

Short Note

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[Josué J. Da Silva](#) *

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Short Note

Recent Advances in Molecular Diagnostic Methods for Foodborne Pathogens with a Focus on *Listeria monocytogenes* and *Salmonella* spp.

Josué J. Silva

Centro de Ciência e Qualidade de Alimentos, Instituto de Tecnologia de Alimentos, Campinas, São Paulo 13070-178, Brazil; josue.biomol@gmail.com

Abstract: This article discusses advances in molecular technologies for detecting foodborne pathogens, specifically *Listeria monocytogenes* and *Salmonella*. Although traditional detection methods are reliable, they are time- and labor-intensive. On the other hand, molecular methods include polymerase chain reaction (PCR), quantitative real-time PCR (qPCR), and next-generation sequencing (NGS), which enable rapid, specific, and sensitive detection of pathogens. The introduction of such procedures has significantly improved food safety testing and monitoring. Although challenges such as accessibility and cost remain, future developments in molecular diagnostics will lead to greater implementation in improving pathogen management throughout the food supply chain.

Keywords: molecular diagnostics; foodborne pathogens; *Listeria monocytogenes*; *Salmonella* detection; NGS

Introduction

Food safety is a worldwide concern; the surveillance of foodborne pathogens is critical for avoiding mass morbidity. Despite their reliability, traditional methods for identification are time-consuming and laborious. Molecular techniques have emerged as powerful tools in recent years, offering faster, more specific and more sensitive modes of detection. This paper reviews molecular techniques that have been developed and applied for the detection of major foodborne pathogens, especially *Listeria monocytogenes* and *Salmonella*.

Molecular Techniques for Pathogen Detection

In food safety laboratories, PCR is commonly used and in most cases, other molecular techniques like real-time quantitative PCR (qPCR) and next-generation sequencing (NGS) are employed as they have proved to be the gold standard. The rapid identification of unique genetic markers from different pathogens assures specificity.

PCR and qPCR are the pioneers of molecular methods that have revolutionized pathogen detection. Polymerase chain reaction amplifies specific DNA targets and can detect even the smallest amounts of pathogen DNA. Quantitative PCR uses fluorescent markers that monitor DNA amplification in real time to determine the amount of pathogen present (Okubara et al., 2005). The sensitivity and specificity observed when using qPCR to detect *L. monocytogenes* makes this method extremely valuable for ensuring food safety (Garrido-Maestu et al., 2018; Jandaghi et al., 2020). Similarly, qPCR assays targeting *invA* and *hilA* have been shown to be well suited for detecting *Salmonella* in a variety of food samples (Xu et al. (2008)).

NGS Technology: NGS represents the ultimate comprehensive approach for pathogen detection, with the ability to sequence the entire genome of a foodborne pathogen. This enables detailed insights into pathogen strains and virulence factors as well as antibiotic resistance profiles. The contribution of NGS in outbreak investigations of *L. monocytogenes* and *Salmonella* has been extremely valuable in tracing the source and pathway of contamination (Lakićević et al., 2022; Jackson et al., 2016; Oakeson

et al., 2018; Portmann et al., 2018). These data are of high resolution and provide insights into pathogen epidemiology beyond what traditional methods can provide.

Molecular Techniques in Food Safety: Their Applications

The rapid and accurate detection of pathogens is the driving force behind the increasing use of molecular techniques in food safety. Such methods are now an important component of routine food testing programs and surveillance measures.

Listeria monocytogenes: The ability of *L. monocytogenes* to grow at refrigerator temperatures makes it a serious hazard for ready-to-eat foods. Molecular techniques enable rapid detection of this pathogen in food processing environments, allowing for timely intervention to prevent contamination. PCR tests targeting the *hlyA* gene, an important virulence factor, are particularly effective in detecting pathogenic strains of *L. monocytogenes* (Liu et al., 2012; Smith et al., 2000; Traunšek et al., 2011).

Salmonella spp.: As one of the most important pathogens of foodborne illness worldwide, significant progress has been made in the detection of *Salmonella* species through molecular techniques and multiplex PCR applied to a variety of foods, such as poultry, eggs, and fresh produce. The efficiency of *Salmonella* surveillance has been improved by the development of multiplex PCR methods, which can detect multiple pathogens simultaneously in large-scale food safety projects (Kawasaki et al., 2009; Nguyen et al., 2016; Zhang et al., 2009).

Despite the many advantages of molecular methods, they require specialized equipment and expertise, making them difficult to implement on a large scale. Advanced bioinformatics—especially when reading complex data from NGS—may not be available in all laboratories.

Future innovations in molecular diagnostics may target technology that is more widely available and less costly. LAMP is a new approach and seems to be very useful as an alternative to PCR; it may have potential applications in point-of-care food safety. In this way, the evolution of microfluidics and lab-on-a-chip technologies will bring miniaturization and automation sure systems for the identification of pathogens, which will contribute to their widespread use.

Conclusions

The detection of foodborne pathogens by molecular means has evolved and positively complements traditional methods. It is thus important to adopt rapid, sensitive, and specific molecular methodologies for detecting these target pathogens so as to assure food safety. Although there are some difficulties that may be encountered, subsequent progress in molecular diagnostics will help enhance the identification and control of such pathogens as *L. monocytogenes* and *Salmonella* from farm to table.

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