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EDITORIAL

Monitoring and analytics of *Listeria monocytogenes*: key facts for authorities, food manufacturers, and retailers

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Recent food recalls triggered by the evidence of Listeria underline the need for reliable measures and quality assurance to minimize a Listeria risk. Those Listeriosis outbreaks have been caused by various contamination points such as freezers, slicers, structural defects, or personal hygiene. Often, the affected companies did not even know that there was a serious persistent *Listeria monocytogenes* presence in the facility. With today's technical prerequisites, it is possible to establish a direct connection between cases of human disease, the products, and companies, and thus also trading companies with high precision and only very slight restrictions. In 2019, a listeriosis case in the federal state of Hessen, Germany, received high media attention because the occurrence of listeriosis in health facilities could be linked to the supplier of parts of these meals. Whole genome sequencing gives the opportunity to get to the bottom of the cause of an infection in the facility. Ideally, a contamination can be traced back to the supplier and compared with previous cases in the company. This makes it possible to draw conclusions about cross-contaminations or entries into the food chain.

1 Consequences of Listeria in foodstuff

Listeriosis is caused by the bacterium *Listeria monocytogenes* that occur ubiquitously, i.e. they are omnipresent and transmitted throughout the entire production chain. In food production, they occur both in raw food material and, above all, through recontamination. However, a transmission by air can be excluded. *Listeria monocytogenes* can trigger very severe diseases in humans, especially in risk groups whose immune systems have been weakened—such as the elderly, pregnant women or patients taking immunosuppressant drugs. The disease is caught by eating contaminated food. In 2018, elderly people over 84 years were most affected by listeriosis in the EU. The case fatality rate in this age group was 18% (vs. 24% in 2017). Across all age groups, the case fatality rate was 16% (vs. 9% in 2017). These facts make listeriosis one of the most serious food-borne diseases, according to EU surveillance (EFSA 2018, 2019). *Listeria monocytogenes* are usually psychrotrophic, meaning that they multiply in food even at cool temperatures (2–4 °C), which plays a decisive role in the calculation of the best-before date and appropriate storing conditions.

Currently, the authorities are directly linking human listeriosis diseases, triggered by Listeria food-borne outbreaks, to food products and environmental samples. So far, the associations between the cases and (potential) sources have only been communicated between authorities or in specialist publications, and are not available as public information.

2 Guidelines on sampling

An ineffective sampling program or ineffective sampling techniques for *Listeria monocytogenes* are not rare scenarios; despite the fact that the European Union Reference Laboratory for *Listeria monocytogenes* (EURL) published guidelines on sampling the food processing area and equipment for the detection of *Listeria monocytogenes*[1] in 2012. Contrary to the EC Regulation 2073/2005 Article 5 (2), the International Standard ISO 18593, which describes surface sampling methods for the detection or enumeration of bacteria in food processing areas and equipment, does not give sufficient guidance to detect persistent *Listeria monocytogenes* in facilities. The EURL Guidelines emphasize that a wrong performed sampling or ineffective sampling

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[1] https://ec.europa.eu/food/sites/food/files/safety/docs/biosafety_fh_mc_guidelines_on_sampling.pdf. Accessed 13 January 2020.

techniques may give false results of non-detection of *Listeria monocytogenes*. *Listeria monocytogenes* is ubiquitous and thus, it can be assumed that no food facility is absolutely free of it. Therefore, not only a detailed sampling plan is necessary, but also a short-term corrective action including re-sampling must be considered. Especially the entry zones for staff, including the hygiene sluices and loading equipment (wooden or plastic) for goods as well as electrolift trucks are risk relevant and need to be checked. Inside a high care or high risk packaging area mainly slicers can be contaminated. But also flooring defects, condensation, as well as dripping water from ceilings can be sources of *Listeria monocytogenes*. The total sampled area should be as large as possible to increase the probability of detection. It is advised to sample between 1000 cm^2 and 3000 cm^2 with sponges. Stick swabs should only be used to sample hard-to-reach or hidden small areas. The well-known sampling area of 100 cm^2 with stick swabs is impracticable.

Once the source of persistent *Listeria monocytogenes* is identified, it is imperative to implement sustainable corrective actions to avoid further food contamination by the pathogenic bacteria. Therefore, different disinfection possibilities exist, e.g. spraying of open areas, nebulizing hard to reach zones, and foaming carpets on crossing paths. Appropriate chemicals must be matched to the area of application. If all of this doesn't reach to sufficient results, construction measures must be taken.

3 Whole genome sequencing

The generic term "next generation sequencing" (NGS) refers to high-throughput sequencing technologies. One option is whole genome sequencing (WGS) that sequences the entire genome. The serotyping of *Listeria monocytogenes* is a classical method to further identify a detected microbiological strain. The additional step of WGS of a bacterium is similar to creating a human DNA profile in forensics. The exact identification of the complete bacterial DNA sequence creates a unique fingerprint of a bacterium. The genome of *Listeria monocytogenes* is completely registered with 2.88 million individual bases. In contrast, PCR methods such as the microbiological qualitative detection of *Listeria monocytogenes* only compare and evaluate short partial DNA sequences.

The German Robert Koch Institute (RKI) gathers *Listeria monocytogenes* isolates from human infections, and the German Federal Institute for Risk Assessment (BfR) collects corresponding isolates from product or environmental samples (e.g. sponges/swabs). Further national reference laboratories for *Listeria monocytogenes* within the EU capture data in a comparable form. WGS data are stored in databases for further evaluation. Currently, different databases are used, e.g. GenomeTrakr (FDA) that is publicly accessible, or closed official databases used by RKI and BfR. In addition, private individually created databases for customers are existing—e.g. for years industries use their own private *Salmonella* WGS data to track their supply chain.

During the recent media-effective cases in Germany, the RKI and BfR used the so-called *Listeria monocytogenes* core genome multilocus sequence typing (cgMLST method). On the basis of WGS of isolated *Listeria monocytogenes* colonies derived from food or surface samples, 1701 gene sequences undergo a bioinformatical analysis before being compared to a public database. The genomic sequence of that isolate defines the complex type. If the complex type matches with the complex types that are already published by the RKI or the European Food Safety Authority or European Centre for Disease Prevention and Control (EFSA/ECDC), this might give more information about the contamination and its source. There are currently more than 13,000 complex types that are publicly known.

An established link between specific strains and defined foods and, if applicable, environmental samples, offer the possibility to identify the source of different foods in relation to specific human infections. The method can also be used in the private sector to reliably identify a source of contamination in the production plant or to limit contamination in the end product for suppliers of raw materials as part of the supply chain control.

4 Conclusion

Sampling of the food processing area and equipment is still often done incorrectly. Besides, a closely sampling plan it is imperative to realize short-term corrective actions including resampling and documentation. Authorities do not accept delaying actions when pathogenic germs are detected in ready-to-eat food and tend to close a company faster now. The economic damage here can be immeasurable. Based on modern techniques like WGS, authorities and food producers now have the possibility to identify sources of contaminations and to find solutions for risk reduction.

Furthermore, public communication plays a considerable role and must be taken into account in the risk management of every company and authorities.

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