to determine whether they are related. Then, another 3-5 isolates sampled after the introduction of corrective measures should be sequenced, to determine whether the problem strain has been eliminated.

BUILDING A STRAIN COLLECTION

Without the physical bacterial isolates, it is not possible to study their properties. After sequencing, it may therefore be appropriate to keep one representative isolate for each sequence variant (if several isolates with the same or nearly the same sequence are detected) from each product, raw material and product contact surface, as well as 1-6 house strains (number depending on sequence diversity, number of reservoirs and sampling period length).

If you use an external supplier of analysis services or research partner, it is important to ensure that they have good systems for managing the isolates and that each isolate can easily be linked to more information in the factory's quality system (e.g. date, time, test site, product, raw material). Ownership and rights to any further use of the isolates (for example for research purposes) should be clarified in an agreement. Please note that some laboratories that offer listeria analyses reserve the right – unless otherwise specifically agreed – to preserve isolates at their own expense and use them later for other purposes, such as research or outbreak investigations.

One challenge arising from the lack of access to isolates or sequences when an outbreak link is suspected is the inability to trace the problem bacterium within one's own company. In order to have an opportunity to follow up on the findings from outbreak investigations as part of the company's own internal controls, you can take parallel samples independently. However, this does not guarantee that the same listeria clones are detected.

SEQUENCING AND ANALYSIS OF SEQUENCE DATA

In practice, whole genome sequencing is a two-step process: 1) a laboratory procedure for obtaining the sequence of an isolate 2) an advanced bioinformatic procedure for placing the sequence into a context (e.g. determining sequence type, looking at genetic relationship with other listeria). Currently, most food companies lack the necessary equipment, software, and/or personnel needed to perform the actual whole genome sequencing or statistical analysis of sequence data. Instead, commercial laboratories or research institutions often handle these tasks.

INTERPRETATION OF SEQUENCE DATA

Interpreting sequence data for problem-solving cannot occur purely in theory; it requires practical knowledge and understanding of listeria as a bacterium, the production premises' conditions, production routines, material and personnel flows, as well as cleaning and processing conditions. It is essential that everyone in the HACCP group and management are familiar with the method's possibilities and limitations. Additionally, the food factory should have at least one person who can put the findings into a larger context and engage in meaningful dialogue both internally and externally (with analysis services, customers, suppliers, and authorities).

RISK EVALUATION

Incorporating information about listeria's virulence (disease ability) and its potential to become a house strain can enhance monitoring and control programs. For example, a 100-fold increase in risk can be attributed to detections of listeria likely to have an elevated ability to cause disease. A corresponding risk increase can be attributed to detection of the listeria types that often form house strains. Points can be systematically assigned based on factors such as detection of listeria on contact surfaces versus the environment (for example a factor of 10) or repeated findings of the same listeria clone (for example, with a factor that indicates the number of days it has probably been present in the facility). An example of how to incorporate potential properties of listeria isolates in a risk evaluation is given in Table 6.

Table 6. Example of how to combine information about listeria isolate with findings in cleaning samples in a risk assessment.

CC	Probable property		Probable contamination			
	Virulence[1]	Establishment[2]	Contact surface[3]	Environment[4]	Repeated finding[5]	Risk
CC1	100	1	-	1	1	100
CC3	100	1	10	1	365	365 000
CC5	1	1	10	-	1	10
CC7	10	10	10	1	1	1 000
CC8	10	10	-	1	1	100
CC9	1	10	-	-	-	0
CC11	10	1	-	1	7	70
CC121	1	10	10	-	10	1000

[1] High virulent isolates are assigned 100 points, medium virulent 10 points and low virulent 1 point; [2] Typical pervasive strains are given 10 points; [3] Assign 10 points when found on contact surface; [4] Assign 1 point for discoveries in the environment; [5] Indicates the number of days from the first to the last time this variant is found at the same equipment/test site.

Analyses of genetic relationships can, in principle, determine whether listeria isolates from one's own factory resemble outbreak isolates. Unfortunately, sequences from clinical outbreak isolates are rarely made public, and even when they are, it takes a long time. As of today, accessing outbreak sequence data is not feasible. If your company is suspected of being linked to an outbreak, you cannot expect to access sequence data from the outbreak to use in your own contact tracing.

SOURCE TRACKING

In order to effectively utilize whole genome sequencing data, it is crucial to define the purpose in advance. This decision influences which isolates to sequence, which bioinformatic analyses to perform, and the potential consequences of different outcomes. For instance, if there is suspicion of a persistent listeria contamination on a conveyor belt, one would select isolates taken from that belt over time and compare them. In order to link sequence information to internal processes and products, tools for visualization like PathoTracker (from Eurofins) and BioMap (from Aquatiq) may be used.

If you want to place your findings in a larger context, interpretation quickly becomes more complicated. A survey of listeria in Norwegian factories and outdoor environments revealed that half of the isolates from the Norwegian food companies closely resembled listeria from other Norwegian companies[vii]. While this suggests a potential common source of contamination, uncertainty remains about whether it occurred recently or in the distant past. This complexity also complicates linking individual companies to outbreaks. Remember that sequence similarity alone does not confirm the source of infection; robust epidemiological data are essential.

CORRECTIVE ACTIONS

In the food safety mangement system, criteria for when and what type of corrective action should be implemented may take into account the information obtained from the whole genome sequences. Some examples:

- For highly virulent strains, apply corrective actions after the first positive detection in the environment or raw material. For low virulent strains, apply corrective actions after two or more detections.
- Prioritize combating listeria variants known to establish themselves permanently in production equipment.
- Consider to change the sanitation standard operating procedures in case of problems with listeria resistant to detergents or disinfectants

Using sequence information, it may be possible to verify the effectiveness of interventions, not only to reduce listeria incidence in general, but to get rid of a particularly problematic type. In order to verify that the measures have the desired effect, procedures for additional sampling followed by sequencing to ensure that problematic bacteria are not detected again within a specified time period should be implemented.

FUTURE

Whole genome sequencing of listeria is expected to become more common among food companies, both due to increased customer demands and the disadvantages being lower than the benefits:

Cost and time for test results: As more companies adopt the technology, costs are expected to decrease. Technological advancements will shorten the time for laboratory analysis and the bioinformatics steps[xxi], but it will probably not be possible to get below 2-3 days from sample collection to results.

Competence: When whole genome sequencing becomes a natural part of food safety education curriculums, the competence of food companies, technology suppliers, authorities and research environments will increase. This will increase the usefulness of using whole genome sequencing in monitoring and problem solving, as well as reduce the risk of misinterpretations. Higher competence can improve communication and contribute to greater trust between different actors.

Knowledge: With a better understanding of listeria populations in the food chain and knowledge of what measures should be taken against listeria with persistence properties, the benefits of whole genome sequencing will be greater. This knowledge will also reduce the likelihood that early adopters of the technology will be mistakenly singled out as sources for listeria in food and outbreaks.

Data sharing: Sharing of sequence information from outbreaks and food will enhance the importance of whole genome sequencing in food companies' food safety management. As of today, only limited and historical information exists, but it is acknowledged that transparency between health authorities, food authorities, researchers and food producers nationally and internationally could improve food safety. There is therefore reason to hope for greater transparency in the future.

Cost-benefit considerations, risk of being at the forefront and distrust in the authorities hinder exploitation of the full potential of the technology. In Austria, there was a major listeria outbreak that initiated significant changes. Today, Austrian food producers are given access to sequence data for listeria found in their own production for free, and they are quickly notified if they may be connected to an ongoing outbreak. Hopefully other countries do not need to wait for major outbreaks before they adopt similar practices.

APPENDIX: OVERVIEW OF OUTBREAKS

Year	*Country*	*Product*	*Category*	*Sick*	*Died*	*CC*	*ST*	*Reference*
1992	France	Meat aspic	Meat	279	85	1		Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992 | Applied and Environmental Microbiology (asm.org)
1985	United States	Cheese	Dairy	142	48	1		Listeriosis outbreak associated with Mexican-style cheese--California - PubMed (nih.gov)
1983	Switzerland	Cheese	Dairy	122	31	1		https://journals.asm.org/doi/10.1128/genomeA.00152-12
2008	Chile	Soft cheese	Dairy	78	14	1	**1**	Molecular epidemiology and genetic diversity of Listeria monocytogenes isolates from a wide variety of ready-to-eat foods and their relationship to clinical strains from listeriosis outbreaks in Chile - PMC (nih.gov)
1981	Canada			41	18	1	**1**	Epidemic Listeriosis — Evidence for Transmission by Food | NEJM
1987	Great Britain	Paté	Meat	366		2	**2**	A possible outbreak of listeriosis caused by an unusual strain of Listeria monocytogenes - ScienceDirect
1983	United States	Pasteurized milk	Dairy	49	14	2	**290**	https://www.nejm.org/doi/full/10.1056/NEJM198502143120704
1994	United States	Chocolate milk	Dairy	45	0	3		An Outbreak of Gastroenteritis and Fever Due to Listeria monocytogenes in Milk | NEJM
1999	France	Rilettes	Meat	10	3	4		Two Consecutive Nationwide Outbreaks of Listeriosis in France, October 1999–February 2000 | American Journal of Epidemiology | Oxford Academic (oup.com)
2014-2015	United States	Ice cream	Dairy	6		5	**5**	Assessing the genome level diversity of Listeria monocytogenes from contaminated ice cream and environmental samples linked to a listeriosis outbreak in the United States | PLOS ONE

Year	*Country*	*Product*	*Category*	*Sick*	*Died*	*CC*	*ST*	*Reference*
2013-2015	Denmark	Smoked salmon	Fish	10	3	6	**6**	Two listeria outbreaks caused by smoked fish consumption—using whole-genome sequencing for outbreak investigations - ScienceDirect
2015-2018	Austria, Denmark, Sweden, Finland, Storbitannia	Frozen vegetables	Vegetables and fruits	47	9	6	**6**	29-11-2017-RRA-Listeria monocytogenes-Austria, Denmark, Finland, Sweden, United Kingdom (europa.eu)
2017-2018	South Africa	Cold meat cuts	Meat	1060	216	6	**6**	Outbreak of Listeriosis in South Africa Associated with Processed Meat | NEJM
1998	United States	Sausages	Meat	40		6	**6**	Whole genome comparisons of serotype 4b and 1/2a strains of the food-borne pathogen Listeria monocytogenes reveal new insights into the core genome components of this species | Nucleic Acids Research | Oxford Academic (oup.com)
1998	United States, Australia, Canada, France	Sausages	Meat	108	14	6		Listeria Outbreaks | Listeria | CDC
2002	United States	Turkey meat	Meat	54	8	6		Multistate Outbreak of Listeriosis Linked to Turkey Deli Meat and Subsequent Changes in US Regulatory Policy | Clinical Infectious Diseases | Oxford Academic (oup.com)
2017-2019	Netherlands, Belgium	Ready-to-eat meat	Meat	21	3	6	**6**	Multi-country outbreak of Listeria monocytogenes sequence type 6 infections linked to ready-to-eat products (europa.eu)
2018-2019	Germany	Blood sausage	Meat	112	6	6	**6**	Large Nationwide Outbreak of Invasive Listeriosis Associated with Blood Sausage, Germany, 2018–2019
2016	Switzerland	Meat paté	Meat	5		6	**6**	Local Outbreak of Listeria monocytogenes Serotype 4b Sequence Type 6 Due to Contaminated Meat Pâté - PubMed (nih.gov)

Year	*Country*	*Product*	*Category*	*Sick*	*Died*	*CC*	*ST*	*Reference*
2018	Switzerland	Cheese	Dairy	34	10	6	**6**	Listeriosis Caused by Persistence of Listeria monocytogenes Serotype 4b Sequence Type 6 in Cheese Production Environment - PMC (nih.gov)
2022	Denmark	Fish meatballs	Fish	11		7	**7**	Denmark – Outbreak of invasive Listeria infection sequence type 7 in Denmark caused by fish meatballs | FoodWorld (kswfoodmicro.com)
2010	United States	Meats	Meat	14		7	**7**	Outbreak of invasive listeriosis associated with the consumption of hog head cheese--Louisiana, 2010 - PubMed (nih.gov)
2015-2018	Italy	Ready-to-eat meat	Meat	25	4	7	**7**	Phylogenetic Analysis and Genome-Wide Association Study Applied to an Italian Listeria monocytogenes Outbreak - PMC (nih.gov)
2007	Norway	Camembert cheese	Dairy	17	3	7	**7**	A large outbreak of Listeria monocytogenes infection with short incubation period in a tertiary care hospital - PubMed (nih.gov)
2014-2019	Denmark, Estonia, Finland, France, Sweden	Cold smoked salmon and trout	Fish	22		8	**1247**	Multi-country outbreak of Listeria monocytogenes clonal complex 8 infections linked to consumption of cold-smoked fish products (europa.eu)
2015-2018	Denmark, France, Germany	Marinated salmon	Fish	12	4	8	**8**	Multi-country outbreak of Listeria monocytogenes sequence type 8 infections linked to consumption of salmon products – 25 October 2018 (europa.eu)
2008	Canada	Ready-to-eat meat	Meat	57	24	8	**120, 292**	https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-11-120
2014-2019	Germany	Ready-to-eat meat	Meat	39	3	8	**8**	Nationwide outbreak of invasive listeriosis associated with consumption of meat products in health care facilities, Germany, 2014–2019 - ScienceDirect
2012-2016	Czech Republic	Meat-turkey	Meat	26	3	8	**8**	An outbreak of listeriosis linked to turkey meat products in the Czech Republic, 2012–2016 | Epidemiology & Infection | Cambridge Core

Year	*Country*	*Product*	*Category*	*Sick*	*Died*	*CC*	*ST*	*Reference*
2009	Chile	Sausages	Meat	73	17	9	**9**	Molecular epidemiology and genetic diversity of Listeria monocytogenes isolates from a wide variety of ready-to-eat foods and their relationship to clinical strains from listeriosis outbreaks in Chile - PMC (nih.gov)
2000	United States	Ready-to-eat meat	Meat	30	5	11	**11**	Multistate Outbreak of Listeria monocytogenes Infection Linked to Delicatessen Turkey Meat | Clinical Infectious Diseases | Oxford Academic (oup.com)
2009	United States	Cheese	Dairy	8		11	**11**	Multistate Outbreak of Listeria monocytogenes Associated with Mexican-Style Cheese Made from Pasteurized Milk among Pregnant, Hispanic Women | Journal of Food Protection (allenpress.com)
2013	Norway	Rakfisk	Fish	3		19	**802**	Listeriosis outbreak in Norway - NIPH
2010	United States	Celery	Vegetables and fruits	10	5	19	**378**	Hospital-acquired listeriosis outbreak caused by contaminated diced celery--Texas, 2010 - PubMed (nih.gov)
2018-2019	Norway	Rakfisk	Fish	13		20	**20**	Listeriosis outbreak in Norway - NIPH
2013-2015	Denmark	Smoked salmon	Fish	10	5	89	**391**	Two listeria outbreaks caused by smoked fish consumption—using whole-genome sequencing for outbreak investigations - ScienceDirect
2012	United States	Cheese	Vegetables and fruits	22	4	101	**101**	Multistate outbreak of listeriosis caused by imported cheese and evidence of cross-contamination of other cheeses, USA, 2012 | Epidemiology & Infection | Cambridge Core
2022	Norway	Smoked salmon	Fish	4		121	**121**	Smoked salmon suspected as source of listeriosis outbreak - NIPH
2019	United States	Hard-boiled eggs	Egg	8	1	155	**372**	Outbreak of Listeria Infections Linked to Hard-boiled Eggs | Outbreak of Listeria Infections Linked to Hard-boiled Eggs | December 2019 | Listeria | CDC

Year	*Country*	*Product*	*Category*	*Sick*	*Died*	*CC*	*ST*	*Reference*
2016-2017	Austria	Meat and fish	Meat and Fish	7		155		Frontiers | Whole Genome Sequencing Based Surveillance of L. monocytogenes for Early Detection and Investigations of Listeriosis Outbreaks (frontiersin.org)
1988	Finland	Butter	Dairy	25	6	155	**155**	Evolutionary Dynamics of the Accessory Genome of Listeria monocytogenes | PLOS ONE
2014	United States	Stone fruit	Vegetables and fruits	2		183	**382**	Listeria monocytogenes in Stone Fruits Linked to a Multistate Outbreak: Enumeration of Cells and Whole-Genome Sequencing | Applied and Environmental Microbiology (asm.org)
2013-2014	Denmark	Rolled sausage	Meat	41	17	224	**224**	Whole-genome Sequencing Used to Investigate a Nationwide Outbreak of Listeriosis Caused by Ready-to-eat Delicatessen Meat, Denmark, 2014 | Clinical Infectious Diseases | Oxford Academic (oup.com)
2018	Australia	Melon	Vegetables and fruits	22		240	**240**	Listeria monocytogenes in ready-to-eat (RTE) food: attribution, characterization and monitoring: meeting report (who.int)
2019	United States	Meat and Cheese	Meat and Dairy	10	1	321	**2041**	Outbreak of Listeria Infections Linked to Deli-Sliced Meats and Cheeses | Outbreak of Listeria Infections Linked to Deli-Sliced Products | April 2019 | Listeria | CDC
2019	Spain	Ready-to-eat meat	Meat	222	3	388	**388**	Listeriosis outbreak caused by contaminated stuffed pork, Andalusia, Spain, July to October 2019 - PMC (nih.gov)
2000	United States	Cheese	Dairy	13	5	388	**558**	Outbreak of Listeriosis among Mexican Immigrants as a Result of Consumption of Illicitly Produced Mexican-Style Cheese | Clinical Infectious Diseases | Oxford Academic (oup.com)
2014	United States	Sprouts	Vegetables and fruits	5	2	554	**554**	Wholesome Soy Products, Inc. Sprouts and Investigation of Human Listeriosis Cases (cdc.gov)
2008	United States	Sprouts	Vegetables and fruits	20	5	573	**573**	Listeriosis outbreaks and associated food vehicles, United States, 1998-2008 - PubMed (nih.gov)

Year	*Country*	*Product*	*Category*	*Sick*	*Died*	*CC*	*ST*	*Reference*
2014-2015	United States	Caramelized apples	Vegetables and fruits	35	7	1,183	**1, 382**	Multistate outbreak of Listeria monocytogenes infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014–2015 | Epidemiology & Infection | Cambridge Core
2009-2010	Austria, Germany, Czech Republic	Cheese	Dairy	14	4	19,403	**398,403**	Genome Sequencing of Listeria monocytogenes "Quargel" Listeriosis Outbreak Strains Reveals Two Different Strains with Distinct In Vitro Virulence Potential | PLOS ONE

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