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Listeria monocytogenes: challenges of microbiological control of food in Brazil

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Abstract

Listeria monocytogenes is a gram-positive bacterium that can survive in food production environments and food products. This microorganism is associated with listeriosis, a serious infection caused by the consumption of contaminated food. The aim of this study was to conduct an integrative literature review on the regulation of *L. monocytogenes* in food in Brazil, its occurrence and methodologies used for identification in studies published from 2001 to 2021, and the epidemiological surveillance of cases of infection. The current regulations for *L. monocytogenes* prohibit ready-to-eat foods of more than 10^2 per gram or milliliter. Officially, only two cases of outbreaks have been identified in the Epidemiological Surveillance of Foodborne Diseases system; however, the circulation of *L. monocytogenes* was observed in foods of different origins, with occurrence ranging from 3.1 to 48.7. The most commonly used identification method was ISO 11290. The isolation and identification methods for this pathogen are expensive and laborious, making it difficult to implement these methodologies in many laboratories. This scenario contributes to the underreporting of cases of listeriosis and therefore represents a risk for the population that is exposed to potentially contaminated food.

Keywords: food safety; sanitary surveillance; foodborne diseases; laboratory methods.

Practical Application: This review provides the epidemiology and laboratory methods for the detection of *L. monocytogenes*.

1 Introduction

The genus *Listeria* consists of non-spore-forming gram-positive bacilli and currently has 28 distinct species (Parte et al., 2020). Among these species, *Listeria monocytogenes* and *Listeria ivanovii* have been associated with diseases in humans and animals, the latter being related to infections in ruminants. *L. monocytogenes* is the most important species in this group because of its clinical relevance (Rocha et al., 2019). *Listeria monocytogenes* is widely distributed in the environment and is found in water, soil, sewage, poultry meat, slaughterhouse waste, and facilities and equipment in food-producing industries. It has the characteristic of growing between 0 °C and 45 °C, has the ability to form biofilm on various surfaces, proliferates in a wide pH range (4.3 to 9.6), and is considered psychotropic, having the ability to multiply at temperatures below 7 °C. As it is psychotropic, the refrigeration used for food preservation does not prevent pathogen multiplication, making ready-to-eat (RTE) foods a common source of contamination (Zunabovic et al., 2011; Forsythe, 2013; Silva, 2019; Dygico et al., 2020). Because of their ability to proliferate under adverse conditions, these microorganisms are difficult to remove from the food production environment and colonize a wide variety of foods, such as dairy products, vegetables, poultry, beef products, and RTE foods (Ruiz-Bolivar et al., 2011; Zavareh & Ardestani, 2020). Its presence is considered a risk because this species is related to listeriosis, a clinical syndrome associated with the consumption of contaminated food (Wing & Gregory, 2002; Orsi & Wiedmann, 2016).

Listeriosis is a serious infection that affects the elderly, pregnant women, newborns, and immunocompromised adults (Maia et al., 2019b). When ingested, *L. monocytogenes* can cross the intestinal mucosal barrier into the bloodstream and colonize target organs such as the liver and spleen. In addition, it can cross the blood-brain and fetoplacental barrier, resulting in severe neurological problems and miscarriage in pregnant women (Vasconcelos et al., 2008; Buchanan et al., 2017; Quereda et al., 2021). Schwab & Edelweiss (2003) identified *L. monocytogenes* in 33.8% of placentas from premature births or abortions in Brazil, exposing the relationship of this pathogen with alterations during pregnancy. Vasconcelos et al. (2008) detected *L. monocytogenes* in the cerebrospinal fluid of a premature newborn, and the newborn's mother presented with fever after consuming gorgonzola cheese.

Gastroenteritis, fever, abdominal cramps, nausea, diarrhea, and vomiting are clinical symptoms present as non-invasive manifestations of the disease (Garrido et al., 2010; Halbedel et al., 2019). Although it is considered rare compared to other foodborne diseases, listeriosis, in its most severe form, has a mortality rate of 20%-30% (Li et al., 2020). Besides being considered a serious public health problem for the food industry, the presence of *L. monocytogenes* can lead to financial loss, since there are costs

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associated with the collection and control of the pathogen in the food production environment. (Jordan & McAuliffe, 2018).

Most outbreaks of listeriosis are associated with RTE foods, which typically contain animal sources, such as meat, eggs, fish, and dairy products. In these cases, the risk is increased by the absence of cooking or other antimicrobial interventions between the end of food production and consumer ingestion. (Jordan & McAuliffe, 2018; Gray et al., 2021). The insertion of *L. monocytogenes* into the food processing area can be caused by contaminated raw materials or by employees and pests that are colonized by bacteria (Gray et al., 2021).

In the United States of America (USA), about 3,000 people die each year from eating contaminated food, with 260 deaths directly associated with listeriosis (Centers for Disease Control and Prevention, 2015). In 2018, the largest outbreak of listeriosis in the world was reported in South Africa, where the consumption of contaminated RTE processed meat led to 937 cases and 216 deaths (Thomas et al., 2020). In view of the problem of foodborne listeriosis, this study aimed to perform an integrative literature review on the regulation of *L. monocytogenes* in foods in Brazil, its occurrence, epidemiological surveillance, and the main compendial methodologies used for the identification of this pathogen.

2 Materials and methods

An integrative literature review was conducted through direct searches of the websites of national and international health agencies and official institutions. Direct consultations with experts in the field were carried out to ensure the consistency and reliability of the findings.

As secondary sources (academic and grey literature search), the databases of PubMed, Cochrane Library, Scopus, Web of Science, Scientific Electronic Library Online (SciELO), Latin American and Caribbean Literature on Health Sciences (LILACS), Capes, and Google Scholar were consulted. Both the gray literature search and academic literature review were conducted using the same search criteria and documents from 2001 to 2021. The search for papers was conducted considering descriptors in both Portuguese and English: "Listeria," "*Listeria monocytogenes*," "foods," "isolation," "Brazil," "methodology," "method," and "identification". Papers were searched and titles and abstracts were evaluated, followed by full reading. Publications that did not respect the delimitation of the theme and the purpose of the study were excluded.

3 Results and discussion

3.1 Regulation concerning Listeria monocytogenes in food in Brazil

In Brazil, until December 2020, Collegiate Board Resolution (RDC) No. 12 of January 2, 2001 established the investigation of *L. monocytogenes* only in medium- and high-humidity cheeses (Brasil, 2001). RDC No. 12/2001 has been repealed, and the new Brazilian regulation on microbiological standards in food has been published as RDC No. 331/2019 and Normative Instruction (IN) No. 60/2019. These legislations establish that foods characterized as RTE foods must comply with microbiological standards for *L. monocytogenes* throughout their shelf life, not exceeding 10^2 mL per g or mL. Foods exempt from this determination are as follows: those with a shelf life of less than 5 days; pH ≤ 4.4; water activity ≤ 0.92; pH ≤ 5.0,; water activity ≤ 0.94; heat-treated; fresh fruits and vegetables; breads; beverages such as water and beer; sugars; honey; chocolate; candies; candy; chewing gum; and live bivalve mollusks. The absence of microorganisms at 25 g or mL is recommended for RTE foods intended for infants or for special purposes (Brasil, 2019a, 2019b, 2020b).

3.2 Occurrence of Listeria monocytogenes in food and epidemiological surveillance of outbreaks and sporadic cases of infection in Brazil

On April 8, 2009, IN No. 9, the Department of Agriculture, Livestock, and Food Supply (MAPA) instituted the control of *L. monocytogenes* in ready-to-consume animal products. According to the most recent data, the occurrence of this bacterium was 0.96% (10/1035) in samples of meat and dairy products in 2019 (Brasil, 2009; Ministério da Agricultura, Pecuária e Abastecimento, 2020). According to the database of the Notifiable Diseases Information System (Sinan) of the Ministry of Health, between 2000 and 2019, only two cases of food outbreaks associated with *Listeria* spp. Were reported in Brazil. In 2007, two people became ill after being exposed to contaminated pork, and in 2009, 17 people were affected by an outbreak caused by the ingestion of egg-based products, both reported in Rio Grande do Sul (Ministério da Saúde, 2022). Despite the low number of cases of foodborne diseases caused by this microorganism, recall and prohibition of the distribution and commercialization of food products contaminated by *L. monocytogenes*, such as Brazilian sausage (Brasil, 2018a), mozzarella (Brasil, 2018b), and curd and prato cheese (Brasil, 2017) have already been determined by the Brazilian Health Regulatory Agency (Anvisa).

After the integrative review, 17 publications reporting the presence of *L. monocytogenes* in foods samples were identified (Table 1). A total of 2,320 samples collected from 2011-2021 were analyzed, and the occurrence of *L. monocytogenes* ranged from 1.2 to 48.7%, with a mean and median of 14.1% and 11.7%, respectively. The samples were collected from at least eight different states, with the majority from São Paulo (n = 1,168; 50.3%) and Rio Grande do Sul (n = 515; 22.0%). The main foods categories were meat (n = 1,135; 48.9%) followed by vegetable products (n = 847; 36.5%). MAPA has a program for the control of *L. monocytogenes* in RTE products of animal origin, with rules to be followed by companies in addition to establishing inspections (Brasil, 2009). Furthermore, these food categories are widely studied due to the international food trade. Importing countries, such the United States of America (USA), stipulate the absence of this pathogen in RTE foods. In addition, the presence of *L. monocytogenes* causes serious financial impacts and even the ban on entry of these products in the countries (Neri et al., 2019; Olanya et al., 2019). Minor et al. (2015) estimated the annual costs per case and totals of foodborne illness in the USA and *L. monocytogenes* was the fourth most expensive on average,

Table 1. Occurrence of *Listeria monocytogenes* in products commercialized in Brazil in the last 10 years (2011-2021).

Food products	No. of samples (% of total)	State/Region	Method used	References
Baked ham	17 (42.5)	Ceará		Fai et al., 2011
Ready-to-eat vegetables	16 (3.1)	São Paulo	ISO 11290	Sant'Ana et al., 2012
Fresh lettuce	2 (5.7)	Rio de Janeiro	BAM/FDA[b]	Brandão et al., 2013
Raw salads	5 (16.7)	Rio de Janeiro	BAM/FDA	Brandão et al., 2013
Meat products	269 (48.7)	São Paulo	ISO 11290	Ristori et al., 2014
Cheeses	1 (2.5)	Rio Grande do Sul	ISO 11290	Marinheiro et al., 2015
Cheese	4 (11.7)	Goiás	BAM/FDA	Lima et al., 2015
Raw, frozen and ready-to-eat vegetables	4 (3.0)	Bahia	ISO 11290	Byrne et al., 2016
Beef carcasses in slaughterhouses	12 (24.0)	Rio Grande do Sul	ISO 11290	Iglesias et al., 2017
Sausages	8 (8.2)	States of the Midwest, Southeast and South	ISO 11290 and VIDAS®	Rodrigues et al., 2018
Frozen and ready-to-eat vegetables	4 (3.0)	Bahia	ISO 11290	Oliveira et al., 2019
Cheese and ham	15 (9.4)	Rio Grande do Sul	ISO 11290	Maia et al., 2019a
Beef	6 (12.0)	Mato Grosso	ISO 11290	Teixeira et al., 2020
Chilled raw meat and fresh sausage	33 (13.9)	Rio Grande do Sul	BAM/FDA	Soares et al., 2021
Salmon sushi	6 (21.4)	Rio Grande do Sul	ISO 11290	Ramires et al., 2021
Canastra cheese	1 (1.2)	Minas Gerais	ISO 11290	Campos et al., 2021
Artisanal Cheese	12 (12.0)	São Paulo	ISO 11290	Allaion et al., 2021

[a] International Organization for Standardization (ISO). [b] Bacteriological Analytical Manual (BAM)/Food and Drug Administration (FDA).

with an average loss of quality-adjusted life days per illness of 23.29 and an average monetary loss of $1,456,676.

The non-inclusion of listeriosis in the national list of compulsory notification of public health problems and events (Portaria n.º 1.061/2020) makes it difficult to identify and investigate the occurrence of outbreaks by health surveillance (Maia et al., 2019a; Brasil, 2020a). The isolation of this pathogen indicates that it circulates throughout the country, which can cause diseases that are not properly identified (Vallim et al., 2015; Silva et al., 2021).

3.3 Identification methods

The methods of isolation and identification of *L. monocytogenes* in food are generally expensive and laborious, making the implementation of these methodologies difficult, thus contributing to the underreporting of the disease in the country (Vallim et al., 2015). Classical methods comprise bacterial cultivation techniques and therefore require a few days to obtain the results. As seen in Table 1, the ISO 11290-1 and ISO 11290-2 standards, published by the International Organization for Standardization, were the most used for the detection and enumeration of *L. monocytogenes* in foods, respectively. This expressive value can be justified by the fact that these techniques constitute the current reference methodologies used in Europe, according to Regulation (EC) 2073/2005. Access to the methodology described in the standard is paid, and the current versions 11290-1:2020 and 11290-2:2020 cost R$174.20 and R$166.85, respectively (last access: 11/20/2021) (Associação Brasileira de Normas Técnicas, 2020a, 2020b).

Initially, two enrichment steps were necessary, using Half Fraser and Fraser broth. Selective cultivation was carried out on a selective base agar for Listeria according to Ottaviani-Agosti (ALOA). As a secondary selective medium, a medium based on esculin, such as Oxford agar and PALCAM agar, was used. The selected colonies were confirmed by biochemical tests such as Gram staining, catalase production, motility test, hemolysis test, and sugar fermentation (Figure 1). This entire process can take up to six days to confirm a positive or negative result (International Organization for Standardization, 2017; Garrido-Maestu et al., 2020).

The detection method described by the Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM), a reference in the USA, has an enrichment step with buffered Listeria enrichment broth (BLEB) incubated for 24 h-48 h, followed by plating selective differential on esculin-based agar, in addition to some chromogenic selective media. Typical colonies were cultured on tryptone soy agar with yeast extract (TSAYE) and confirmed by biochemical tests (Figure 2). The methodology described in the BAM is publicly available on the FDA website (Food and Drug Administration, 2017).

The absence of a fully efficient method to differentiate *L. monocytogenes* from other species of the genus, associated with the emergence of strains with atypical characteristics, hinders identification by conventional methods, requiring the use of alternative methods such as those based on molecular and immunochemical techniques (Reis, 2020). An alternative validated method for the identification of *L. monocytogenes* is VIDAS®, an automated system based on detection by immunoenzymatic reaction from antigens using enzyme linked fluorescent assay (ELFA) technology. After the enrichment step, the methodology used depended on which guide was chosen, and the enriched broth was added to the well of the VIDAS® tube and inserted into the equipment. A major benefit was obtaining a negative result on the day after starting the analysis. Other alternative methods based on chemical reactions include API® Tape Range,

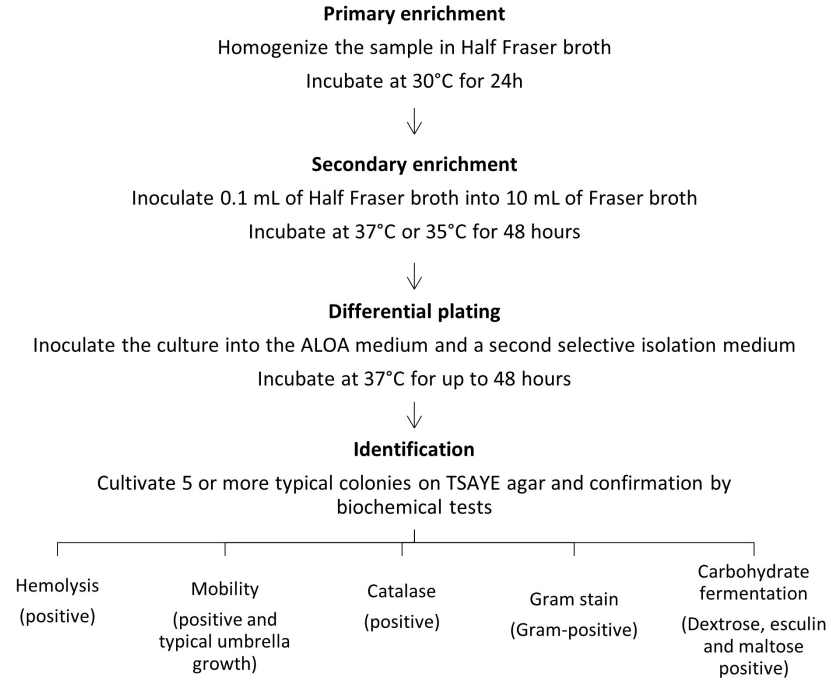

Figure 1. Scheme of isolation and identification of *Listeria monocytogenes* in foods by methodology described in ISO 11290-1.

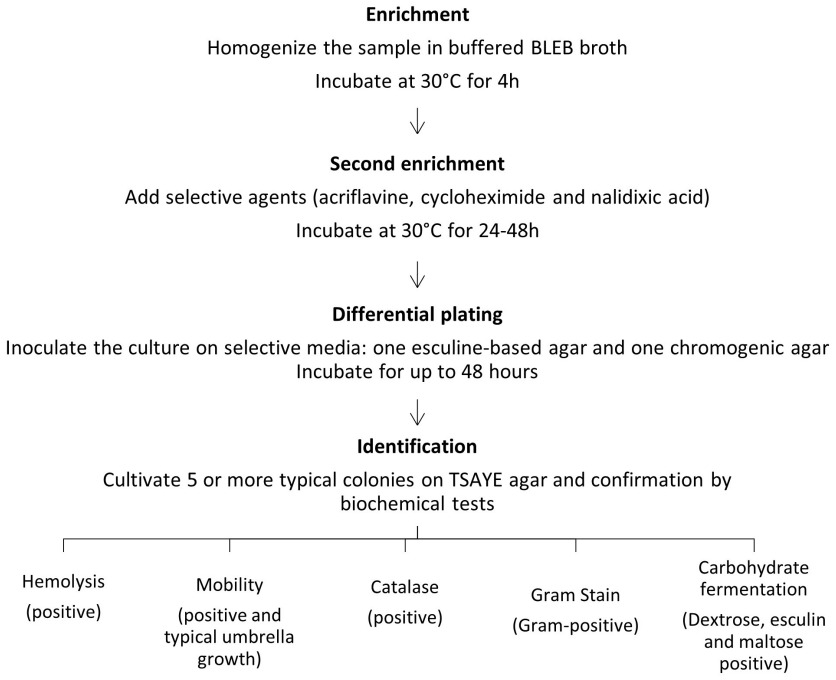

Figure 2. Scheme for the isolation and identification of *Listeria monocytogenes* in foods using the methodology described in the BAM/FDA.

Micro-ID, and VITEK 2 (Food and Drug Administration, 2017; Gonçalves et al., 2017; Biomérieux, 2021).

Traditional methods require long and laborious steps, which are against the urgency of control during food outbreaks. Thus, techniques based on polymerase chain reaction (PCR) can be used to identify pathogens (Andrade et al., 2010). To carry out PCR-based methodologies, it is necessary to carry out the steps of extraction of deoxyribonucleic acid (DNA), amplification of the target gene, and separation of the amplified products in agarose gel after the stage of cultivation in selective media for Listeria (Chen et al., 2017). Molecular techniques are considered more sensitive, accurate, and rapid for the detection of *L. monocytogenes* (Shamloo et al., 2019). The inlB gene is considered to be specific for *L. monocytogenes* and is absent

in other species of the genus. InlB is a surface protein that aids in the invasion of pathogens into mammalian cells. Gene amplification has been used to detect these bacteria in foods, even in very low amounts (Khelef et al., 2006).

Faced with an investigation of an outbreak of foodborne infection, information on the strains involved in the contamination is needed, allowing an investigation of the source of the outbreak and its distribution in the environment. Therefore, molecular typing methods, such as those based on restriction enzymes, sequencing of genetic material, and DNA hybridization, have been used (Gasanov et al., 2005; Rosas et al., 2017). Eventually, these techniques are necessary in laboratories that perform analyses for the National Health Surveillance System (SNVS) and have a quality system in place. However, the validation of these techniques in laboratories is more expensive than other traditional techniques (Leite et al., 2009).

3.4 Laboratories of the National Health Surveillance System

According to Law No. 9782/99 of January 26, 1999, SNVS comprises a set of actions carried out by institutions of the direct and indirect Public Administration of the Union, States, the Federal District, and Municipalities, which carry out activities of regulation, standardization, control, and inspection in the area of sanitary surveillance (Brasil, 1999). The SNVS is composed of Anvisa, the National Institute for Quality Control in Health (INCQS), the Central Laboratories (Lacen), and the State Health Departments and municipal health surveillance services (Conselho Nacional de Secretários de Saúde, 2011).

The National Network of Health Surveillance Laboratories (RNLVISA) is composed of Lacen in each of the 27 states of the federation and the Federal District, the INCQS, and five municipal laboratories, and is responsible for the analysis of products, such as water, food, medicines, and pharmaceutical inputs (Lopes, 2017).

According to the analytical profile of RNLVISA, in 2019, 11 laboratories distributed in five regions of Brazil carried out research on *L. monocytogenes* in food, with the test characterized as microbiological or research for toxins (Agência Nacional de Vigilância Sanitária, 2018). These data contrast with the initiative to use techniques based on molecular biology in the detection of *L. monocytogenes*, which are useful during investigations in the food industry that require fast and sensitive methodologies to monitor the pathogen's incidence in production (Andrade et al., 2010).

Currently, 103 laboratories are in the catalog of the Brazilian Network of Testing Laboratories, and are classified as accredited for *Listeria* spp. research. including private, public, and university laboratories (Instituto Nacional de Metrologia, Qualidade e Tecnologia, 2019).

ISO/IEC 17025:2017 (International Organization for Standardization, 2017c) describes the general requirements for competence, impartiality, and operation of testing laboratories. Accredited laboratories periodically participate in proficiency tests (EPs), which are used as an external quality tool, allowing the assessment of whether the results obtained in the tests are reliable. The quality of RNLVISA tests is important to ensure that the analyzed products are correctly evaluated and do not cause harm to the consumer (Rocha, 2019).

One study evaluated the results obtained during eight years of EP rounds in the field of food microbiology. During this period, an assay was carried out for *L. monocytogenes* in the cheese matrix with 11 participating laboratories, only one of which was accredited, in contrast to the rounds for researching other bacteria, such as *Salmonella* spp., which had a total participation of 160 laboratories, 18 of which were accredited (Rocha, 2019). The low number of laboratories able to correctly identify this pathogen contributes to underreporting of the disease in the country, representing a risk mainly for the groups most susceptible to serious infections.

4 Conclusions

The presence of *L. monocytogenes* in food poses a danger to consumers, especially individuals in the risk group. Despite the rare outbreaks described in Brazil, studies have demonstrated the circulation of this pathogen in several food products. In Brazil, the number of laboratories belonging to the RNVISA trained to carry out the correct identification and epidemiological research of *L. monocytogenes* is still scarce, hindering the efficient control of this bacterium. Thus, there is a need to encourage the implementation of new techniques for the diagnosis and identification of *L. monocytogenes*, especially for laboratories belonging to the SNVS, to assist in research and scale the real frequency of this pathogen in cases and outbreaks of listeriosis. In addition, it is necessary to strengthen the communication between the public health network and food surveillance, helping to monitor cases and strengthen epidemiological investigations.

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