



# Whole genome sequencing as the ultimate genomic subtyping tool for the identification and control of *Listeria monocytogenes* in the RTE food chain

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## ABSTRACT

*Listeria monocytogenes*, a saprophytic facultative anaerobic Gram-positive pathogen, has been involved in numerous outbreaks worldwide along with high mortality rate. Food contamination related to listeriosis occurs during primary production or more commonly, in food production facilities. The use of advanced diagnostic technologies such as whole genome sequencing (WGS) support surveillance, foodborne outbreak investigation as well as pathogen source tracking in food industry. WGS can also be useful for determining traits such as different genetic markers including virulence and antimicrobial resistance associated genes, which can be of great benefit for enhancing public health protection and for effective food safety management system. This is a very affordable, fast and powerful tool for obtaining high quality genomic data which can also be used in the regulatory field to differentiate more and less pathogenic *L. monocytogenes* clones.

## 1. Introduction

*Listeria monocytogenes* is a pathogenically heterogeneous species, comprising 14 serotypes and four evolutionary lineages that have been grouped into multiple clonal complexes (CCs) and sequence types (ST) according to multilocus sequence typing (MLST) (Yin *et al.*, 2019; Orsi *et al.*, 2011; Ragon *et al.*, 2008). *L. monocytogenes* CCs consist of: (a) infection-associated isolates, which belong to hypervirulent lineage I (e.g. CC1, CC2, CC4, and CC6), (b) food-associated isolates, which belong to hypovirulent lineage II and are mainly found within food processing environments (e.g. CC9 and

CC121), and (c) intermediate-associated isolates that are greatly connected to both human and food settings (Maury *et al.*, 2016). Listeriosis is a relatively rare disease but causes a large proportion of deaths and severe cases mainly in vulnerable populations, including immunocompromised individuals, infants, pregnant women and elderly persons affecting those over 60 years of age. This is a predominantly foodborne disease where the people usually become ill after eating food contaminated by different clones of *L. monocytogenes*. In Europe, a total of 1876 listeriosis cases were reported in 2020 (European Food Safety Authority and Euro-

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pean Centre for Disease Prevention and Control, 2021). Even low numbers of colony forming units (CFU) can be pathogenic for the susceptible people. The largest listeriosis outbreak was reported from a South African manufacturer in January 2017 lasting until July 2018 with a total of 1060 associated cases. Ready-to-eat processed meat products (polony) were pointed out as the source of infection (Smith *et al.*, 2019).

## 2. Transmission routes and WGS

Nastasijevic *et al.* (2017) illustrated a proof of concept for the practical application of WGS to provide insights into contamination pathways of *L. monocytogenes* in a pork processing factory. The authors used WGS to characterize and track 8 positive environmental isolates (out of 53 *Listeria* spp.) of *L. monocytogenes* that originated from critical sampling locations (CSLs) in pork meat production environment such as slaughter line, chilling chamber, deboning, modified atmosphere packaging (MAP) and dispatch. WGS analysis classified these isolates into three serotypes and clonal complexes (CC26, CC9 and CC1). The isolates of *L. monocytogenes* that originated from the above mentioned CSLs were either genetically close and/or identical to isolates from the slaughter line. Accordingly, Nastasijevic *et al.* (2017) deduced that the contamination derived from the slaughter line and emphasized WGS as a powerful tool of food safety management system. Furthermore, using WGS, Demaître *et al.* (2021) concluded that the persistence of *Listeria* contamination on the slaughter line was due to the presence of hypovirulent lineage II CC9 strain in the carcass splitter for longer than one year. However, Burnett *et al.* (2022) found hypervirulent I strains of *L. monocytogenes* in two meat processing facilities during 2011–2015. These strains originated from raw and environmental samples, representing serotypes 4b, 1/2b and 3b and belonged to clonal complexes: CC1, CC2, CC5 and CC288. Only a single lineage II isolate of serotype 1/2c was discovered in raw meat and belonged to CC9. The authors indicated that hypervirulent strains of *L. monocytogenes* can also well colonize, persist and predominate in food establishments. In that regard, colonization and persistent contamination of food and production establishments represents a high risk for human health.

## 3. Outbreak investigations and WGS

Large-scale listeriosis outbreaks investigated by WGS were reported in the US and EU.

In November 2014, WGS data showed that one restaurant in Rhode Island was the likely cause of a small outbreak. The establishment was also linked to a listeriosis case that happened in 2013 (Berkeley *et al.*, 2016). In a multistate outbreak linked to Blue Bell Creamery, the ice cream was likely contaminated from the manufacturing facility (Anonymous, 2017). Due to the fact that ten cases from four separate states were found between 2010 and 2015, this outbreak was both complex and uncommon. Also, this product contained very low levels of contamination and did not support the growth of *L. monocytogenes* (Chen *et al.*, 2016; Pouillot *et al.*, 2016). In another U.S multistate outbreak associated with the soft cheese distributed by Karoun Dairies in 2015, 30 persons in 10 states were affected and three deaths were recorded from California and Ohio (Centers for Disease Control and Prevention, 2015). In 2020, FDA and CDC analysed by WGS a multistate outbreak linked to contaminated fresh enoki mushrooms imported from Republic of Korea (Centers for Disease Control and Prevention, 2020). Some other listeriosis reports involve a 2018 multistate outbreak linked to pork products produced by Long Phung Food Products and an outbreak associated with hard-boiled eggs produced at the Almark Foods Gainesville, Georgia (Centers for Disease Control and Prevention, 2018, 2019). Interestingly, in the listeriosis outbreak reported in a 2020, deli meats were suspected as the cause, but the specific supplier and type of deli meat have never been confirmed (Centers for Disease Control and Prevention, 2020). In each of these outbreaks, WGS was utilized to depict genetic relatedness between outbreak strains and isolates suspected as an infection source. However, there are no WGS data for the latest listeriosis reports which include different RTE products such as packaged salads, cooked chicken and cheese.

In Denmark, WGS was used to solve outbreaks caused by the consumption of smoked fish, which led to deaths of seven persons and one stillborn baby (Lassen *et al.*, 2016). Also, two multi-country outbreaks caused by *L. monocytogenes* ST8 and ST1247 that affected Germany, France, Denmark, Estonia, Finland, France and Sweden were likely caused by consumption of ready-to-eat salmon products (European Food Safety Authority European Centre for

*Disease Prevention and Control*, 2018; 2019). An outbreak of listeriosis that occurred in Austria was caused by *L. monocytogenes* IVb-CC4-ST4-CT7652 strain which has not been detected anywhere before. Liver pâté was identified as the likely source of this outbreak (Cabal et al., 2019). Lüth et al. (2020) used WGS to investigate one of the largest listeriosis outbreaks in Germany associated with consumption of meat products from a single producer. According to the *European Food Safety Authority* (2018), another multi-country outbreak of *L. monocytogenes* serogroup IVb, ST6 infection was traced to frozen corn that was produced in Hungary and packed in Poland. According to all of the aforementioned findings, WGS-based typing methods are a powerful tool for outbreak investigations and source tracking of *L. monocytogenes* in the food industry. As the amount of WGS data for *L. monocytogenes* increases, it is now possible to simultaneously identify some other genetic components, such as accessory genes associated with virulent and antibiotic-resistant phenotypes. This primarily refers to *Listeria* pathogenicity islands, (LIPI)-3 and (LIPI)-4, which carry genes conferring higher virulence (Maury et al., 2016; Tavares et al., 2020; Centorotola et al., 2021;

Roedel et al., 2019) and antibiotic target-modifying enzymes. WGS analysis showed that antibiotic target-modifying enzymes (*mprF* and *fosX*) were detected in clonal complexes CC21 and CC121, while complexes CC8 and CC1 exclusively harboured *mprF* that changes cell wall charge (Zuber et al. 2019).

Overall, cooperation between epidemiologists, molecular microbiologists and bioinformaticians is essential for properly conducted outbreak investigations at both national and international level. Moreover, WGS has become an invaluable tool for discovering transmission routes and various antimicrobial resistance genes. In recent years, the cost of WGS has decreased significantly, allowing its routine use in the food industry which will benefit food safety management systems, public health, and food agencies. On the other hand, WGS can help in the revision of existing regulation because, in terms of quantitative microbiological risk assessments, not all *L. monocytogenes* CCs are considered to be equally pathogenic. In this way, CCs would be ranked according to their degree of virulence and association with different RTE foods (Lakićević et al., 2022).

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