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Annals of Bacteriology Research

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Challenges and Consequence of Listeria Monocytogens in Food Industry

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Received: Aug 12, 2023

Accepted: Aug 29, 2023

Published Online: Aug 31, 2023

Journal: Annals of Bacteriology Research

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Bacteria; Diseseses; Foodborne; Food.

Abstract

Foodborne listeriosis is one of the most serious and severe foodborne diseases. It is caused by the bacteria Listeria monocytogenes. It is a relatively rare disease, with 0.1 to 10 cases per 1 million people per year, depending on the countries and regions of the world. Although the number of cases of listeriosis is small, the high rate of death associated with this infection makes it a significant public health concern. Listeria monocytogenes (L. monocytogenes) can be found in moist environments, soil, water, decaying vegetation, and animals. It can also survive and even grow under refrigeration and other methods used for food preservation. A condition known as listeriosis may manifest in people who consume food contaminated with L. monocytogenes. Food that has been harvested, processed, prepared, packed, transported, or stored in environments contaminated with L. monocytogenes is typically a source of L. monocytogenes transmission. Raw materials, water, soil, and incoming air can all contaminate an environment. Pets that consume L. monocytogenes-contaminated food can also spread the bacteria in the home environment.

Background, Etiology, and morphological characteristics of Listeria monocytogenes

Listeria species are microaerophilic, belonging to the Firmicutes phylum [1]. Members of the *Listeria* genus are *L. monocytogenes, L. ivanovii, L. innocua, L. seeligeri, L. welshimeri, L. grayi, L. marthii and L. rocourtiae*. While *L. monocytogenes* is pathogenic for humans and animals, L. ivanovii is pathogenic to ruminants, and the other species are nonpathogenic [2]. *Listeria* species are Gram-positive, non-spore-forming, rod-shaped bacteria that are naturally found in the environment, including soil, sewage, feces from animals and birds, and surface water [3].

History

Listeria monocytogenes is described first in 1926 by Murray *et al*. (1926) [4] and colleagues during an outbreak involving rabbits and guinea pigs in a laboratory in Cambridge (Great Britain) [5]. Murray isolated Gram-positive rods from the blood of laboratory animals; he could not attribute these harmful pathogens to any bacterial species known at that time. Thus, he dubbed this novel agent Bacterium *L. monocytogenes*. Likely, other bacteriologists have already cultured this exact bacterium without having a definite categorization before Murray [4].

Epidemiology

Foodborne infections have been recognized as major sources of morbidity and mortality, especially in poorer countries, and a large amount of money is spent on social and medical costs [6]. Foodborne diseases cause up to two million deaths are recorded in developing countries. *L. monocytogenes* are detected in different types of food and its prevalence rate differs from place to place, based on the hygiene, food content, and environmental contamination rates. *L. monocytogenes* has been listed among the most important foodborne pathogens around the world [7].

Cite this article: Banu MG. Challenges and Consequence of Listeria Monocytogens in Food Industry. Ann Bacteriol Res. 2023; 6(2): 1002.

Host range

Listeria monocytogenes is a widespread zoonotic pathogen that infects a variety of species including mammals, birds, fish, and crustaceans [8]. *L. monocytogenes* is also a multi-host bacterium that can cause listeriosis in many domestic animals and wild animals including cows, goats, pets, wild rodents, and wild birds with similar pathological manifestations [9,10]. *Listeria monocytogenes* is a well-known saprophytic bacterial pathogen found in cattle production environments. Listeriosis can be fatal in sheep, causing encephalitis (circling disease) with brainstem and cranial nerve dysfunction, miscarriage with placentitis in the final trimester (from 12 weeks on), and gastroenteritis with septicemia [11]. Pets are capable of carrying pathogenic bacteria in their intestines, such as *L. monocytogenes*, and can disseminate those pathogens into the natural environment and to where human activity occurs [12]. *L. monocytogenes* have been reported in the intestines of seemingly healthy goats and sheep. This organism is often excreted into the environment regularly. Feces containing *L. monocytogenes* can contaminate the host as well as the farm or slaughterhouse's surroundings [13,14].

Source of infection

Foodborne pathogens are a worldwide concern for human disease and public health [15] and can cause acute and chronic diseases and produce a wide range of symptoms. Since the consumption of raw meat is a risk factor for infections with some bacterial pathogens [16]. *Listeria* is commonly isolated most from animal feces, human feces, farm slurry, sewerage sludge, soil, farm water troughs, surface water, plants, animal feed, and the walls, floors, drains of farms and other environments since *Listeria* has ubiquitous nature in the environment [8]. Major changes in food production, processing, and distribution, increased use of refrigeration as a primary preservation method, changes in the eating habits particularly towards ready-to-eat foods, and an increase in the number of people considered to be at high risk for the disease are suggested as possible reasons for the emergence of human food-borne Listeriosis [17,16].

Transmission

Listeria monocytogenes is the leading cause of death among bacterial pathogens acquired primarily through the consumption of contaminated foods [18]. *L. monocytogenes* is mainly transmitted to humans by ingestion of contaminated food, especially Ready To Eat (RTE) food products such as dairy, meat, and fish products [19]. *L. monocytogenes* contamination may develop as a result of inadequate quality control procedures during food processing/handling and packaging [11].

Risk factors

The most important aspect of food hygiene is the ability of the bacteria to survive in a wide range of temperatures and to make biofilms on solid surfaces, including food processing facilities, which are more resistant to disinfectants and sanitizing agents [20]. The increase is mainly because of changing consumer behaviors, as many individuals consume RTE foods. Furthermore, globalization of food trade and demographic changes such as an increase in susceptible populations because of aging and the existence of other immune-compromising infections have augmented the risk of listeriosis [21].

The cases of human listeriosis and the number of high-profile outbreaks that resulted in many deaths had significantly increased in many countries. *Listeria* possesses unique virulence factors to invade the host, evade immune cells, and cause infection [20]. The most common risk factors for *Listeria* species is that reduce host immune response includes poor nutritional status, transport, sudden changes of weather to very cold and wet which are favorable for the growth of the organisms, late pregnancy, and parturition stresses, long periods of flooding with resulting poor access to pasture and overcrowding and insanitary conditions with poor access to feed supplies [21,15]. The other risk factors of the organism are pathogenic infection increases due to a massive multiplication of *Listeria* species in the feed or environment [11].

Toxins and Virulence Factors

Listeria monocytogenes pathogenicity is fueled by a complex and well-coordinated intracellular life cycle that includes numerous critical phases such as host cell attachment and invasion, intracellular multiplication and motility, and intercellular dissemination. Virulence factors play a specific role in the infection process and influence host cell signal transduction in ways that aid infection spread [22].

Internalins

Internalin A was first discovered as a listerial surface protein necessary for *L. monocytogenes* to penetrate non-phagocytic cells including epithelial cells [23]. Internalin A, a surface protein on the surface of listerial cells, binds to E-cadherin, a surface protein on the surface of host epithelial cells [24]. The phagocytosis of *L. monocytogenes* cells appears to be stimulated by this interaction. Internalin B, a similar protein, is involved in the invasion of hepatocytes in the liver [25].

Listeriolysin O

Listeria is encased in a vacuole that is surrounded by a membrane when they are ingested [26]. Professional phagocytic cells begin killing *Listeriae* inside the vacuoles almost immediately, and *L. monocytogenes* survival is dependent on escaping the vacuole [27]. Listeriolysin O (LLO), a bacterial pore-forming toxin, is required for lysing the vacuolar membrane and allowing *L. monocytogenes* to enter the cell cytoplasm [22].

ActA protein

Listeria monocytogenes replicate in the cytoplasm after escaping from the vacuole [26]. A surface protein called ActA causes globular actin molecules to polymerize and form polarized actin filaments, allowing these bacteria to travel directly to another cell [22]. Listeriopods are formed as bacterial cells migrate through these filaments to the cell membrane, causing parts of the membrane to bulge outwards, allowing *L. monocytogenes* to spread without being exposed to antibodies or other immunoactive molecules [23].

Phospholipases

Phosphatidylinositol-specific phospholipase C (PI-PLC) and a broad-range or phosphatidylcholine-specific phospholipase C (PC-PLC) are two different phospholipase C produced by *L. monocytogenes* (PCPLC) [27]. Since bacteria with mutations in the genes coding for these enzymes are less virulent to mice than wild-type bacteria, both seem to play a role in the invasion and dissemination of *L. monocytogenes* [23]. In certain cell types, such as epithelial cells, PC-PLC damages vacuolar membranes and can thus be used instead of LLO [25].

During cerebral listeriosis, PC-PLC is also involved in the cell-to-cell distribution of *Listeria* in the brain Phosphatidylcholine-specific phospholipase C (PC-PLC) is generated as an inactive precursor, while PI-PLC is synthesized in an active form [26]. To cleave off part of the precursor and activate the phospholipase, a bacterial zinc-dependent metalloprotease and a host cell cysteine protease are needed [22].

Pathogenesis of *Listeria monocytogenes*

Adhesin proteins are found in the *L. monocytogenes*, which aid attachment to host cells and contribute to virulence [28]. *L. monocytogenes* reach both phagocytic and nonphagocytic cells, and Internalin A and B, a listerial surface protein, interact with E-cadherin, an epithelial cell receptor, inducing phagocytosis into epithelial cells [29]. Following phagocytosis, the bacterium is enclosed in a phagolysosome, where the bacterium is activated to produce listeriolysin O which lyses the phagolysosome membrane, allowing listeriae to escape into the epithelial cell's cytoplasm and proliferate [30]. ActA induces host cell actin polymerization, which propels the bacteria to the cell membrane [28].

The tendency of a surface protein (ActA) to polymerize actin selectively on the older pole of the *Listeria* cell subverts the cytoskeleton of the host cell and confers intracellular motility to the bacterium [31]. The bacterium is pushed into an adjacent mammalian cell by the resulting 'comet tail'-like structure, where it is encapsulated in a vacuole once more. Filopods are elongated protrusions that develop as they push against the host cell membrane. The listeriae are released after these filopods are absorbed by neighboring epithelial cells, macrophages, and hepatocytes, and the cycle starts again [29].

Clinical manifestations

In humans

In vulnerable patients, such as immunosuppressed patients, those at the extremes of age (neonates and older people), and pregnant women, *L. monocytogenes* causes invasive infections such as meningitis, meningoencephalitis, and bacteremia [32]. In immunocompetent persons, it can also cause severe disease (attributed by some investigators to ingestion of high infective doses), as well as outbreaks of benign febrile gastroenteritis [33].

Another form of human disease is a perinatal infection, which is associated with a high rate of fetal loss (including full-term stillbirths) and serious neonatal disease [34]. *L. monocytogenes* is a foodborne pathogen with a high mortality rate in humans. Septicemia, neurolisteriosis, and a maternal-fetal infection are all symptoms of this infection. Maternal fever, early birth, fetal death, and neonatal systemic and central nervous system diseases are also possible complications during pregnancy [35]. Maternal listeriosis is most often recorded in the second and third trimesters of pregnancy, as isolated cases or outbreaks [32].

In Animals

Listeriosis can cause encephalitis (circling disease) with brainstem and cranial nerve dysfunction in sheep, as well as abortion with placentitis in the last trimester and gastroenteritis with septicemia. The septicaemic form is seen in young lambs (under 5 weeks of age), while the encephalitic form is seen in older lambs (4-8 months) [12]. Individual sheep show different signs, but incoordination, tilting of the head, pacing in circles, propelling themselves forward before they reach a stable object such as a wall or fence, and unilateral facial paralysis (causing saliva drooling, drooping of eyelid and ear) are all common [34].

Respiratory failure causes death in 2-3 days. Sheep, goats, and cattle all show identical symptoms [36]. The disease course in cattle, on the other hand, is long and takes around 1-2 weeks. In areas where genital tract infections are normal, buffaloes are also susceptible to listeriosis [37]. Camels are also susceptible to the cerebral type of listeriosis. While there are few gross lesions in aborted ruminant fetuses, there can be autolysis if the fetus is retained. Gross lesions in aborted fetuses contain thin yellow foci of necrosis in the liver and superficial abomasal erosions [33].

Diagnosis

The diagnosis of Listeriosis depends upon detecting the growth of the microorganisms in corporal fluids normally considered as sterile-blood, amniotic, and cerebrospinal fluids [38]. *L. monocytogenes* detection and identification in foods traditionally involve culture methods based on selective pre-enrichment, enrichment, and plating. This is followed by the characterization of *Listeria* species using colony morphology, sugar fermentation, and hemolytic properties [18].

For identification and isolation of *L. monocytogenes*, ISO 11290 advises the use of Oxford and Polymyxin Acriflavin Lithium-Chloride Ceftazidime (PALCAM) agar. The USDA (United States Department Of Agriculture) uses chromogenic media such as Agar *Listeria* Ottaviani [20] and Agosti and RAPID-L. mono, while the One Broth *Listeria* process calls for *Listeria* Brilliance green agar. *Listeria's* -glucosidase activity cleaves the chromogenic substrate, resulting in blue or green colonies [39,40].

The methodology for detecting and isolating *L. monocytogenes* has advanced from cold enrichment to molecular approaches [41]. Antibody-based tests, enzyme-linked immunosorbent assays, culture-based approaches, and immune-capture techniques are all used to isolate *L. monocytogenes*. Culture-based tests are typically favored among these approaches for a variety of reasons, including their sensitivity, low cost, and status as the "gold standard" when opposed to other validated methods [42].

Furthermore, culture-based assays yield pure colonies of the desired cells, which can be used for epidemiological surveillance and disease control. Selective agents and an enrichment procedure are used in the isolation and detection of *L. monocytogenes* using culture-based methods [43]. The selective agents are used to prevent other microflora from competing, while the enrichment technique enables *L. monocytogenes* to reach measurable levels and damaged or stressed cells to recover [41]. The broth is usually plated into selective or differential media after primary and secondary enrichment [44].

The Polymerase Chain Reaction (PCR) approach has been widely used for the identification of *L. monocytogenes.* The most important and unique genes of *L. monocytogenes* are targeted by conventional PCR. Since they are straightforward and have fast outcomes, traditional PCR methods are used more commonly than cultural procedures [45]. PCR, on the other hand, is unable to differentiate between living and dead cells, as well as healthy but non-cultivable cells, metabolically damaged, depressed cells, and low levels of *L. monocytogenes* [46].

Treatments

Listeriosis can be treated by using conventional antibiotics like ampicillin and penicillin following diagnosis. However, the antimicrobial profile of the organism at a particular place, like within the country, and the individual level has to be known to be effective in treating patients and in reducing blind antimicrobial treatment, which may lead to the emergence of antimicrobial drug-resistant strains of *L. monocytogenes* [47].

The intracellular nature of *Listeria* makes its effective treatment difficult. Many antibiotics have been demonstrated to be active in vitro against *Listeria* [48]. Antibiotics that are effective against *L. monocytogenes* include Penicillin, Amoxicillin, and Ampicillin, which are the most often used and recommended by existing recommendations and professional opinions. When picked up by cells, these antibiotics bind to PBP-3 with high affinity and are retained in the cytosol [47].

Ampicillin is currently the medication of choice for the treatment of listeriosis. Cotrimoxazole may be used as an experimental treatment; it has been linked to a positive effect, most likely due to its superior brain penetration [49]. Quinolones have good tissue and cell penetration and are quickly bactericidal, but their clinical efficacy is not as high as the experimental model predicts [48].

Prevention and Control

The disease may be avoided by identifying and eliminating potential sources of infection, as well as maintaining a high degree of cleanliness and effective management practices on the farm and in the food-processing sector [50]. Because of the widespread nature of the causal organism, the lack of a simple way of detecting *Listeria* contamination in the environment, and a lack of awareness of risk factors other than silage, listeriosis is difficult to control [51]. To manage the disease in animals, thorough sanitary and sanitation methods, feed treatment as well as isolation of the sick animal, should be implemented. The capacity of *L. monocytogenes* to attach to a variety of surfaces adds to the complexity and difficulty of removing this bacterium. As a result, the slaughtering area must be kept clean and disinfected [52]. The presence of *L. monocytogenes* in cooked food typically indicates poor cooking or post-cooking cross-contamination. Contamination can be avoided by using methods such as managing the pH, water activity, using preservatives, and limiting the shelf life [51]. Identification of high-risk foods and education of high-risk persons must be prioritized at the consumer level. Keep raw meat apart from other food substances to reduce the danger of cross-contamination. Listeriosis requires the implementation of effective food safety control measures and ensuring that these control strategies are consistently met [52].

Food Safety practice and Contaminations at restaurants

Microbial foodborne disease is a major concern in terms of public health due to the high risk of microbial contamination of foods by several types of biological hazards Contamination by pathogenic microorganisms is one of the most important challenges faced by producers of processed meat products. Factors such as climate, farm, holding conditions, and method of transportation to the slaughter facility determine the cleanliness of the animal before slaughter [53].

Unhygienic slaughter equipment and houses are the main factors that contribute to microbial contamination in raw meat (Afzaal *et al*., 2019). The health status of butcher shop workers, cloths and knives, wooden boards, and weighing scales can act as a source of microbial contamination [53]. The types of microorganisms present on meat and meat products are influenced by the sanitary conditions in the environment from which the food came, the properties and microbiological quality of any added ingredients, the extent to which the product was processed and handled, and the conditions involved in subsequent storage, handling, and distribution (Afzaal *et al*., 2019). Also, contamination of raw meat easily occurs from external sources during bleeding, handling, and processing via knives, tools, clothes, hands, and air [54].

The shelf life of food decreases due to microbial contamination, which promotes foodborne illness. Foodborne pathogens like *Salmonella* species, *L. monocytogenes, Campylobacter* species, and verocytotoxin producing *Escherichia coli O157*, originating from the animal during slaughter, contaminate the carcass and spread to the cut or raw meat intended for further processing (Ahmed and Sarangi, 2013). Contamination can occur during processing, by contact with facility equipment, by contact with food handlers (e.g., hand contact, knives), and exposure to other environmental sources (e.g., air, water) [55]. Ensuring good quality raw materials, adequate lethality treatment, and effective sanitation of both the equipment and processing environment is crucial in preventing contamination of RTE meats [51]. In Ethiopia, like other African countries, the risk of acquiring foodborne illness is high because of prevailing poor food handling and sanitation practices, inadequate food safety regulation, weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of education for food-handlers [56].

Public Health Impacts

Foodborne pathogens have been identified as significant causes of morbidities and mortalities, particularly in developing countries [6]. Cattle and many other mammals including humans can be asymptomatic shedders. The aetiological factor of *Listeriosis* in humans is the bacteria *L. monocytogenes* [57].

The first human case of Listeriosis was reported by Nyfeldt in 1929 [8]. *L. monocytogenes* is transmitted from man to man or from animal to man mostly through the ingestion of the organism with contaminated food. It was reported that approximately 99% of human listeriosis appears to be food-borne. The contamination sources are animal or human fecal matters. The incubation period of listeriosis in humans is 4 to 21 days [20]. Listeriosis can be asymptomatic or cause febrile gastroenteritis in healthy individuals; however, cases of invasive infection can result in conditions such as septicemia, meningoencephalitis, and fetal loss [58].

Listeria monocytogenes cause both perinatal and adult listeriosis in humans. Perinatal human listeriosis may cause an intrauterine infection resulting in intrauterine sepsis and death before or after delivery. *L. monocytogenes* is an intracellular bacterium and can penetrate the intestinal, blood-brain, and placental barriers [10], and cause a series of clinical conditions that can range from mild illness such as gastroenteritis in healthy individuals to severe infections of the central nervous system [59] such as meningitis, meningoencephalitis, cerebral abscesses, cerebritis, and bacteremia in immunocompromised, elderly, and young individuals [55].

Adults can develop meningoencephalitis, bacteremia, and sometimes focal infections. Following the consumption of an infectious dose of *L. monocytogenes*, listeriosis can manifest twofold- either as non-invasive gastrointestinal listeriosis, which is observed among the immunocompetent people [10], or the invasive Listeriosis which is observed mostly among the immunocompromised, making them come down with serious health issues like gastroenteritis, encephalitis, meningitis, septicemia, stillbirths, and abortion [6].

Antimicrobial resistance of *L. monocytogenes*

The first antimicrobial-resistant *L. monocytogenes* strains were discovered in 1988 [60]. Resistance to antimicrobials and particularly multidrug resistance is an emerging problem in developing and developed countries. Resistant microorganisms have emerged because of the improper use of antibiotics in human health as well as in agricultural practices [61].

Antimicrobials used by veterinarians in food-producing animals for illness treatment, prevention, and growth promotion may also contribute to the establishment of resistant strains. Antibiotic resistance, particularly multi-resistance, is a public health concern since it can lead to treatment failure [62]. Irrigation water and agricultural soil play a huge role in the incidence of antibiotic-resistant *L. monocytogenes* in fresh produce and the subsequent outbreak of farm produce-related listeriosis [61,62]. A growing number of antibiotic-resistant strains have been discovered from humans, animals, and food. The acceleration in antimicrobial resistance of *L. monocytogenes* is linked to selective pressure exerted by over-prescription of drugs in clinical settings and their heavy use as promoters for growth in farm animals and increased global trade and travel, which favor the spread of antimicrobial resistance between countries and continents [63].

Listeria monocytogenes are susceptible to most antibiotics commonly used against Gram-positive bacteria, having a cure rate of approximately 70%. However, the gravity of listeriosis can only be averted by the early administration of the right set of antibiotics. The resistance of *L. monocytogenes* has been increased to the antibiotics used for therapeutic purposes in humans [64]. Although most *L. monocytogenes* strains are resistant to antimicrobial agents used in human and animal medicine, multidrug-resistant strains are increasingly frequently isolated [65]. Resistant *L. monocytogenes* strains have been reported against first-line antibiotics [66]. Gentamicin-resistant clinical strains of *L. monocytogenes* were also reported [67]. *L monocytogenes* strain resistant to ampicillin was identified in the US [68]. *L. monocytogenes* resistant to streptomycin, erythromycin, kanamycin, sulfonamide, and rifampin were also reported in clinical isolates in different countries [69].

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