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LISTERIA MONOCYTOGENES IN THE FOOD CHAIN – RECENT ADVANCEMENTS IN TECHNOLOGIES FOR RAPID DETECTION AND IDENTIFICATION

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ABSTRACT

Listeria monocytogenes (*Lm*) is an opportunistic non-sporing foodborne pathogen that has been responsible for several outbreaks in India and other countries due to consumption of contaminated food and causes a disease called listeriosis. This disease is rare but life-threatening with severe symptoms and a high mortality rate of 20-30%. *Lm* can easily adapt to adverse environmental and stress conditions, making it a causative agent for major foodborne diseases. The classical culture methods for detection of *Lm* are simple and inexpensive but they are time consuming, laborious, and slow in providing results. Several alternative methods for identification of this pathogen are being used, which take short time for analysis, require less sample, cost effective and helpful in routine food sample testing. More sophisticated detection approaches are also needed to genetically discriminate strains for epidemiological investigations and to study listeriosis infections. In this review, we provide latest information on rapid and analytical methods for the detection and identification of *Lm* in foods. An emphasis is also given on techniques for subtyping of *Lm* strains, which are essential to determine evolutionary relationships between different strains and to track the source of food contamination. To the best of our knowledge, this is the first review paper to compile the most recent insights for testing presence of *Lm* in foods.

Keywords: *Listeria monocytogenes*, food, detection, PCR, identification, alternative methods, NGS

INTRODUCTION

The genus Listeria as of 2020 is known to contain 21 species which includes *L. monocytogenes* (Lm), *L. innocua*, *L. welshimeri*, *L. ivanovii*, *L. marthii*, *L. seeligeri*, *L. grayi*, *L. rocourtiae*, *L. fleischmannii*, *L. costaricensis*, *L. weihenstephanensis*, *L. floridensis*, *L. newyorkensis*, *L. aquatica*, *L. cornellensis*, *L. thailandensis*, *L. riparia*, *L. goaensis*, *L. booriae*, *L. grandensis* and *L. valentina* **(Quereda et al., 2020)**. This classification was made using sequence analyses including deoxyribonucleic acid (DNA) sequencing, 16S ribosomal ribonucleic acid (rRNA) information and multilocus enzyme analysis. It is reported that Lm is the only species of *Listeria* genus that can cause listeriosis in humans, though L. ivanovii is pathogenic to ruminants and occasionally causes human infections **(Snapir et al., 2006)**.

In 1929, Nyfeldt was the first one to isolate Lm from humans presuming that it was the causative agent of infectious mononucleosis **(Nyfeldt, 1937)**. The organism is Gram-positive, facultative anaerobic, motile at 20–28°C but non-motile at above 30°C. Biochemically, *Listeria* spp. are catalase-positive but oxidase, urea, and indole negative. Lm grows at pH 4.5-9.5, and can replicate at temperatures between -0.4 to 45°C **(Bucur et al., 2018)**. The ability to tolerate cold stress is one of the factors responsible for this bacterium to be detected frequently in refrigerated foods. Lm is one of the leading foodborne pathogens that causes sporadic infections and primarily causes a disease called listeriosis, which occurs in two forms; a non-invasive (febrile listerial gastroenteritis) and a more severe invasive listeriosis **(Buchanan et al., 2017)**. However, the expression of infection depends on age of the infected individual, infectious dose and virulence properties of the strain. The infective dose of Lm for causing listeriosis is difficult to assess, but the studies have shown that 10^4 to 10^7 live cells in susceptible people and more than 10^7 in healthy individuals should be enough to contract the disease. The symptoms of the invasive listeriosis are meningitis, endocarditis, encephalitis and meningoencephalitis which occur mainly in immunocompromised individuals.

LM IN FOOD AND ENVIRONMENT

Lm is ubiquitous and mainly found in soil, polluted water, and feed though it is present in very low number in these commodities **(Cox *et al.*, 1989)**. Several domesticated animals, particularly ruminants carry the bacterium and contaminate breeding environments. The organism can survive for longer periods in sewage sludge and river water, which may subsequently lead to potential risk of food contamination **(Dhama *et al.*, 2015)**. Studies have clearly shown that the pathogen is widely distributed and persisting longer periods in food production facilities are responsible for the formation of biofilms **(Buchanan *et al.*, 2017; Rodríguez-Campos *et al.*, 2019)**. These biofilms can tolerate sanitizers and other disinfectants that finally may lead to food contamination. *Lm* is present in various kinds of raw and ready-to-eat minimally processed foods **(Välimaa *et al.*, 2015; Madad *et al.*, 2023)** and it remains a hazard to the food handlers and food processing industries. The pregnant women, infants, the elderly and immuno-compromised individuals are at greatest risk of severe listeriosis. A list of major *Listeria* outbreaks happened in different countries is outlined in table 1. This information is gathered as per Centers for disease control and prevention (CDC) reports, previous publications and internet resources.

Table 1 Major reported *Listeria* outbreaks in different countries

Country	Year	Associated food product	No. of Cases/Deaths
Germany	1949-1957	Raw milk	~100
Switzerland	1983-1987	Soft cheese	122/33
Austria	1986	Raw milk/vegetables	28/5
Denmark	1989-1990	Blue-mold cheese/hard cheese	26/6
France	1995	Soft cheese	37/11
France	1997	Soft cheese	14/0
Finland	1998-1999	Butter	25/6
France	1999-2000	Meat	42/0
Sweden	2001	Soft cheese	33/0
Canada	2008	Meat	57/23
USA	2010	Pre-cut celery	10/0
USA	2011	Cantaloupe/melon	147/33
USA	2012	Cheese	22/4
USA	2013	Cheese	6/1

USA	2014	Packed caramel apple, sprouted bean, dairy products	5/2
USA	2015	Cheese, ice cream	10/3
USA	2016	Frozen vegetables/packaged salads	19/1
USA	2017	Raw milk, cheese	8/2
South Africa	2017-2018	Processed meat	1060/216
USA	2018	Deli ham, pork products	4/0
Australia	2018	Cantaloupe	18/6
USA	2019	Deli meats, mushrooms, Hard-boiled egg	18/2
Spain	2019	Ready to eat pork	207/3
USA	2020	Mushrooms, Deli meat	48/5
USA	2021	Packaged salads, cooked chicken	18/3
USA	2022	Ice cream, mushroom, cheese	6/0
USA	2023	Ice cream, leafy greens, packed peaches, nectarines, plumps	32/1
USA	2024	Deli meat	57/9
Denmark, Germany, Italy	2024	Smoked fish products	20/5

PATHOGENICITY OF *LISTERIA*

Listeria is a facultative intracellular parasite. When the food contaminated with *Listeria* ingested, it is taken up by enterocytes or M-cells in the small intestinal lining and multiply in underlying phagocytic cells. After entering the cytosol, this organism induces the polymerization of host actin filaments using ActA protein and by the force generated by actin polymerization, it moves first intracellularly, and then from cell to cell. *Lm* enters the gastrointestinal tract, small intestine and from there the bacteria are carried to liver and spleen through the macrophage cells. At this stage most of the cells are destroyed by neutrophils and Küpffer cells but some can escape into the cytosol and disseminate to main target organs via blood **(Quereda *et al.*, 2021)**. All these events are highly complex and involved several virulence factors initially with host cell adhesion, invasion, multiplication and finally spreading intracellularly. The intracellular presence in phagocytic cells also permits the bacteria access to the brain and transplacental entry to the foetus in pregnant women. An excellent review on pathogenicity of *Listeria* was published by **Quereda *et al.* (2021).**

SAMPLE PREPARATION

Microbiological testing typically comprises efficient preparation of food samples, sample homogenization and testing by microbiological or molecular methods. For the detection of target microorganisms from any matrix, sample preparation is a key step before testing by PCR or any other analytical method. Ideally, this is achieved by the separation of target cells from the food matrix ideally by membrane filtration, increase their concentration by pre-enrichment in suitable media, separate and purify them from background microflora, concentrate the target cells from bulk sample and minimise or reduce inhibitory substances **(Rohde *et al.*, 2015).**

Conventional methods for the detection of target pathogens can be divided into qualitative and quantitative. The qualitative method defines presence/absence of test pathogen in a given sample. The method includes adding an enrichment step with antimicrobial supplements to get rid of background flora as often the pathogenic microorganisms are present in very low numbers in bulk food samples. The enrichment culture is predominantly help to revive microbial cells that may have been stressed or injured by environmental conditions or during food processing by chemical or physical treatments. Basically, in the enrichment step, a 1:10 dilution of the food matrix is made (e.g., 25 g of food in 225 ml of enrichment medium) in order to reduce the background flora and inhibitory substances. After enrichment, a few biochemical tests are needed to perform to discriminate the bacteria of interest from other cells. In the quantitative method, the bacterial cells are counted and expressed as the number of organisms present per unit weight of sample. Culture methods are generally used for enumeration of bacteria and is performed using plate counts **(Foddai and Grant 2020).** A selective agar media is preferred for plating and enumeration of bacteria.

The conventional extraction of bacteria is performed by applying mechanical forces to homogenize the food matrix by simple vortexing or ultrasonication. Commercially available laboratory blenders like bag mixers, Stomacher or Pulsifier are preferred for homogenization **(Wu *et al.*, 2003).** Stomacher, the most widely used for this purpose consists of two movable paddles that continuously macerate the food in few seconds. The adjustable blending power allows the blending of most complex samples. The peristaltic movement of the paddles allows optimal bacterial extraction during blending, without risk of cross-contamination. Bead mill homogenizers are also used for sample extraction, wherein the beads are vigorously shaken to break up tissue and disrupt cells. These techniques are essentially used for the disruption of cells to release nucleic acids or proteins for molecular detection or other downstream applications. Other novel systems such as FastPrep®-24 is used to homogenize biological samples by lysing thoroughly and quickly any tissues and cells and thus allows easy and reproducible isolation of stable RNA, active proteins and full-length genomic DNA.

CULTURE BASED METHODS

As *Lm* is mainly found in various kind of foods, several culture-based methods have been reported for identification and characterization of the bacterium **(Gasanov *et al.*, 2005)**. This pathogen may sometimes be sub-lethally injured by various environmental stresses like temperature, pressure, and antimicrobial substances and enter into viable but non-culturable state (VBNC). But once the cells are resuscitated, they become active and can cause infection **(Zhang *et al.*, 2023)**. For the resuscitation of VBNC or sub-lethally injured states, the enrichment process divided into a pre-enrichment and an enrichment step in a selective medium that contains supplements (usually antibiotics) to suppress the background microflora. Presumptive positive colonies are further confirmed by various biochemical and serological tests **(Jasson *et al.*, 2010; Dwivedi and Jaykus 2011)**. An excellent review on isolation and identification of this organism was published by **Gasanov *et al.* (2005)**.

Most regulatory agencies specify that isolation of *Listeria* by culture media must be able to detect one live cell per sample unit (25 g). Hence, enrichment methods are to be employed using various antimicrobial agents to reduce background microflora before confirmation of target species **(Gasanov *et al.*, 2005)**. *Listeria* enrichment media also contains esculin and ferric ion resulting in the formation of black colour (esculin hydrolysis). Other antimicrobial agents like ceftazidime, moxalactam and lithium chloride are also rarely added into the media. The other selective plating media such as PALCAM and Oxford are recommended by various regulatory agencies for isolation of *Lm* **(Liu *et al.*, 2017)**. Various selective agents are added in PALCAM agar for inhibition of background flora and the differentiation can be done by hydrolysis of esculin and mannitol fermentation. The *Listeria* colonies in the PALCAM agar appear as grey-green with a black center and a black halo due to esculin hydrolysis. Oxford agar is also an important medium for detection of this organism from foods **(Pinto *et al.*, 2001)**. *Lm* colonies on the Oxford medium, are brown-green with a black halo, and upon further incubation the colonies turn into dark colour with a black centre and surrounded by black zones **(Magalhães *et al.*, 2014)**.

A breakthrough in the isolation of *Lm* took place with the development of chromogenic media. Agar *Listeria* Ottaviani and Agosti medium (ALOA), contains a chromogenic substrate called X-glucoside, which can detect β-glucosidase (**Ottaviani *et al.*, 1997**). *Listeria* species can hydrolyse the substrate and produce blue to green coloured colonies. *Lm* can be differentiated from other *Listeria* by phosphatidyl inositol specific phospholipase C (PIPLC) activity. Upon hydrolysis of phospholipase C enzyme, *Lm* colonies appear as green-blue in colour with an opaque halo, but the other species lacking phospholipase C, appear same colour but without halo. Rapid'L.mono agar, an improved ALOA medium utilizes same enzyme but with different substrate (X-IP). In this medium, *Lm* appear as blue colour colonies without halo (**Janzten *et al.*, 2006**). An improved chromogenic agar used for *Lm* identification is CHROMagar™Listeria (Becton Dickson Diagnostics). Colonies of *Lm* and *L. ivanovii* on this medium appear blue colour with a white halo, while other *Listeria* spp. colonies are also blue but with no halo (**Hegde *et al.*, 2007**). A flowchart of isolation and detection of *Listeria* from food and environmental samples by various regulatory bodies is shown in Figure 1.

Figure 1 Flowchart of isolation and detection of *Listeria* from food and environmental samples by various regulatory bodies

RAPID DETECTION/IDENTIFICATION METHODS

Owing to the limitations of the culture methods, morphological and biochemical tests for identification of *Lm*, rapid or alternative detection and/or identification methods such as culture, immunoassays, aptamers, and nucleic acids have been developed. The principle, advantages, and limitations of these assays are depicted in Table 2. However, most of these rapid methods do not show much specificity and sensitivity for direct identification of the target microorganisms unless a short pre-enrichment step is introduced before analyzing the food samples. Summary of various methods for detection of foodborne pathogens is depicted in Figure 2. A recent progress on point-of-care testing of food pathogens including *Lm* by rapid methods has been outlined by several authors (**Liu *et al.*, 2018, 2022**).

Immunological assays

Cell surface molecules are often chosen as target antigens for the development of immunological assays as the antibodies generated against those antigens can be used without the need of cell separation or fractionation. An immunological assay is basically a reaction between antigen and antibody. These assays are developed based on recombinant, monoclonal, and polyclonal antibodies to specific antigen of target organism (**Jasson *et al.*, 2010; Kalinin *et al.*, 2023**).

Among the several immunological assay platforms, enzyme linked immunosorbent assay (ELISA), lateral flow immunoassay (LFIA), and immunomagnetic separation (IMS) are widely used methods in food pathogen testing (**Xiao *et al.*, 2021**). The ELISA method is more powerful for analysing huge numbers of test samples (proteins, whole cell, or its components) yet the method involves reagent manipulations and a microplate reader to obtain quantitative data. Monoclonal antibodies with high sensitivity are generally used in ELISA systems to identify a specific epitope of a target antigen, whereas polyclonal antibodies are used rarely as they show high cross-reactivity among other microorganisms. However, for the improved sensitivity of these assays, pre-enrichment of the sample may be needed to eliminate or reduce the background microflora and to increase the target cell number (**Xiao *et al.*, 2021**). Various ELISA based commercial kits are available in market for rapid detection of target antigens in food sample. TRANSIA™ PLATE *Listeria monocytogenes*, a commercial ELISA kit for specific *Lm* identification is developed by BioControl Systems. It is a monoclonal antibody-based sandwich type of reaction specific to *Lm*. Another commercial system, VIDAS® LMO2 (bio-Mérieux) is also available for food sample analysis (**Vaz-Velho *et al.*, 2000**). These ELISA systems are certified by ISO and validated by AFNOR. Recently, a chemiluminescent based detection platform was reported for rapid detection of *Lm* from dairy products. The authors demonstrated the test system using milk and yogurt with a good detection limit with 100% relative specificity and the proposed method was also evaluated with qPCR (**Bromberger and Mester 2023**).

LFIA

With the principle of antigen-antibody interaction, these assays are most popular because of low-cost user-friendly formats, very short assay times (usually less than 10 min), no need of reagent manipulation and in most cases, results can be visualized by naked eyes. The advantages and dis-advantages of LFIA technique are described in Table 2. A novel strip test method combining IMS with LFIA was developed for accurate detection of *Lm* (**Shi *et al.*, 2015**). A pair of monoclonal antibodies was used to construct sandwich format, where superparamagnetic particles were coupled with one of the antibodies. The LOD of this LFIA system was found to be 10^4 CFU/ml. **Wang *et al.* (2017)** developed a LFIA for identification and quantification of eight *Lm* serotypes including 1/2a, 1/2b, and 4b using monoclonal antibodies developed against P60 protein of *Lm*. Rapid point-of-care nucleic acid based lateral flow assays based on PCR are also popular for detection of clinical, food, and waterborne pathogens (**Kim *et al.*, 2020**). The working principle, typical configuration, critical components of the assay, procedure, and diagnostic applications are not provided in this review as these have already been published elsewhere (**Mirica *et al.*, 2022; Younes *et al.*, 2023**).

IMS

IMS is widely used for concentrating the target cells, wherein the paramagnetic beads are coated with antibodies to capture the cells. IMS has essentially been used to capture the target cells for improved detection by ELISA and LFIA. The application of IMS technology in food microorganisms' detection has been reviewed by **Wang *et al.* (2020).** A magnetic concentration step combined with a lateral flow immunoassay was developed to detect *Lm* in spiked milk with a limit of detection (LOD) of 10^2 CFU/ ml using *Lm* -specific monoclonal antibodies (**Cho and Irudayaraj 2013**). An immunochromatography (ICG) test combined with an IMS was developed for qualitative detection of *Lm* in naturally contaminated meat samples (**Shim *et al.*, 2008**). **Capo et al. (2020)** developed a novel sandwich fluorescence linked immunosorbent assay to detect *Lm* on simulated bench-working surfaces. The assay was based on the immobilization of the *Lm* monoclonal antibody on the chitosan-cellulose nanocrystal (CNC) membrane, with an LOD of 10^2 CFU/ml (2 log). A rapid point of care test (POCT) was developed to detect *Lm* based on LFIA, using anti-Internalin antibodies with biotin-streptavidin system. This system showed an LOD of 10^2 CFU/ml in pure culture; 10^2 CFU/g in artificially inoculated food products (**Lopes-Luz *et al.*, 2023**). An immunomagnetic bead-based fluorescence immunochromatographic assay (FICA) was developed to detect *Lm* in pork, sausage, and milk with an LOD of 1×10^4 CFU/ml in 3 hr (**Li *et al.*, 2017**). **Cho *et al.* (2014)** developed a *Lm*-specific and sensitive monoclonal antibody-based IMS method detecting the pathogen in spiked milk sample in 4 hr with an LOD of less than 5 CFU/ml.

Aptamers

Aptamers are single-stranded DNA or RNA molecules of very short length that can selectively bind to a specific target, including proteins, peptides, carbohydrates, toxins, and even live cells. Aptamers for desired target are selected from a large oligonucleotide library by a process called SELEX (Sequential Evolution of Ligands by Exponential Enrichment). By using this procedure, non-binding aptamers are discarded and those aptamer molecules binding to the specific

target are expanded further. Positive selection rounds are usually followed by negative selection to improve the selectivity of the resulting aptamer candidates (**Banerjee and Nilsen-Hamilton 2013**).
For *Lm* detection, several candidate DNA aptamers have been identified showing high specificity (**Bruno and Sivils 2017; Du *et al.*, 2022**). Though all the reported aptamers showed affinity towards *Lm*, their binding targets are different. **Suh *et al.* (2014)** reported four aptamers specific to cell surfaces of *Lm.* **Feng *et al.* (2018)** selected a specific aptamer that has been used for magnetic capture of *Lm* and detection using loop-mediated isothermal amplification at an LOD of 5 CFU/ml within 3 hr. **Guo *et al.* (2020)** reported aptamer-coupled magnetic beads for specific capture of *Lm* with an LOD of 10 CFU/ml.

Bacteriophage based detection

Bacteriophages are the viruses that infect bacteria and used for biocontrol of pathogens as well as efficient separation and detection of pathogens (**Klumpp and Loessnor 2013**). Bacteriophages are host specific and can be found naturally where the hosts are abundant. About 500 listeriophages (phages that attack on *Listeria* spp.) have been isolated from different environmental sources (**Stone *et al.*, 2020**). Commercial sources of listeriophages have been used by the food industry for application either on food matrices or on surfaces of food processing plants (**Kawacka *et al.*, 2020**). Paramagnetic bead-based phage proteins have been used for the separation of *Listeria* (**Zhou and Ramasamy 2019**). The beads coated with phage protein were used for separation of *Lm* in experimentally inoculated raw milk and later detected by real-time PCR with an LOD of 10^2 to 10^3 CFU/ml (**Walcher *et al.*, 2010**). **Elsayed *et al.* (2023)** isolated and characterized bacteriophages for combating 22 multidrug-resistant *Lm* in dairy cattle forms alone and in conjugation with silver nanoparticles. Several authors have already reviewed on application of bacteriophages in food safety and detection (**Kawacka *et al.*, 2020; Romero-Calle *et al.*, 2023**).

Microfluidics

Microfluidics allows the precise control and manipulation of fluids on an exceedingly small scale. Combined with sensing and actuating capabilities, this results in compact devices that are also known as lab-on a-chip or miniaturized total analysis systems (μ-TAS). This system combines sample separation, concentration, preparation, and detection on a small chip with microchannels enabling researchers to perform a variety of lab functions on minute amounts of fluid (such as droplets). Microfluidics is a combination of chemistry, physics, biotechnology and engineering.
Microfluidic devices have been developed as rapid detection platforms for testing various foodborne pathogens (**Wang *et al.*, 2023; Jiang *et al.*, 2024**). These platforms can be coupled with either culture, immunological or molecular detection for testing of analytes. Colorimetric detection is an attractive format commonly used by chromogenic agars and can be paired with paper-based microfluidic devices (μPADs) for enzymatic detection. **Jokerst *et al.* (2012)** developed a paper-based microspot assay using wax printing on filter paper. Detection was achieved by measuring the colour change when an enzyme of specific pathogen reacts with its chromogenic substrate. With a combination of the paper based analytical device and sample enrichment, the authors detected *E. coli* O157:H7, *Salmonella Typhimurium*, and *Lm* in meat at a detection limit of 10 CFU/cm^2 in 12 hr.
A rapid and sensitive microfluidic device integrated with an electrochemical impedance analysis and urease catalysis was developed to measure *Listeria* (**Chen *et al.*, 2016**). The *Listeria* cells, the anti-*Listeria* monoclonal antibodies modified magnetic nanoparticles (MNPs), and the anti-*Listeria* polyclonal antibodies and urease modified gold nanoparticles (AuNPs) were incubated in a fluidic separation chip to form sandwich complexes. The capture efficiency of the *Listeria* cells in the separation chip was ~93% in 30 min with an LOD of 1.6×10^2 CFU/ml in one hour. Recently, **Xing *et al.* (2023)** developed a microfluidic biosensor for detection of multi-foodborne bacteria single stranded (ss) DNA simultaneously within 5 min using a smart phone. This technology was based on the fluorescence resonance energy transfer (FRET) between the graphene oxide (GO) and fluorescence molecules modified capture ssDNA of the target bacteria ssDNA (ctDNA) which were coated on the microfluidic chips. The fluorescence recovery was recorded by a smartphone fluorescent detector. By using this device, trace amounts of foodborne bacteria ssDNA in milk and water samples were successfully detected with very low detection limits. A review on recent advances in microfluidic devices for food pathogens detection was published by **Gao *et al.* (2022).**

Biosensors

The term "biosensor" refers to powerful analytical device involving biological sensing element with wide range of applications such as biomedicine, drug discovery, diagnosis, food safety and defense. This device converts a biological response (microorganism, enzyme, antibody, nucleic acid, or hormone) into an electrical signal. This sensor consists of a bioreceptor and a transducer. The transducer may be mass-based (piezoelectric, magnetic), optical (Fourier transform spectroscopy, surface-plasmon resonance, optic fibres), electrochemical (amperometric, potentiometric, impedimetric, conductometric). Application of biosensors in food pathogen detection has several advantages as these systems are portable, simple to use, can be used in the field. Furthermore, they have many features such as robust, accurate, sensitive, and specific (**Bhunia 2008**).
Fiber optic biosensors are most popular ones that are widely used for the food pathogens detection including *Listeria* spp. They have been used for the detection of *Lm* in artificially inoculated ready-to-eat products at a detection level of 10^2 to10^3 CFU/ml (**Ohk *et al.*, 2010**). A bio-photonic immunosensor has been developed with biofunctionalization of integrated photonic circuits to detect *Lm* in vegetables, meat, and ready-to-eat products with an LOD of 5 CFU/ml (**Fernández Blanco *et al.*, 2023**). **Huang and co-workers (2015)** reported a light scattering immunoassay using gold nanoparticles that bind to the cell surface epitopes of *Lm* at a detection limit of 3.5×10^1 CFU/ml. **Armstrong and co-workers (2021)** developed a novel flow-through electrochemical biosensor for specific detection of *Lm* from whole cell lysates with a detection limit of less than 10^5 CFU in 5 ml (or $<2 \times 10^4$ cells/ml). **Zhang *et al.* (2022)** developed a simple paper-based multi-biocatalyst platform to identify *Lm* by detecting multiple biomarkers using two different modified working electrodes with a lower detection limit at 10^4 CFU/ml. Biosensors for *Listeria* detection have excellently been reviewed by **Soni *et al.* (2018).** Recently, **Guk *et al.* (2024)** reported a PoreGlow system based on split green fluorescent protein (GFP) for rapid and accurate detection of *Lm.* This approach was able to identify *Lm* at a level of 10 CFU/ml and an LOD of 0.17 μg/ml LLO toxin.

MOLECULAR DETECTION/IDENTIFICATION METHODS

Polymerase chain reaction (PCR)

As an alternative to classical methods, molecular assays, particularly PCR based methodologies have been developed for detection and identification of clinical, food and environmental microorganisms (**Gasanov *et al.*, 2005**). Basically, PCR requires two synthetic oligonucleotides to amplify specific DNA target with the help of a thermostable polymerase. Later, these PCR amplified products are separated by gel electrophoresis and visualized using a nucleic acid stain. Many variations of PCR have been developed for various applications in molecular biology. These include multiplex PCR (mPCR), quantitative PCR (qPCR), real-time PCR, loop-mediated isothermal amplification (LAMP) (**Notomi *et al.*, 2000; Law *et al.*, 2015; Matle *et al.*, 2020; Wang *et al.*, 2020; Song *et al.*, 2023; Cheng *et al.*, 2024**). Despite of several advantages of these molecular methods, they usually suffer from low sensitivity when the pathogen is detected from complex food matrices and they cannot distinguish between live and dead bacteria. Hence to multiply target species, enrichment of food sample is often needed to dilute the PCR inhibitors. Classical PCR method has been used as a principal identification method for detection of *Lm* by targeting various genes such as 16S rRNA sequence and pathogenic markers (**Jadhav *et al.*, 2012; Law *et al.*, 2015**).

Multiplex PCR (mPCR)

Simultaneous amplification of two or more target genes in a single reaction tube with the same PCR conditions can be achieved by mPCR assay. Though this method is a variant of conventional PCR, certain key features like primers' design, primer concentration and fixing similar annealing temperature and template quantity are crucial for reliable amplification. **Cooray *et al.* (1994)** developed a mPCR targeting three virulence-associated genes (*prfA*, *hlyA*, and *plcB*) with specific primers for successful identification of pathogen in milk. **Li *et al.* (2021)** developed an mPCR for rapid identification of *Lm*, *L. ivanovii*, and other non-pathogenic *Listeria* in fresh mushroom *(Flammulina velutipes*) by targeting LMxysn_1095 and *lmo1083* genes involved in glycosylation modification. **Kumar *et al.* (2015)** reported a *Lm* specific mPCR format by targeting 16S rRNA and virulence associated genes (*iap*, *hly* and *prf)*. **Doumith *et al.* (2004)** developed an mPCR assay for differentiation of major *Lm* serovars.

Real-time PCR

Real-time PCR has proven to be highly reliable for the detection of clinical, environmental, food pathogens, and in gene expression studies (**Deer and Lampel 2010; Köppel *et al.*, 2021**). By this method one can test whether the target DNA is present or absent in a test sample (qualitative) and/or quantify the number of gene copies (quantitative) (qPCR). Addition of DNA binding fluorescent dyes or dual-labelled probes in the real-time PCR mix allows increased fluorescence during amplification of target sequence in a specialized thermocycler. The procedure allows monitoring the progress of amplification in real-time by measuring the fluorescence generated by dual-labeled probes or dyes (**Mackay and Landt 2007**). Hence the fluorescence intensity is directly proportional to the amount of DNA accumulated and is monitored on screen without the use of time-consuming gel electrophoresis. SYBR® Green is one of the most widely used double stranded DNA binding fluorescent dyes, which exhibits a strong fluorescent signal and able to detect very small amount of target DNA. But it has certain limitations as it inhibits the PCR reaction and shows low reproducibility (**Buh Gasparic *et al.*, 2010**). An advancement to SYBR® Green approach is the

TaqMan® probe chemistry. The probe is a short single chain oligonucleotide that contains a 5′ reporter dye and 3′ quencher. In qPCR, the annealing temperature of the TaqMan® probe should be higher than the annealing temperature of primers to hybridize the template DNA during polymerization with the help of *Taq* DNA polymerase (**Patel *et al.*, 2006**).

A real-time PCR was designed according to the EN UNI ISO 16140-3:2021 for specific detection and characterization of *Listeria* spp. and *Lm* contamination. This rapid approach overcomes the limitations of culture-based techniques, meets all the criteria as per ISO guidelines and offers a powerful approach to the real-time assessment of food safety, useful for industry self-monitoring and regulatory inspection (**Bolzon *et al.*, 2024**). Real-time PCR based detection of *Listeria* spp. and *Lm* in food and feed has been reported by several authors (**Heo *et al.*, 2014; Köppel *et al.*, 2021; Azinheiro *et al.*, 2023; Félix *et al.*, 2023**). A good number of commercial real-time PCR based kits for *Lm* detection are available in market with varied specificities and LOD (**Välimaa *et al.*, 2015; Osek *et al.*, 2022**). An excellent review on rapid detection methods for foodborne pathogen based on nucleic acid amplification including digital PCR was published by **Ndraha *et al.* (2023).**

Droplet digital PCR (ddPCR)

Droplet digital PCR (ddPCR) is another variant of conventional PCR, by which accurate quantification can be achieved without the need of higher quantity of target DNA (**Hindson *et al.*, 2013**). The ddPCR also showed its superiority over qPCR as the latter one is frequently inhibited by different substances present in sample matrices, leading to a reduced sensitivity (**Costa *et al.*, 2022**). This method utilizes a water-oil emulsion droplet system. Droplets are formed in a water-oil emulsion to form the partitions that separate the template DNA molecules. These droplets act as the same function as individual test tubes or wells in a plate in which the PCR reaction takes place. This method has been used for identification of number of food and waterborne microorganisms, soil bacteria, and genetically modified organisms (**Cooley *et al.*, 2018**). The ddPCR has been used to assess the number of *Lm* in biofilm production by targeting *hlyA* gene (**Klančnik *et al.*, 2015**). **Ricchi and co-workers (2017)** compared qPCR, ddPCR and culture methods for the quantification of *Lm* and it was found that ddPCR might be a valid alternative to the other two methods. **Grudlewska-Buda *et al.* (2020)** evaluated the results of ddPCR and culture method of the study conducted to recover *Lm* cells from the biofilms on steel and polypropylene using *hlyA* gene.

Isothermal amplification

Isothermal amplification is a process in which nucleic acid sequences are rapidly and efficiently accumulated at a fixed temperature without the requirement of thermocycling. Loop mediated isothermal amplification (LAMP) is one of the isothermal amplification methods that has largely been employed for identification of *Lm* from food samples with good specificity and LOD (**Tirloni *et al.*, 2017; Nathaniel *et al.*, 2019; Ledlod *et al.*, 2020; Fiore *et al.*, 2023**). LAMP uses 4-6 primers recognizing 6-8 distinct regions of target DNA. A strand-displacing DNA polymerase initiates synthesis and two specially designed primers form "loop" structures to facilitate subsequent rounds of amplification through extension on the loops and additional annealing of primers. The advantages and limitations of LAMP are shown in Table 2. **Fiore *et al.* (2023)** reported a simple and rapid colorimetric LAMP assay for *Lm* detection in RTE meat samples. A sensitive and specific duplex lateral flow dipstick test combined with LAMP assay was reported for the identification of *Lm* in meat products (**Ledlod *et al.*, 2020**). A novel visual assay for ultrasensitive detection of *Lm* in milk and chicken was developed using helix loop-mediated isothermal amplification (HAMP) (**Prasad *et al.*, 2024**). The authors tested the HAMP system in artificially inoculated milk with and without pre-enrichment with an LOD of 12 CFU/ml (3 hr) and 1.2 CFU/ml (6 hr) was found, whereas in chicken an LOD of 150 CFU/g (3 hr) and 15 CFU/g (6 hr) was observed.

CRISPR/Cas-based detection

One of the advanced methods that has gained great importance in nucleic acid detection is clustered regularly interspaced short palindromic repeats (CRISPR) associated systems (CRISPR/Cas). To achieve higher detection sensitivity, the CRISPR/Cas system is frequently associated with PCR and isothermal nucleic acid amplification techniques. This system is a unique adaptive immune system that functions by nucleic acid recognition guided by simple CRISPR RNA (crRNA) (**Chakraborty *et al.*, 2022**). The shearing activity of the Cas protein is triggered when the crRNA binds to a complementary DNA/RNA target. Cas nucleases such as Cas9, Cas12 and Cas13 have been reported to be used for the nucleic acid detection of *Listeria*, *Salmonella*, *S. aureus*, and other pathogens (**Chakraborty *et al.*, 2022**). Detection of *Lm* based on CRISPR/Cas9-triggered isothermal exponential amplification reaction (CAS-EXPAR) was developed using *hly* gene (**Huang *et al.*, 2018**). It utilizes the target-specific nicking activity of Cas9 and nicking endonuclease (NEase)-mediated amplification. This method combines the benefits of Cas9/sgRNA site-specific cleavage and EXPAR fast amplification kinetics. This method does not require exogenous primers for amplification and chances of nonspecific amplification is minimal with a high detection sensitivity of 0.82 amol of purified ssDNA. In another experiment, CRISPR/Cas9 system integrated with lateral flow nucleic acid (CASLFA) was reported to detect *Lm* using *hly* gene as target was developed by **Wang *et al.* (2020)**. The detection limit was found to be as low as 150 copies and the authors reported that the method is low cost, user friendly and can be completed within 40 min.

DNA microarrays

Gene or DNA microarray technology is based on the ability to deposit numerous (tens of thousands) different DNA sequences on a small surface, usually a glass slide, often referred to as a "chip." Microarray has two broad classifications viz., gene expression microarray and tissue microarray (TMA) based on its mode of preparation and on the types of probes used. Microarrays have been used for direct and indirect pathogen identification. In the latter case, pathogen-specific host gene expression signatures are selected as surrogate markers for detection or diagnostic purposes (**Palmer *et al.*, 2006**). The principle, types, advantages, limitations, and future prospects of microarray technology has excellently been reviewed by **Bumgarner (2013).**

Several researchers developed and validated the microarrays for successful identification of *Lm* from various clinical and food samples (**Laksanalamai *et al.*, 2012; Sarengaowa *et al.*, 2020). Bang *et al.* (2013**) developed a DNA microarray for the detection of *Lm* in milk with an LOD of 8 log CFU/ ml. Recently, an *in-situ* synthesized gene chip was developed for detection of five important foodborne pathogens in lettuce and fresh cantaloupe (**Sarengaowa *et al.*, 2020**).

Table 2 Principle, advantages, and limitations of methods used for identification of *L. monocytogenes*

Method	Principle	Advantages	Limitations	Detection limit*	References
Culture-based methods • Pre-enrichment • Enrichment • Chromogenic agar • Biochemical tests	Use of culture media including enrichment and selective media for cultivation of *L. monocytogenes*	• Reliable and cost effective • Used to isolate viable cells • Used for isolation of specific pathogen • Approved by international regulatory authorities	• False- positive or false- negative results • Slow growth of injured or stressed cells • Labour intensive and time consuming • Depend on the nutritional components and environmental conditions	<1 CFU/25 g	**Ottaviani *et al.*, 1997; Gasanov *et al.*, 2005; Hegde *et al.*, 2007; Dwivedi and Jaykus, 2011**
Alternative methods • Immunological assays • ELISA • LFD	The affinity between microbes or their antigens and antibodies (monoclonal or polyclonal) is exploited for the detection purposes	• Easy to perform and the process can be automated • Sensitive and reproducible • Large number of samples can be assayed • Commercial kits available • Toxins can be detected	• Pre-enrichment step is needed to increase the viable cell number • Cross reactivity with other closely related antigens • Specificity and sensitivity are based on the quality and affinity of antibodies	10 CFU/g to 10^2 CFU/g	**Capo *et al.*, 2020; Xiao *et al.*, 2021; Lopes-Luz *et al.*, 2023**
Biosensors • Electrochemical • Optical	To convert a biologically induced recognition event (e.g., enzyme,	• Rapid and reliable detection	• Highly expensive	10^2 CFU/25 g to <10 CFU/g	**Soni *et al.*, 2018; Fernández**

• Cell based	antibody) into a detectable signal, via a transducer and processor	• Sensitive and reproducible • Portable and easy to handle • Field based detection	• Results depend on type of sample and its purity • All the results should be validated with the microbiological methods		**Blanco *et al.*, 2023; Zhang *et al.*, 2022**
Molecular methods • Conventional PCR • Multiplex PCR	Invitro amplification of nucleic acid target using DNA polymerase in the presence of a template	• Reliable and widely used • Sensitive and specific • Can be automated • Multiple organisms can be identified simultaneously	• Can not differentiate viable and non-viable cells • Sensitivity depends on the purity of DNA template • Reaction can be inhibited by contaminants	1 CFU/g to 10 CFU/25 g	**Jadhav *et al.*, 2012; Law *et al.*, 2015; Osek *et al.*, 2022; Cheng *et al.*, 2024**
• Real-time PCR • Quantitative PCR	Measurement of accumulated amplification product in real time as the reaction progresses and product quantification after each cycle	• Detection of multiple organisms • More rapid than conventional PCR • Highly sensitive and specific • No need of agarose gel electrophoresis • DNA/organisms can be quantified	• Very sensitive to contaminants present in food • High cost • Needs skilled technicians • Results should be validated against conventional methods	1 CFU/25 g	**Heo *et al.*, 2014; Köppel *et al.*, 2021; Azinheiro *et al.*, 2023; Félix *et al.*, 2023; Bolzon *et al.*, 2024**
Aptamers	Single stranded oligonucleotides with their three-dimensional structure can specifically bind to corresponding target molecules	• Generation by SELEX process is easy • Less manufacturing time and cost • Thermostable	• Quick degradation in biological media • Interaction with incorrect target • Susceptible to nuclease degradation	10 CFU/ml to 10^3 CFU/ml	**Bruno and Silvis, 2017; Feng *et al.*, 2018; Guo *et al.*, 2020**
Isothermal amplification (LAMP)	Auto cycling and DNA strand displacement activity mediated by *Bst* polymerase under isothermal conditions	• Rapid than PCR • Simple to perform • Thermal cycling not required • Cost effective • Higher sensitivity than PCR	• High chances of false positive and false-negative results • Complicated primer design • Results should be validated against conventional methods	<10 CFU/25 g	**Notomi *et al.*, 2000; Fiore *et al.*, 2023; Prasad *et al.*, 2024**
Microarrays (Gene chip)	Each DNA strand in a complex sample has the capacity to recognize and hybridize to its complementary sequence immobilized onto a specific region of the DNA microarray	• High throughput analysis • Rapid • Sensitive and specific	• High cost • Needs skilled technicians • Difficult to distinguish between the viable and non-viable cells	$<10^3$ CFU/g	**Law *et al.*, 2015; Sarengaowa *et al.*, 2020**
Microfluidics	It is the science of manipulating and controlling fluids, usually in the range of microliters (10^{-6}) to picolitres (10^{-12}), in networks of channels with dimensions from tens to hundreds of micrometers	• Require very less sample and reagents • Multiple analytes can be processed at a time • Reduced turnaround time, low cost	• More complex fabrication process • Adsorption issues • Limited chemical compatibility • Poor mechanical strength, unsuitable for upscaling	$<10^2$ CFU/ml or 6.3×10^{-2} pmol/L	**Jokerst *et al.*, 2012; Wang *et al.*, 2023; Jiang *et al.*, 2024**
CRISPR/Cas-based detection	Systems that can be programmed to target specific stretches of genetic code and to edit DNA at precise locations, as well as for other purposes, such as for new diagnostic tools.	• Gene editing • Exceptional sensitivity • Pathogen detection • Low cost • Ease of use • Point of care	• Can cause unintended genetic changes • Induction of chromosomal alterations • Regulatory challenges • Ethical issues	Femtomolar range	**Chakraborty *et al.*, 2022; Huang *et al.*, 2018; Wang *et al.*, 2020**

*Detection limit is based on enrichment time (2 hr to 24 hr). For culture methods the time taken is < 7 days.

BACTERIAL TYPING METHODS

Bacterial typing methods (also known as 'finger printing') provide tools to track the sources of contamination in foods and to trace out listeriosis outbreaks. These typing methods can also be used to preliminary understand the epidemiology and genetics of *Lm* (**Moura *et al.*, 2016**). These methods are available in two major categories viz., phenotypic, and genotypic. The phenotypic methods include serotyping and phage typing. The genetic subtyping approaches include PCR-based subtyping methods [random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), PCR-restriction fragment length polymorphism (PCR-RFLP) and repetitive element PCR (REP-PCR)] and DNA sequencing-based subtyping techniques [e.g. multilocus sequence typing (MLST)], pulsed-field gel electrophoresis (PFGE) and ribotyping]. Combination of phenotyping and genotyping techniques is recommended for more specific epidemiologic investigation of *Lm* outbreaks. The advantages and dis-advantages of typing methods are described in table 3.

Serotyping by antisera

Serotyping is generally the first choice for investigators to characterize *Lm* isolates during epidemiological surveillance. It is based on an agglutination reaction of somatic [O] or flagellar [H] antigens of a particular organism with mono or polyvalent antisera. At present 13 serotypes have been described for *Lm* (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4ab, 4c, 4d, 4e, and 7). Out of these serotypes, 4b, 1/2b, and 1/2a are responsible for listeriosis infections in humans (**Swaminathan and Gerner-Smidt 2007**). The application of serotyping method is very limited in epidemiological investigations as it provides poor discriminative capability of isolates involved in outbreak situations and shows inconsistent results (Gasanov et al. 2005). The availability of high-quality sera is also one of the drawbacks of this method. A commercially available serotyping kit by Denka Seiken Co., Tokyo, Japan is being used frequently to serotype *Listeria* isolates. Strain, serotype, and virulence profiles of *Lm* was reported by **Muchaamba *et al.* (2022).**

Molecular serotyping

With the limitations associated with conventional serotyping, molecular serotyping is extensively used for *Lm* typing (**Kérouanton *et al.*, 2010, Matle *et al.*, 2020**). Basically, these methods are dependent on PCR /multiplex PCR with specific primers of virulence genes. At present, the isolates of *Lm* cluster into at least four lineages (I, II, III, and IV), divided into thirteen serotypes. Isolates of serotypes 1/2b and 4b, belong to lineage I, are predominantly associated with human listeriosis and possess the genes encoding *Listeria* pathogenicity island (**Cotter *et***

al., 2008). Lineage II isolates fall into serotype 1/2a, are found in the environment, and frequently cause outbreaks of listeriosis. These isolates often harbour plasmids and provide resistance to heavy metals. The isolates of lineages III and IV are rarely isolated.

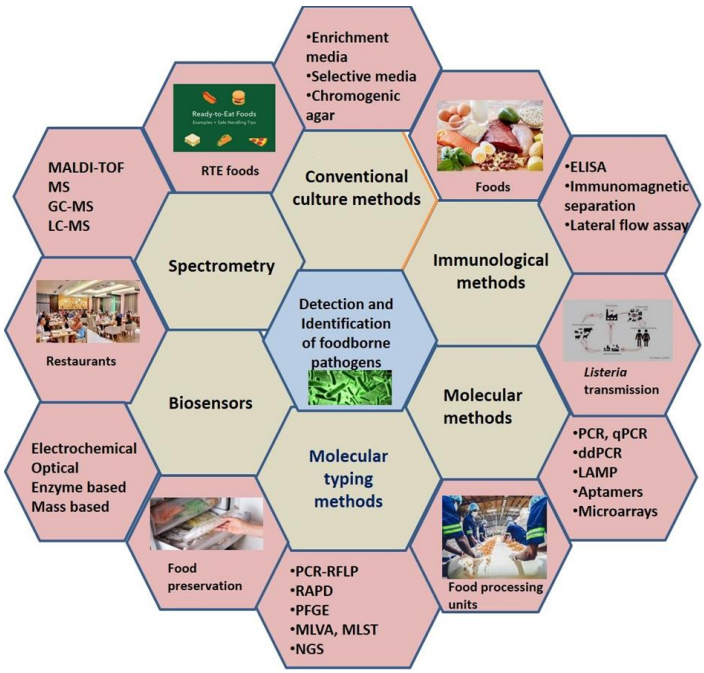

Figure 2 Summary of various methods for detection and identification of foodborne pathogenic bacteria

Molecular subtyping

Due to the occurrence of diverse strains of *Lm*, several typing approaches are in use for differentiation of various strains, tracking the source of the contamination and to investigate the disease outbreaks (**Law *et al.*, 2015; Matle *et al.*, 2020**). Various sub-typing methods that can be used for characterization of strains of *Lm* are briefly summarized below.

RAPD: This technique is based on simple PCR to detect DNA polymorphisms (**Penner *et al.* 1993**). It utilizes short primers with random sequences of 8–15 nucleotides in length. The amplicons thus obtained are separated by agarose gel electrophoresis. Generally, a single colony, a cell lysate or purified DNA can be used as the PCR template. RAPD is more cost effective, easy to perform, requires very small quantity of temple DNA and quicker than other typing methods. RAPD-PCR technique has been used to determine the effectiveness in typing *Lm* (**Zeinali *et al.*, 2017; Yoshida *et al.*, 1999**).

RFLP: This typing method is used to recognize specific variations in the bacterial DNA sequence. In this method, the DNA is cleaved with restriction endonucleases to generate short fragments and later visualized by agarose gel electrophoresis. After separation, DNA fragments are transferred to nitrocellulose or nylon membranes through southern blotting, followed by hybridization with one or more labelled DNA probes and visualized with the help of a photographic film. This method has been reported for identification of *Listeria* spp from food and environmental sources (**Rip and Gouws 2020; Osek *et al.*, 2022**).

Ribotyping (rRNA gene restriction pattern analysis): It is a variant of RFLP method useful for subtyping strains of *Lm* particularly in outbreak situations. This method is based on the restriction endonuclease digestion of genomic DNA with restriction enzymes to generate DNA fragments, followed by a Southern blot hybridization (**Bouchet et al., 2008**). This method has been used to investigate *Lm* strains in foods and food processing plants (**Vongkamjan *et al.*, 2013; Matloob and Griffiths, 2014**). The DuPont Qualicon RiboPrinter® automated microbial characterization system that provides speed, accuracy, and good resolution in few hours and can be used to characterize clinical pathogens, spoilage organisms and pathogens.

PFGE: It is a powerful and gold standard genotyping technique that discriminates bacterial strains for generating specific DNA pattern after digestion with a restriction enzyme. The digested products are then analyzed by agarose gel by using alternating electric fields. The DNA pattern thus obtained on agarose gel is referred to as 'DNA fingerprint' or 'PFGE pattern' (**Lopez-Canovas *et al.*, 2019**). *ApaI*, *AscI*, and *SmaI* are most frequently used restriction enzymes in PFGE (**Aarnisalo *et al*. 2003**). PFGE has been used in epidemiological and outbreak investigations for subtyping large number of bacterial species, including *Lm* (**Lopez-Canovas *et al.*, 2019; Hunt and Jordan 2021**). Recent developments of PFGE technique and common PFGE workflows are excellently reviewed by **Neoh *et al.* (2019).**

MLVA: Multiple-locus variable-number tandem-repeat analysis (MLVA) has emerged as a highly discriminatory molecular typing method, which is based on repetitive DNA elements organised in tandem, which is called variable number of tandem repeats (VNTR analysis). VNTRs are short segments of DNA that have hypervariable copy numbers within the genome. The tandem repeats are in stable regions of the genome and usually not to be associated with mobile genetic elements, such as plasmids (**Lunestad *et al.*, 2013**). Several web-based platforms with databases are available to compare MLVA profiles of various strains. This method has been used for typing *Lm* isolates from different sources (**Martín *et al.*, 2018; Andrews *et al.*, 2023; Manqele *et al.*, 2023**).

MLST: It is a molecular typing approach that refers to systematic sequencing of six or seven conserved house-keeping genes or loci of bacterial genome. For each house-keeping gene, the different sequences present within a bacterial species are assigned as distinct alleles and, for each isolate, the alleles at each of the seven loci define the allelic profile or sequence type (ST). Each isolate of a species is therefore unambiguously characterized by a series of seven integers which correspond to the alleles at the seven house-keeping loci. For *Lm* typing by MLST, the various genes are in use viz., *abcZ* (ABC transporter), *bglA* (beta-glucosidase), *dat*dat (D-amino acid aminotransferase), *dapE* (succinyl diaminopimelate desuccinylase), *cat* (catalase), *ldh* (lactate dehydrogenase), and *lhkA* (histidine kinase) (**Kurpas *et al.*, 2020**). **Knudsen *et al.* (2017)** reported genome wide analyses of *Lm* clonal diversity from food processing plants. **Wei and coworkers (2024)** conducted whole-genome sequencing of various isolates *Lm* and *L. innocua* obtained from different levels of the dairy supply chains across different regions in Ethiopia.

Next-generation sequencing (NGS): NGS is a technology for determining the sequence of a nucleic acid to study genetic variation associated with diseases. Traditional Sanger sequencing is a gold standard for analyzing gene targets of short length in a single working day, whereas NGS enables to find different genomic features and can analyze thousands of genes in multiple samples in a single sequencing run. The accuracy and speed of NGS has revolutionized in the field of genetic analyses and in vast areas of research such as clinical, food, environmental, agricultural, reproductive, and forensic science (**Levy and Myers 2016; Vincent *et al.*, 2017**). Recently, **Lakicevic *et al.* (2023)** have excellently reviewed on whole genome sequencing for control of *Lm* in food chain. **Yu *et al.* (2023)** reported the NGS for diagnosis of *Lm* causing meningoencephalitis in patients. Similarly, NGS has been reported by several researchers for sequencing of *Lm* isolates recovered from food and clinical samples (**Drali *et al.*, 2019; Unrath *et al.*, 2021; Zhang *et al.*, 2021**).

Table 3 Principle, advantages, and limitations of typing methods for discrimination of isolates of *L. monocytogenes*

Typing method	Principle	Advantages	Limitations	References
Serological typing • O and H antigen • Polyclonal antibodies • Monoclonal antibodies	Antigen-antibody reaction when a microbial culture is mixed with a specific antiserum directed against the cell surface components	• Antisera available commercially • Easy to perform	• Generation of antisera is expensive • Cross-reactivity • Low discriminatory power • Laborious and time-consuming	**Swaminathan and Gerner-Smidt, 2007; Jadhav *et al.*, 2012; Muchaamba *et al.*, 2022**
Molecular serotyping: • PCR • Multiplex PCR	Depends on the size difference between PCR products after amplification of the crude DNA template by serotype-specific primers	• Rapid • Easy to perform • Low-cost	• Unable to distinguish certain serotypes from each other	**Cotter *et al.*, 2008; Kérouanton *et al.*, 2010; Matle *et al.*, 2020**

Amplification-based typing: • PCR-RFLP	Restriction enzymatic digestion of amplified DNA to compare band patterns for identification and speciation	• Easy to perform • Low-cost	• Low discrimination conditions • Sensitive • Difficult to interpretation	**Rip and Gouws, 2020; Osek *et al.*, 2022**
• RAPD	Amplification of unnamed regions of the genome with short primers	• Need small amounts of DNA • Low cost, easy to perform • No need for previous information about the genome	• Difficult to achieve reproducibility	**Yoshida *et al.*, 1999; Zeinali *et al.*, 2017**
DNA-Restriction based typing • PFGE	Based on total DNA restriction patterns. The results are analyzed by comparison of the bands' patterns of each sample	• Standard protocols available • Ability to separate large DNA fragments (>100 kb) • High resolution and discriminatory power	• Requires trained manpower • Time-consuming • High equipment cost	**Lopez-Canovas *et al.*, 2019; Hunt and Jordan, 2021**
Sequence based typing: • MLVA	Genetic analysis of a particular microorganism detects the copy numbers of repetitive DNA sequences throughout the genome	• Simple and cost-effective • Highly discriminative • Results can be stored • Useful in phylogenetic analysis	• Require skilled technician • Not practical for routine subtyping	**Martín *et al.*, 2018; Andrews *et al.*, 2023; Manqele *et al.*, 2023**
• MLST	Based on PCR and sequencing of fragments within several housekeeping genes in the entire bacterial chromosome to study genetic diversity	• Preferred for epidemiological studies • Web-based analysis platforms available	• High price • Prolonged • Low discrimination	**Knudsen *et al.*, 2017; Kurpas *et al.*, 2020; Wei *et al.*, 2024**
• NGS	Parallel sequencing of multiple small fragments of DNA to determine sequence. Whole genome or markers targeted and sequenced	• Enables multi pathogen detection • High-throughput • Enables analysis of whole genome	• Skilled personnel are needed • Cost-related • Time-consuming • Bioinformatics are required for data analysis	**Drali *et al.*, 2019; Unrath *et al.*, 2021; Zhang *et al.*, 2021; Yu *et al.*, 2023**

CONCLUSION

Contaminated food in general contains low levels of spoilage and/or pathogenic microorganisms and hence the selective enrichment of target pathogen is paramount for rapid detection. Rapid identification methods have already been existing to target *Lm* that can be concluded in 48 hrs. But any method which is simple, low-cost, and can precisely identify the target pathogen in food commodities in a single working day with or without use of pre-enrichment is the need of the hour and this can help to report to the concerned food agencies for discard of contaminated or suspicious foods. All the reference methods are mainly based on isolation of target bacteria using suitable culture media. Many of the developed alternative methods are rapid and sensitive but the results must be in concurrence with standard microbiological tests. Most of the quick tests (PCR, real-time PCR, ELISA, LFA) are usually preferred by consumers and food business operators as these methods are cost effective and high sample throughput for assessing product quality.

Currently, the nucleic acid-based approaches are most widely used for laboratory identification of *Lm* because they are most sensitive and reliable in detection from various food matrices. Combination of two or more existing detection approaches is an ideal choice for accurate detection of target pathogen. Such methods should always be simple, specific, reproducible, fast, cost-effective, and user friendly. Several reliable and robust molecular subtyping methods are useful to differentiate the causative agents at strain level and help during epidemiological investigations of human listeriosis outbreaks.

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Data Availability: The data that support the findings of this study are available from the first author upon reasonable request.

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