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Emerging Diagnostic Tools for *Listeria* Infections Challenges and Perspective

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Abstract: Listeriosis, a severe infectious disease characterized by meningoencephalitis, abortion, septicemia, and a high fatality rate. Listeriosis predominantly affects certain risk groups, including pregnant women, newborns, elderly people, and immunocompromised patients. In animals, it causes abortion, endometritis, repeat breeding, encephalitis, septicemia, and mastitis. Therefore, Precise detection of *Listeria* pathogen is indispensable in veterinary and human clinical diagnostics and consider a global honorable aim for effective early treatment and improving prognosis. Therefore, this review emphasizes emerging techniques for diagnosis of Listeriosis and touch upon the conventional, molecular and proteomic techniques used in the diagnosis of these pivotal pathogens.

Key words: *Listeria* · Proteomic technique · Molecular techniques · Conventional tools

INTRODUCTION

Bacteria of the facultative anaerobic gram-positive genus *Listeria* are widely distributed in the environment, particularly the closely related species *Listeria monocytogenes* and *L. innocua*. Both of these *Listeria spp.* are frequently found in food products, where they can grow over a pH range of 4.39 to 9.40, even at refrigeration temperatures. Ingestion of foods contaminated with *L. monocytogene*s can result in listeriosis, a severe infectious disease characterized by meningoencephalitis, abortion, septicemia, and a high fatality rate (30%). Listeriosis predominantly affects certain risk groups, including pregnant women, newborns, elderly people, and immunocompromised patients. *L. innocua*, in contrast, is nonpathogenic, and its presence in foods is no hazard to human health [1-5]. In animals, it causes abortion, endometritis, repeat breeding, encephalitis, septicemia, and mastitis [6]. *Listeria monocytogenes* is an opportunistic pathogen and a major concern especially in food industries [7].

Relevance of Early Accurate Diagnosis of *Listeriosis:* *Listeria monocytogenesis* an important foodborne pathogenic bacterium that is explicitly threatening public health and food safety. Rapid, simple, and sensitive detection methods for this pathogen are of urgent need for the increasing on-site testing demands [8]. Moreover, accurate diagnosis is vital for avoiding broad-spectrum antimicrobial medicines and implementing focused treatment due to antimicrobial resistance [9, 10]**.**

Conventional Diagnosis of *Listeriosis*: The process of isolation and identification by biochemical standard methods are laborious, time consuming and can take up to 7-10 days. Moreover, because of high phenotypic similarity in genus *Listeria*, differentiation between spp. is not always reached [11-13] The differentiation of pathogenic and non pathogenic *Listeria* remains a time consuming and tedious task, beside it hazards to lab stuff. [14]. Detection of food-borne bacteria involve the use of pre-enrichment and /or specific enrichment, followed by the isolation of the bacteria in solid media and a final confirmation by biochemical and/or serological tests are less sensitive and unreliable due to undecisive results comparing to recent PCR and real time PCR techniques thus one or more molecular methods needs to be carried out for typing and subtyping the involved strains [15, 16]. PCR has been offer several advantages over the classical microbiological methods, such as shorter time of analysis, low detection limits, specify and potential for automation [17]. Many authors developed PCR assay for

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identification of *L. welsheimeri* and *L. monocytogenes*, this PCR could be important to study the occurrence of these agents in food, environment, animals and human carriers [18].

Listeria spp. Could be isolated from bovine mastitis cases; and detected of multiple virulence associated genes in isolated *L. monocytogenes* using the PCR assay [14] and in pregnant women [19]. Even in table eggs, Sayed *et al*. [20] identified *Listeria monocytogenes* in 7% of egg shell using PCR assay which revealed the degree of contamination and public health hazards.

Multiplex PCR: Many of m. PCR assays are used for simultaneous detection of several pathogenic *B. cereus, S. aureus* and *L. monocytogenes* [21] the high through output and cost effective m- PCR method developed in this study provided a powerful tool for simultaneous, rapid and reliable detection and proved the possibility of simultaneous identification and differentiation of pathogenic *Listeria monocytogenes* and other *Listeria* spp. during one reaction performed [22]. Also, Kerouanton *et al*. [23] defined a scheme for multiplex molecular serotyping of *L. monocytogenes* based on a previously described PCR assay, then evaluated and compared this new procedure with conventional serotyping by agglutination; they concluded this molecular serotyping scheme could be considered as a useful and rapid method for characterization of *L. monocytogenes*. Jofre *et al*. [24] achieved a mPCR for simultaneous detection of *L. monocytogenes* and *Salmonella* in cooked ham; in addition to saving time and effort, the m PCR proved to be a simple and ready to go method for simultaneous confirmation of *Listeria* and *Salmonella* colonies directly from agar plate without any DNA extraction steps. Moreover, mPCR could detect and identify all spp. of genus *Listeria* within 2-3 days from food samples and within 3-4 hours from colonies on agar plate using the iap gene which encoding to the major extra cellular protein that is common to all members of *Listeria* spp. [12, 25].

Real Time PCR: Real time PCR was recommended in strategy, of screening to considerably reduce time, costs and giving more sensitive and specific results [16]. Additionally, an effective multiplex real time PCR (RT-PCR) method was presented by Suo *et al.* [26] for simultaneous detection of *Salmonella* spp., *E. coli* O157 and *L. monocytogenes*, Piednoir *et al*. [27] proposed a new validation procedure of qualitative SYBER green real time (q PCR) assays for detection and discrimination of *Listeria* spp. and *L. monocytogenes* with high accuracy (98% and 100% for *Listeria* spp. and *L. monocytogenes* respectively). In other study of Vitullo *et al.* [28] used real time PCR assay for serogrouping *L. monocytogenes* and differentiation from other *Listeria* spp., the assay is a rapid method for categorization of suspected *L. monocytogenes.*

The DNA was submitted to real time PCR targeting the *prs* gene for *Listeria* spp., the gene *inlA* for *Listeria monocytogenes* and the gene *iap* for *Listeria innocua by* Salza *et al*. [29] the results revealed the prevalence of *Listeria* spp. and *Listeria monocytogenes* was 18.6 and 10.0%, respectively. Samples enrichment coupled with quantitative PCR (qPCR) showed a greater sensitivity than the conventional culture method with an overall prevalence of 12.1% for *L. monocytogenes*. The present study supports the use of a culture enrichment coupled with qPCR for routine monitoring of contamination in food premises.

In a study of Kim *et al*. [30] aimed to develop, identify, and validate a SYBR Green qPCR-based genetic marker designed to detect *L. monocytogenes* and *L. innocua.* Because of the close genetic resemblance between *Listeria monocytogenes* and *Listeria innocua*, combined with their presence in similar environments, poses challenges for species-specific detection in food products. Ensuring food safety through microbiological standards necessitates reliable detection of pathogens like *L. monocytogenes* and *L. innocua* throughout the food chain using appropriate analytical techniques. Therefore, this direct qPCR method offers significant advantages for the rapid and precise detection of *L. monocytogenes* and *L. innocua*, potentially enhancing the efficiency of diagnostic and monitoring processes within food and vegetable distribution systems.

Emerging Molecular Assays: Nowadays, Application of the isothermal recombinase polymerase amplification (RPA) and the lateral flow strip (LFS) in the detection is promising for fast speed, high sensitivity, and little dependency on equipment and trained personnel. However, the simplicity comes with an intrinsic and non-negligible risk, the false-positive signals from primer-dimers. In this study, an improved RPA-LFS system was established for detection of *L. monocytogenes* that eliminated false-positive signals from primer- dimers. The intelligent use of the probe successfully linked the positive signal to the actual amplification product.

This RPA-LFS system was highly specific to *L. monocytogenes* and was able to detect as low as 1 colony-forming unit of the bacterium per reaction (50 ml) without DNA purification, or 100 fg of the genomic DNA/50 ml. The amplification could be conducted under the temperature between 37 and 42°C, and the whole detection finished within 25 min. Test of artificially contaminated milk gave 100% accuracy of detection without purification of the samples [8]. The global food demand is expected to increase in the coming years, along with challenges around climate change and food security. Concomitantly, food safety risks, particularly those related to bacterial pathogens, may also increase. Thus, the food sector needs to innovate to rise to this challenge. Therefore, Hadi *et al*. [31] discuss recent advancements in molecular techniques that can be deployed within various foodborne bacteria surveillance systems across food settings. Also, they provide updates on nucleic acid-based detection, with a focus on polymerase chain reaction (PCR)-based technologies and loop-mediated isothermal amplification (LAMP). These include descriptions of novel genetic markers for several foodborne bacteria and progresses in multiplex PCR and droplet digital PCR. Beside they provide an overview of the development of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins systems, such as CRISPR-Cas9, CRISPR-Cas12a, and CRISPRCas13a, as tools for enhanced sensitive and specific detection of foodborne pathogens. The final section describes utilizations of whole genome sequencing for accurate characterization of foodborne bacteria, ranging from epidemiological surveillance tomodel-based predictions of bacterial phenotypic traits throughgenome-wide association studies or machine learning.

Despite the recent advances in food preservation techniques and food safety, significant disease outbreaks linked to foodborne pathogens such as bacteria, fungi, and viruses still occur worldwide indicating that these pathogens still constitute significant risks to public health. Despite the recent advances in food preservation techniques and food safety, significant disease outbreaks linked to foodborne pathogens such as bacteria, fungi, and viruses still occur worldwide indicating that these pathogens still constitute significant risks to public health [32].

*Listeria monocytogene*s clonal complex (CC) 8 strain is a common clone in food and clinical cases. Cheng *et al*. [33**]** aimed in their study to develop multiplex PCR (m PCR) and high-resolution melting (HRM) qPCR to simultaneously detect L. monocytogenesCC8 and the other *L. monocytogenes* strains based on pan-genome analysis. A novel multiplex PCR and HRM qPCR targeted for the genes LM5578_1180 (specific for CC8) and LM5578_2262 (for *L. monocytogenes*) were developed. The specificity of this multiplex PCR and HRM qPCR were verified with other CCs of *L. monocytogenes* and other species strains and concluded this multiplex PCR and HRM qPCR could accurately detect CC8 strains and the feasibility of these methods were satisfactory in terms of sensitivity, specificity, and efficiency was consistent with that of the National Standard Detection Method (GB4789.30-2016). Therefore, the developed assays could be applied for rapid screening and detection of *L. monocytogenes* CC8 strains both in food and food production environments, providing accurate results to adopt monitoring measures to improve microbiological safety.

Proteomic Identification: Microorganisms can be quickly found using matrix-assisted laser desorption ionization time of flight mass spectrometry [34]. This method is thought to be both effective and inexpensive. Although the initial acquisition cost of the mass spectrometer is substantial, the operational cost per sample is minimal (less than $1/sample), rendering the technique appealing for high-throughput laboratories [35].

Application of MALDI-TOF in Detection of Listeria: *Listeria monocytogenes* is a food-borne pathogen that is the causative agent of human listeriosis. MALDI-TOF MS fingerprinting may have potential for *Listeria* identification and subtyping and may improve infection control measures [36]. Similarly, Thouvenot *et al.* [37] This study aimed to evaluate MALDI-TOF MS for species discrimination of *Listeria* in the context of routine surveillance. MALDI-TOF MS yielded 100% accuracy for the identification of *L. monocytogenes, L. innocua, L. ivanovii, L. fleischmannii, L. grayi, L. seeligeri, L. weihenstephanensis* and *L. welshimeri*, as confirmed by whole genome analyses. a simple and rapid proteomics-based MALDI-TOF MS approach was developed to detect *L. monocytogenes* directly from selective enrichment broths. Unlike the routine application of MALDI-TOF MS for identification of bacteria from solid media, this study proposes a cost-effective and time-saving detection scheme for direct identification of *L. monocytogenes* from broth cultures [38]. Similarly, Araújo

et al. [39] evaluated the detection of *Listeria* spp. using MALDI-TOF MS directly in enrichment broths, without isolated colonies, with naturally contaminated food and stool samples. The success rate was 77%. Considering the reduced time for diagnosis and the success rate, this is a promising screening tool, recent applications of MALDI-TOF MS to foodborne pathogen detection, emphasizing the applicability for routine microbiological analysis in the food sector. Many studies confirmed the higher discriminatory potential of MALDI-TOF MS at the species and strain level, thus making it a competent bacterial typing tool that could give information about the pathogenic character. It could also be of crucial importance for the risk assessment in the food chain, as well as for epidemiological studies [40]. The protocol describes a MALDI-TOF MS workflow as a single tool for simultaneous identification and subtyping of *L. monocytogenes* directly from solid culture medium [41]. Classification of Listeria species by incorporating denoising autoencoder (DAE) and machine learning algorithms in matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was attempt by Li *et al*. [42] the identification accuracy of Listeria species was 100%. Reis *et al*. [43] evaluate the effectiveness of the automated methods VITEK® 2 and MALDI-TOF/MS in identifying 94 strains of the genus *Listeria* with atypical identification profile. The results demonstrate that automated methodologies could not discriminate atypical strains of the *Listeria* genus and point to the need for the use of complementary tests, such as PCR and chromogenic media, for the correct identification of these strains.

The Future Prospective: The advancement of bioinformatic tools and proteomic-based methods in the future, together with user-implemented or updated databases and libraries, can improve MALDI-TOF MS by tackling the drawbacks associated with the poor differentiation of closely related species.

A promising future is the development of a MALDI-TOF MS-molecular-based approach that doesn't require sequencing for genetic information. This technique could identify microbial clusters.

CONCLUSION

1. Recent advancements have made MALDI-TOF MS an attractive microbiological diagnostic tool with promising future uses, and
2. m- PCR assay proved to be a valuable method for the diagnosis of co-infection for its accuracy, specificity and low cost in comparison to traditional techniques.

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