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Review paper

Use of whole genome sequencing as routine typing method — improvements in the investigation of foodborne outbreaks of *Listeria monocytogenes*

Ariane Pietzka[a], Brankica Lakićević[b], Lazar Milojević[b] and Werner Ruppitsch[a]*

[a] *Austrian Agency for Health and Food Safety — AGES, Institute of Medical Microbiology and Hygiene Division for Public Health, Beethovenstraße 6, 8010, Graz, Austria*
[b] *Institute of Meat Hygiene and Technology, Department for Microbiological and Molecular-biological Testing, Belgrade, Kaćanskog 13, 11040, Republic of Serbia*

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ABSTRACT

Sequencing technologies have revolutionized the characterization of microorganisms in the recent years to a level that was previously unimaginable. Whole-genome sequencing (WGS) techniques have evolved from an expensive luxury typing method affordable only to a few institutions to a common tool for routine analysis in public health microbiology. In particular, improvements in pathogen source tracking, determination of phylogenetic relationship, antibiotic resistance and virulence-traits have improved outbreak investigation tremendously. In addition, WGS allows the easy establishment of global databases based on standardized nomenclatures facilitating international data exchange, cross-border outbreak investigation strain tracking and source identification.

1. Introduction

International trade in fresh and frozen foods has become a major food — and public health — safety challenge today (*Allerberger et al.,* 2022). The contamination of food and feed with pathogenic microorganisms has become a major global health threat. Therefore, a well-functioning surveillance system is a necessary tool for early detection of microbial threats to prevent or at least stop outbreaks and prevent further transmission and morbidity.

In recent years, whole genome sequencing (WGS) has become the new standard in public health surveillance worldwide, allowing the most accurate and detailed characterization of microorganisms that significantly improved outbreak investigation, surveillance, and infection prevention and control in public health microbiology. For zoonotic foodborne pathogens, *Listeria (L.) monocytogenes, Escherichia (E.) coli* and *Salmonella (S.) enterica* were used as model organisms for a European pilot project with the aim to implement and evaluate WGS for routine analysis. Similar projects have been carried out in the US and elsewhere (*Jackson et al.,* 2016; *Van Walle et al.,* 2018; *ECDC et al.,* 2019).

L. monocytogenes is one of the most important foodborne pathogens because of the severity of certain clinical manifestations, i.e., infections of the central nervous system, septicaemia and abortion and the high case-fatality rate of up to 30% of cases (*Halbedel et al.,* 2020). *L. monocytogenes* causes inva-

***Corresponding author:** Ariane Pietzka, *ariane.pietzka@ages.at*

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sive illness mainly in certain well-defined high-risk groups, the so called YOPIs (Young-Old-Pregnant and Immunocompromised). However, listeriosis can also occur in otherwise healthy individuals, especially during an outbreak (*Allerberger et al.,* 2022). Given the strong impact of listeriosis on human health and the challenges of controlling *L. monocytogenes* along the food supply chain, listeriosis has been classified as high priority for molecular surveillance in the European Union/European Economic Area (EU/EEA) over the last two decades (*Gattuso et al.,* 2022).

L. monocytogenes is ubiquitous in the environment and has been isolated from soil, dust, food products for humans (both of animal and vegetable origin), feed, water and sewage, and it can be carried by almost any animal species, including asymptomatic humans. The principal reservoirs of the organism are said to be soil, forage, water, mud, livestock feed and silage (*Heymann,* 2015). Due to this environmental ubiquity, *Listeria* strains are also frequently detected in food products. Strains causing illnesses are mainly found in foods that are packaged and prepared commercially, rather than in home cooked foods. The change in lifestyle with less time for home cooking and more ready-to-eat (RTE) and take-away foods increased the risk of listeriosis (*Carpentier and Cerf,* 2011). Changes in food production and technology have led to the production of foods with a long shelf life that are typical "*Listeria* risk foods," because the bacteria have time to multiply, and the food does not undergo a bactericidal/listericidal process such as cooking before consumption.

Listeriosis outbreaks have historically been difficult to resolve, and only a small proportion of cases could be linked to the food products responsible for an outbreak. This was also due to the fact that previous molecular typing methods, such as pulsed-field gel electrophoresis (PFGE), had limited genetic resolution compared to WGS-based analyses.

2. Whole genome sequencing-based surveillance of *Listeria monocytogenes*

An almost gapless characterization of *L. monocytogenes* isolates is desirable, and the European Centre for Disease Prevention and Control and the European Food Safety Authority (ECDC-EFSA), recommend using WGS-based typing methods (*Van Walle et al.,* 2018; *ECDC et al.,* 2019) along with a European-wide molecular typing database to improve the identification and investigation of multi national outbreaks (*Ruppitsch et al.,* 2015a; *Pietzka et al.*, 2019; *Cabal et al.,* 2022; *Lakicevic et al.,* 2023). There are different analysis pipelines for WGS data, such as analysis of single-nucleotide variants (SNP) or a gene-by-gene allelic profiling using core genome (cgMLST) (*Ruppitsch et al.,* 2015b; *Moura et al.,* 2016) as well as whole-genome multilocus sequence typing (wgMLST) (*Hyden et al.,* 2016a; *Brown et al.* 2019; *Jagadeesan et al.,* 2019; *Lakicevic et al.,* 2023). For backward compatibility with datasets obtained with traditional methods, information on serotype, classical multilocus sequence type (MLST) or MLVA data can be extracted from WGS data (*Hyden et al.,* 2016b).

A multi-country outbreak of *L. monocytogenes* was investigated in a joint ECDC-EFSA rapid outbreak assessment in 2018. The outbreak was ongoing in five EU member states (Austria, Denmark, Finland, Sweden and the United Kingdom) with 32 human cases involved. Six closely related non-human *L. monocytogenes* isolates from frozen corn, frozen vegetables and surfaces on which vegetables had been processed were detected by WGS analysis. WGS analysis provided a strong microbiological link between the human and the non-human isolates. Consumption of frozen corn was later confirmed by several patients in different countries. Contaminated batches of frozen corn and vegetable mixes could be traced back to a company in Poland that packaged frozen vegetables produced and processed in Hungary (*Joint ECDC-EFSA,* 2018).

In Austria, WGS-based surveillance has been successfully implemented and used in combination with analysis of epidemiological data for surveillance and outbreak investigation in recent years. Since 2016, whole genome sequence-based typing is performed at the National Reference Laboratory for all *L. monocytogenes* isolates from different sources such as isolates from patients, foods and food-associated material as well as isolates from the environment and the veterinary sector. This isolate-based surveillance allows successful investigation and confirmation of local and multi-country outbreaks. In 2018, a listeriosis outbreak likely due to contaminated liver pâté was investigated. A group of 32 individuals celebrated at a tavern in Austria, where traditional food was served. Eleven individuals developed gastrointestinal symptoms, including one case with severe sepsis. Human, food and environmental samples taken from the tavern and a local production facility (where some of the served meat products originated) were tested for *L. monocytogenes* and isolates were analyzed. A novel *L. monocytogenes* strain was detected in twelve human, two food and one environmental samples from the meat processing company.

Active case finding identified, from the same region in Austria, two further cases which tested positive for the outbreak strain. These two cases had not visited the tavern, but confirmed regular consumption of locally produced liver pâté. Based on WGS analysis, liver pâté produced by company X was identified as the likely source of the outbreak (*Cabal et al.* 2019).

Lachmann et al. described a listeriosis outbreak in Germany, most likely associated with the consumption of smoked and graved salmon products. In a national surveillance program in Germany, WGS was used for typing and cluster detection of *L. monocytogenes*. In the frame of this programme, twenty-two independent listeriosis outbreaks with 228 cases, occurring between 2010 and 2021, were identified. Listeriosis outbreaks can affect several countries and last for several years, making it difficult to link affected patients without the use of WGS. Systematic WGS-based typing of *L. monocytogenes* isolates from food products enables the identification of outbreak vehicle. Many of these twenty-two outbreaks were cross-border outbreaks with further cases in other countries. WGS analysis revealed closely related *L. monocytogenes* isolates from different salmon products. Interviews on food consumption and shopping behaviour confirmed the WGS results (*Lachmann et al.* 2022).

Advances in outbreak investigation after implementation of WGS based typing have been reported worldwide. Jackson et al. described how WGS has improved the detection and investigation of listeriosis outbreaks in the U.S., and demonstrated significantly more clusters and outbreaks of foodborne listeriosis were identified and resolved thereafter (19 outbreaks) than before the use of WGS (two outbreaks) (*Jackson et al.,* 2016).

The so far biggest reported listeriosis outbreak, with 1060 confirmed cases and 216 confirmed deaths, in South Africa from 2017–2018, was finally successfully terminated with the aid of WGS. Contaminated ready-to-eat meat from a South African producer was identified as the infection source. After withdrawal of contaminated meat products from the market the outbreak ended (*Smith et al.,* 2019). As a consequence, listeriosis has been added to the list of mandatory notifiable diseases in South Africa and surveillance systems have been optimized to improve prevention and early detection of future listeriosis outbreaks.

3. Discussion

WGS enables efficient tracking and distribution of microorganisms on a "farm to fork" principle. Subpopulations of bacterial pathogens can be transferred into food processing facilities from a variety of sources outside processing facilities, including animals, incoming raw materials, soil, dust and water (*Zuber et al.,* 2019; *Lakicevic et al.,* 2023). Within production facilities, microorganisms can persist for long periods of time on various surfaces, equipment, floors and cold rooms (*Stessl et al.,* 2022) and can be transferred to food and ultimately to consumers via aerosols, contaminated contact materials and food processing workflows (*Pightling et al.,* 2018; *Elson et al.,* 2019; *Stessl et al.,* 2019). WGS allows characterization of bacterial subpopulations with the highest discriminatory power at every stage, from the environment to suppliers and food processing facilities, to final products and consumers (*Pightling et al.,* 2018; *Zuber et al.*, 2019). Thus, WGS allows the identification of the responsible source of infection or contamination with a high level of confidence, which has improved correct decision making (*Zuber et al.,*2019; *Stessl et al.,* 2022) and enables authorities and also food companies the timely and selective implementation of appropriate control and preventive measures to stop further transmission and to terminate outbreaks.

4. Conclusion

In conclusion, the use of WGS offers several advantages, namely superior discriminatory power for strain characterization, robustness and stability, which are critical for cluster detection, tracing the source and reservoir of the causative strain (*Ruppitsch et al.,* 2019). In addition, the high resolution of WGS allows public health agencies to take action at a lower level of epidemiologic evidence, which is a critical advantage for reducing disease and resolving outbreaks (*Jackson et al.,* 2016). WGS technologies offer benefits not only to public health and food authorities, but also to the food industry in the context of the farm-to-fork principle and upcoming improvements in technology and bioinformatics, with the perspective of metagenomic sequencing applied directly to samples (*Ruppitsch et al.,* 2019).

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References

Allerberger, F., Bagó, Z., Huhulescu, S., Pietzka, A. & Pleininger, S. (2022). Listeriosis: The Dark Side of Refrigeration and Ensiling. In: Sing, A. (eds) Zoonoses: Infections Affecting Humans and Animals. Springer, Cham, https://doi.org/10.1007/978-3-030-85877-3_10-1

Brown, E., Dessai, U., McGarry, S. & Gerner-Smidt, P. (2019). Use of Whole-Genome Sequencing for Food Safety and Public Health in the United States. *Foodborne Pathogens and Disease,* 16 (7), 441–450, doi: 10.1089/fpd.2019.2662

Cabal, A., Allerberger, F., Huhulescu, S., Kornschober, C., Springer, B., Schlagenhaufen, C., Wassermann-Neuhold, M., Fötschl, H., Pless, P., Krause, R., Lennkh, A., Murer, A., Ruppitsch, W. & Pietzka, A. (2019). Listeriosis outbreak likely due to contaminated liver pâté consumed in a tavern, Austria, December 2018. Euro surveillance : bulletin Europeen sur les maladies transmissibles = *European Communicable Disease Bulletin*, 24(39), 1900274, https://doi.org/10.2807/1560-7917.ES.2019.24.39.1900274

Cabal, A., Rab, G., Daza-Prieto, B., Stöger, A., Peischl, N., Chakeri, A., Mo, S. S., Bock, H., Fuchs, K., Sucher, J., Rathammer, K., Hasenberger, P., Stadtbauer, S., Caniça, M., Strauß, P., Allerberger, F., Wögerbauer, M. & Ruppitsch, W. (2022). Characterizing Antimicrobial Resistance in Clinically Relevant Bacteria Isolated at the Human/Animal/Environment Interface Using Whole-Genome Sequencing in Austria. *International Journal of Molecular Sciences,* 23 (19), 11276, https://doi.org/10.3390/ijms231911276

Carpentier, B. & Cerf, O. (2011). Review — Resistance of *Listeria monocytogenes* in food industry equipment and premises. *International Journal of Food Microbiology,* 145,1–8.

Elson, R., Awofisayo-Okuyelu, A., Greener, T., Swift, C., Painset, A., Amar, C. F. L., Newton, A., Aird, H., Swindlehurst, M., Elviss, N., Foster, K., Dallman, T. J., Ruggles, R. & Grant, K. (2019). Utility of Whole Genome Sequencing To Describe the Persistence and Evolution of *Listeria monocytogenes* Strains within Crabmeat Processing Environments Linked to Two Outbreaks of Listeriosis *Journal of Food Protection,* 82 (1), 30–38. doi: 10.4315/0362-028x.jfp-18-206

European Centre for Disease Control, European Food Safety Authority, Van Walle, I., Guerra, B., Borges, V., Carriço, J. A., Cochane, G., Dallman, T., Franz, E., Karpišková, R., Litrup, E., Mistou, M. -Y., Morabito, S., Mossong, J., Alm, E., Barrucci, F., Bianchi, C., Costa, G., Kotila, S., Mangone, I., Palm, D., Pasinato, L., Revez, J., Struelens, M., Thomas-López, D. & Rizzi, V. (2019). Technical report on the collection and analysis of whole genome sequencing data from food-borne pathogens and other relevant microorganisms isolated from human, animal, food, feed and food/feed environmental samples in the joint ECDC–EFSA molecular typing database. *EFSA Support. Publ. 16*:1337E. Available at: https://doi.org/10. 2903/sp.efsa.2019.EN-1337

Gattuso, A., Cella, E., Fillo, S., Ortoffi, M. F., Angeletti, S., Ciccozzi, M., De Medici, D., Lista, F. & Fiore, A. (2022). Phylogenetic and Evolutionary Genomic Analysis of *Listeria monocytogenes* Clinical Strains in the Framework of Foodborne Listeriosis Risk Assessment. *Frontiers in Microbiology,* 13, 816880, doi: 10.3389/fmicb.2022.816880

Halbedel, S., Wilking, H., Holzer, A., Kleta, S., Fischer, M. A., Lüth, S., Pietzka, A., Huhulescu, S., Lachmann, R., Krings, A., Ruppitsch, W., Leclercq, A., Kamphausen, R., Meincke, M., Wagner-Wiening, C., Contzen, M., Kraemer, I. B., Al Dahouk, S., Allerberger, F., Stark, K. & Flieger, A. (2020). Large Nationwide Outbreak of Invasive Listeriosis Associated with Blood Sausage, Germany, 2018-2019. *Emerging Infectious Diseases,* 26 (7)*,* 1456–1464, https://doi.org/10.3201/eid2607.200225

Heymann, D. (ed). (2015). Control of Communicable Diseases Manual, 20th edn. American Public Health Association, Washington, D.C.

Hyden, P., Grim, C., Pietzka, A., Blaschitz, M., Indra, A., Sensen, C. W., Allerberger, F., Rattei, T. & Ruppitsch, W. (2016a). Comparison of SNP based and cgMLST based typing of *Listeria monocytogenes* isolates from Seeliger's historical "Special *Listeria* Culture Collection". Poster; Boston, USA; ASM Microbe.

Hyden, P., Pietzka, A., Lennkh, A., Murer, A., Springer, B., Blaschitz, M., Indra, A., Huhulescu, S., Allerberger, F., Ruppitsch, W. & Sensen, C. W. (2016b). Whole genome sequence-based serogrouping of *Listeria monocytogenes* isolates. *Journal of Biotechnology,* 235*,* 181–186, https://doi.org/10.1016/j.jbiotec.2016.06.005

Jackson, B. R., Tarr, C., Strain, E., Jackson, K. A., Conrad, A., Carleton, H., Katz, L. S., Stroika, S., Gould, L. H., Mody, R. K., Silk, B. J., Beal, J., Chen, Y., Timme, R., Doyle, M., Fields, A., Wise, M., Tillman, G., Defibaugh-Chavez, S., Kucerova, Z., Sabol, A., Roache, K., Trees, E., Simmons, M., Wasilenko, J., Kubota, K., Pouseele, H., Klimke, W., Besser, J., Brown, E., Allard, M. & Gerner-Smidt, P. (2016). Implementation of Nationwide Real-time Whole-genome Sequencing to Enhance Listeriosis Outbreak Detection and Investigation. *Clinical Infectious Diseases : an official publication of the Infectious Diseases Society of America,* 63(3), 380–386, https://doi.org/10.1093/cid/ciw242

Jagadeesan, B., Gerner-Smidt, P., Allard, M. W., Leuillet, S., Winkler, A., Xiao, Y., Chaffron, S., Van Der Vossen, J., Tang, S., Katase, M., McClure, P., Kimura, B., Ching Chai, L., Chapman, J. & Grant, K. (2019). The use of next generation sequencing for improving food safety: Translation into practice. *Food Microbiology, 79,* 96–115, https://doi.org/10.1016/j.fm.2018.11.005

Joint ECDC-EFSA — Rapid outbreak assessment, 2018. Multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections probably linked to frozen corn. 22 March 2018.

Lachmann, R., Halbedel, S., Lüth, S., Holzer, A., Adler, M., Pietzka, A., Al Dahouk, S., Stark, K., Flieger, A., Kleta, S. & Wilking, H. (2022). Invasive listeriosis outbreaks and salmon products: a genomic, epidemiological study. *Emerging Microbes & Infections*, 11 (1), 1308–1315, https://doi.org/10.1080/22221751.2022.2063075

Lakicevic, B., Jankovic, V., Pietzka, A. & Ruppitsch, W. (2023). Whole genome sequencing as the gold standard approach for control of *Listeria monocytogenes* in the food chain. *Journal of Food Protection,* 86(1)*,* 100003, https://doi.org/10.1016/j.jfp.2022.10.002

Moura, A., Criscuolo, A., Pouseele, H., Maury, M. M., Leclercq, A., Tarr, C., Björkman, J. T., Dallman, T., Reimer, A., Enouf, V., Larsonneur, E., Carleton, H., Bracq-Dieye, H., Katz, L. S., Jones, L., Touchon, M., Tourdjman, M., Walker, M., Stroika, S., Cantinelli, T., Chenal-Francisque, V., Kucerova, Z., Rocha, E. P., Nadon, C., Grant, K., Nielsen, E. M., Pot, B., Gerner-Smidt, P., Lecuit, M. & Brisse, S. (2016). Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. *Nature Microbiology,* 2*,* 16185, https://doi. org/10.1038/nmicrobiol.2016.185

Pietzka, A., Allerberger, F., Murer, A., Lennkh, A., Stöger, A., Cabal Rosel, A., Huhulescu, S., Maritschnik, S., Springer, B., Lepuschitz, S., Ruppitsch, W. & Schmid D. (2019). Whole Genome Sequencing Based Surveillance of L. monocytogenes for Early Detection and Investigations of Listeriosis Outbreaks. *Frontiers in Public Health*, 7, 139, doi: 10.3389/fpubh.2019.00139

Pightling, A. W., Pettengill, J.B., Luo, Y., Baugher, J.D., Rand, H. & Strain, E. (2018). Interpreting Whole-Genome Sequence Analyses of Foodborne Bacteria for Regulatory Applications and Outbreak Investigations. *Frontiers in Microbiology,* 9, 1482, doi: 10.3389/fmicb.2018.01482

Ruppitsch, W., Prager, R., Halbedel, S., Hyden, P., Pietzka, A., Huhulescu, S., Lohr, D., Schönberger, K., Aichinger, E., Hauri, A., Stark, K., Vygen, S., Tietze, E., Allerberger, F. & Wilking, H. (2015a). Ongoing outbreak of invasive listeriosis, Germany, 2012 to 2015. *Euro Surveillance* 20(50) 30094, https://doi.org/10.2807/1560-7917.ES.2015.20.50.30094

Ruppitsch, W., Pietzka, A., Prior, K., Bletz, S., Fernandez, H. L., Allerberger, F. & Mellmann, A. (2015b). Defining and evaluating a core genome multilocus sequence typing scheme for whole-genome sequence-based typing of *Listeria monocytogenes*. *Journal of Clinical Microbiology,* 53*,* 2869–2876, https://doi.org/10.1128/ JCM.01193-15

Ruppitsch, W. , Pietzka, A., Cabal, A., Chakeri, A., Schmid, D., Lakicevic, B., Lepuschitz, S. & Allerberger, F. (2019). Advances in foodborne outbreak investigation and source tracking using whole genome sequencing *IOP Conf. Series: Earth and Environmental Science 333* 012010, doi:10.1088/1755-1315/333/1/012010

Smith, A. M., Tau, N. P., Smouse, S. L., Allam, M., Ismail, A., Ramalwa, N. R., Disenyeng, B., Ngomane, M. & Thomas. J. (2019). Outbreak of *Listeria monocytogenes* in South Africa, 2017–2018: Laboratory Activities and Experiences Associated with Whole-Genome Sequencing Analysis of Isolates. *Foodborne Pathology and Disease*, 16 (7), 524–530.

Stessl, B., Ruppitsch, W. & Wagner, M. (2022). *Listeria monocytogenes* post-outbreak management — When could a food production be considered under control again? *International Journal of Food Microbiology,* 379*,* 109844, https://doi.org/10.1016/j.ijfoodmicro.2022.109844

Van Walle, I., Björkman, J. T., Cormican, M., Dallman, T., Mossong, J., Moura, A., Pietzka, A., Ruppitsch, W., Takkinen, J. & European *Listeria* WGS typing group (2018). Retrospective validation of whole genome sequencing-enhanced surveillance of listeriosis in Europe, 2010 to 2015. *Euro Surveillance : bulletin Europeen sur les maladies transmissibles = European Communicable Disease Bulletin,* 23 (33)*,* 1700798, https://doi.org/10.2807/1560-7917.ES.2018.23.33.1700798

Zuber, I., Lakicevic, B., Pietzka, A., Milanov, D., Djordjevic, V., Karabasil, N., Teodorovic, V., Ruppitsch, W. & Dimitrijevic, M. (2019). Molecular characterization of *Listeria monocytogenes* isolates from a small-scale meat processor in Montenegro 2011–2014. *Food Microbiology,* 79*,* 116–122, doi: 10.1016/j.fm.2018.12.005