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Review On: *Listeria monocytogenes* Epidemiology, Pathogenesis, Detection and Management Strategies

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Abstract: *Listeria monocytogenes* is a zoonotic food-borne pathogen that is associated with serious public health and economic implications. In animals, *L. monocytogenes* can be associated with clinical *listeriosis*. *Listeria monocytogenes* (commonly called *Listeria*) is a Gram-positive facultatively intracellular foodborne pathogen often found in food and elsewhere in nature. This review aimed to through light on the *Listeria* epidemiology, Pathogenesis, Detection and management strategies. Genus *Listeri*a has six species that include *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria seeligeri*, *Listeria innocua*, *Listeria welshimeri* and *Listeria grayi*. In human beings, *listeriosis* symptoms include encephalitis septicemia and meningitis. In addition, *listeriosis* may cause gastroenteric symptoms in human beings and stillbirths or spontaneous abortions in pregnant women. This zoonotic pathogen can be diagnosed using both classical microbiological techniques and molecular-based methods. Control is a more practical approach. Such control can be achieved by attention to detail in hygiene strategies, monitoring the occurrence of the organism, or using novel control methods such as bacteriocins (Are ribosomally synthesized peptides that are pore-forming agents, which act by disrupting the integrity of the target cell membrane) and bacteriophage (Are viruses that infect and can kill bacteria) and are logical candidates for bio control of *Listeria monocytogenes* in food.

Key words: *Listeria monocytogenes* · Epidemiology · Pathogenesis · Management Strategies

INTRODUCTION

Listeriosis is a severe foodborne disease commonly caused by eating food contaminated with the *Listeria* species. Genus *Listeria* has six species that include *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria seeligeri*, *Listeria innocua*, *Listeria welshimeri* and *Listeria grayi* [1]. Two types of species, *Listeria monocytogenes* and *L. ivanovii*, are capable of causing disease in humans and animals [2]. *Listeria monocytogenes* is an intracellular pathogen; it is referred as a main cause of human food-borne infections across the globe [3]. Food-borne *listeriosis* induced by *Listeria monocytogenes*, is a relatively rare, yet serious condition with greater mortality (20-30%) than other food-borne microbial pathogens, such as *Salmonella* spp. While *Listeria monocytogenes* commonly cause mild gastroenteritis in healthy adults, it may be associated with high severity in susceptible individuals. Basically, *Listeria monocytogenes* frequently affect immunocompromised individuals, pregnant women and elderly people. The signs and symptoms of *Listeria monocytogenes* infection range from a flu-like illness to meningitis and septicemia and it may lead to spontaneous abortion or *listeriosis* of the newborn [4].

Listeria causes illness in humans which is present in dairy products including raw milk, cheeses, deli meats and hotdogs. Illness is caused by eating contaminated food which causes the infection [5]. *Listeria monocytogenes* is the causative agent of foodborne human listeriosis. *Listeria monocytogenes* emerged as an important foodborne pathogen in the latter part of the 20th century. The primary clinical picture manifests as intestinal infection - diarrhea, nausea and vomiting [6]. Clinical syndromes caused by this microorganism include also sepsis in the immunocompromised patient, meningoencephalitis in infants and adults and febrile gastroenteritis [7]. The presence of *listeriosis* is usually associated with group that includes young, old, pregnant and immunocompromised (YOPI) individuals.

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Human *listeriosis* is severe, with a high mortality rate and infection during pregnancy may lead to abortion or stillbirth [8].

The first reported outbreak of human *listeriosis* associated with consumption of cheese occurred in the USA in 1985 [9] and was caused by a fresh cheese. Since then, several outbreaks associated with the consumption of cheese have occurred worldwide and 72 fatalities continue to be reported. Listeria is commonly present in the dairy environment, on the farm and in the processing plant. On the farm, animals have some pathogens themselves, but Listeria is frequently present in manure and fermented silage. It is most abundantly present in humid areas and stable water including drains, coolers, washing areas and floors [10]. A strong relationship between the occurrence of *Listeria monocytogenes* in raw milk and the infection of the disease frequency was observed. When unpasteurized milk was tested [11]. With increasing the consumption of manufactured ready-to-eat foods in the whole world, it has become known as an important opportunistic human foodborne pathogen [12].

Listeria monocytogenes is intrinsically resistant to cephalosporins (cefazolin, ceftiofur and cefpirome), quinolones (nalidixic acid and early fluoroquinolone such as ofloxacine), fosfomycine and clindamycine. Acquired resistance has been rarely identified. Most of the isolates are susceptible to Penicillin G, amoxicillin, aminoglycosides (gentamycin), tetracycline, phenicols, trimethoprim and sulfonamides, rifampin, glycopeptides (vancomycin) [13]. At very low frequencies, resistance to tetracycline has been identified from various sources: beef meat, beef processing plants, pork cheeks and sheep. Resistance to erythromycin was also identified in environmental and food samples. Remarkably, no resistance to penicillins has been evidenced to date [13]. This review aimed to through light on the *Listeria monocytogenes,* epidemiology, Pathogenesis, Detection and management strategies.

Literature Review

Etiology: Listeria species (spp.) are short Gram-positive rods [14] that belong to the phylum Firmicutes, class Bacilli, Order Bacillales [15]. The Listeria spp. are facultatively anaerobic, non-spore-forming, about 0.5 ìm in width and 1 ìm – 1.5 ìm in length [16] and belong taxonomically to the Clostridium-Bacillus-Lactobacillus sub-branch with Brochothrix Thermosphacta [17] *Listeria* spp. are generally motile because of peritrichous flagella at a temperature range of 24°C – 28°C but non-motile above 30°C [18] . These species are also catalase-positive; however, exceptions have been reported [19]. Listeria spp. is oxidase; urea and indole negative and hydrolyze aesculin [20]. *Listeria spp*. also has the ability to tolerate salt conditions (NaCl) up to 20% (weight/volume [w/v]) and grows in a pH range of 4.4–9.6 [21]. The growth temperature for these species ranges from –0.4°C to 45°C, with an optimum growth temperature of 37°C and can survive at relatively low water activities (aw < 0.90) [3] .These growth conditions contribute to their versatility to grow and survive under extreme environmental conditions posed at food-processing facilities and become a serious problem for food industry [22]

Listeria monocytogenes can cause a variety of diseases, including infections in pregnancy, ranging from a mild chill to a severe illness which may precipitate premature birth or miscarriage and meningitis in newborn children. Septicemia and meningitis occur in adults, whose immunity to infection is impaired, such as those suffering from cancer or leukaemia or transplant patients. Infection does occur in otherwise healthy adults and children, although this is extremely rare. Immunocompromised individuals, such as those being treated for cancer, those with organ transplants and those with acquired immunodeficiency syndrome are at increased risk [23].

Growth and Survival Characteristics: The growth and survival of *Listeria monocytogenes* is influenced by a variety of factors. In food these include temperature, pH, water activity, salt and the presence of preservatives. The temperature range for growth of *Listeria monocytogenes* is between -1.5 and 45°C, with the optimal temperature being 30-37°C. Temperatures above 50°Care lethal to *Listeria monocytogenes*. Freezing can also lead to a reduction in *Listeria monocytogenes* numbers. As *Listeria monocytogenes* can grow at temperatures as low as 0°C, it has the potential to grow, although slowly, in food during refrigerated storage. *Listeria monocytogenes* will grow in a broad pH range of 4.0-9.6. Although growth at pH <4.0 has not been documented, *Listeria monocytogenes* appears to be relatively tolerant to acidic conditions. *Listeria monocytogenes* becomes more sensitive to acidic conditions at higher temperatures [24]. Like most bacterial species, *Listeria monocytogenes* grows optimally at a water activity (aw) of 0.97. *Listeria monocytogenes* is reasonably tolerant to salt and has been reported to grow in 13-14% sodium chloride [25].

Listeria monocytogenes can also be isolated from the surface and underground waters, improperly fermented silage, sewage sludge, slaughter wastes, animal and human faeces, foodstuffs and food industry plants. The occurrence of *L. monocytogenes* in surface waters seems to be related to direct upstream land use, specifically, crop land and proximity to a dairy farms. Silage is the most common feed to harbor *Listeria monocytogenes*. Chemical quality of silage, i.e. its PH and aerobic deterioration, affects the presence of *Listeria monocytogenes* and the pathogen iscommonly found on poor quality silage [26].

Epidemiology

Reservoir and Mode of Transmission: Contamination may also occur via tools or hands, footwear and gloves and aprons of the personnel involved in manufacturing. All food contact surfaces can be a source of contamination, but concern especially rests on complex machinery that is hard to clean and equipment that has contact with large production like dairy processing plant. Because of the ubiquitous nature of *Listeria monocytogenes*, it is unrealistic to eliminate the organism from the food chain and possible contamination sites will exist throughout the chain [26] . Calves which develop septicemic are disease may acquire infection from contamination on the cow with subclinical bacteremia, through the navel from the environment and also as a congenital infection. The encephalitic form of the disease results from infection of the terminals of the trigeminal nerve consequent to abrasion of the buccal mucosa from feed or browse or from infection of tooth cavities. Spinal myelitis is believed to result from growth to spinal nerves subsequent to body area infections [27]

Virulence and Infectivity: When *Listeria monocytogenes* is ingested, it may survive the stomach environment and enter the intestine where it penetrates the intestinal epithelial cells. Furthermore, acidic conditions in the gastrointestinal tract and in macrophages following phagocytosis can be encountered by the pathogen and acid tolerance thus enhances the virulence [28]. The organism is then taken up by macrophages and non-phagocytic cells. The *Listeria monocytogenes* surface protein internal in is required for this uptake by non-phagocytic cells, as it binds to the receptors on the host cells to instigate adhesion and internalization. The bacterium is initially located in a vacuole after uptake by a macrophage or non-phagocytic cell. *Listeria monocytogenes* secrete listeriolysin O protein, which breaks down the vacuole wall and enables the bacteria to escape into the cytoplasm. Any bacteria remaining in the vacuole are destroyed by the host cell. Once located in the cytoplasm of the host cell, *L. monocytogeneis* able to replicate. *Listeria monocytogenes* is transported around the body by the blood, with most *Listeria monocytogenes* being inactivated when it reaches the spleen or liver. *L. monocytogeneis* able to utilize the actin molecules of the host to propel the bacteria into neighboring host cells. In the case of invasive listeriosis, this ability to spread between host cells enables *Listeria monocytogenes* to cross the blood-brain and placental barriers [29]

Pathogenesis: Infection with *Listeria monocytogenes* usually follows ingestion of contaminated feed and may result in septicaemia, encephalitis or abortion. Organisms probably penetrate the M cells in Payer s patches in the intestine. Spread occurs via lymph and blood to various tissues. In pregnant animals, infection results in transplacental transmission. There is evidence that the organism can invade through breaks in the oral or nasal mucosa. From this site, migration in cranial nerves is thought to be the main route of infection in neural Listeriosis. Lesions in the brain stem, often unilateral, are composed of microabscesses and perivascular lymphocytic cuffs. *Listeria monocytogenes* have the ability to invade both phagocytic and non-phagocytic cells, survive and replicate intracellular and transfer from cell to cell without exposure to humoral defence mechanisms. Specific surface proteins, internalise and facilitated both the adherence of organisms to host membranes and their subsequent uptake. Virulent strains also possess a cytolytic toxin, listeriolysin, which destroys the membranes of phagocytic vacuoles allowing *Listeria* to escape into the cytoplasm. In the cytoplasm, the organisms utilize cellular microfilaments to generate tail-like structures which confer motility. The motile Listeria contacts the internal surface of the cytoplasmic membrane and induces pseudopod-like projections. These projections containing the bacteria are taken up by adjacent cells. The entire process is then repeated following replication of species in domestic animals [30].

Clinical Listeriosis: In susceptible people and animals, *Listeria monocytogenes* can cause a life- threatening, invasive disease. The main predisposing factor is decrease in cell- mediated immunity because of underlying disease or pregnancy and the risk of Listeriosis is increased also in neonates and the elderly. About 20% of invasive Listeriosis cases are fatal [31].

Human Listeriosis: In humans, 99% of Listeriosis cases are food-borne. The clinical symptoms of invasive Listeriosis typically begin 20-30 days after the ingestion, even though the incubation period can be up to 72 days. Most cases are sporadic, leading to meningitis, encephalitis, sepsis and abortion and reported in people with another severe underlying disease [32]. Physiological reduction in cell-mediated immunity in pregnant women may result in Listeriosis with influenza-like symptoms and miscarriages. In people with no predisposing factors, invasive Listeriosis is rare and the most typical symptom is mild gastro-enteritis with fever, headache, nausea, diarrhoea and abdominal pain [33].

Cutaneous and eye infections have rarely been reported, mainly in farmers and veterinarians in direct contact with afterbirths and infected foetuses About 1% of asymptomatic humans occasionally excrete *Listeria monocytogenes*in their faces [34]. In healthy humans, the immune system destroys *Listeria monocytogenes* in the liver. Disturbed cell-mediated immunity may enable the passage of the bacterium from the liver to the central nervous system and placenta, leading to the appearance of symptoms of invasive *Listeriosis* [35].

Listeriosis in Animals: Most Listeriosis cases have been reported in sheep, among which *L. ivanovii* is also a significant cause of Listeria infections also in cows and goats, causing encephalitis, abortion, or septicaemia. In sheep and cows, subclinical mastitis and gastroenteritis caused by *Listeria monocytogenes* have also been reported [36]. In monogastric animals, listeriosis is rare and large epidemics with generalized listeriosis and acute deaths have been reported only in farmed chinchillas. In swine, the primary manifestation of Listeriosis is septicemia, whereas in horses, abortions and encephalitis is also typical [37].

Methods of Detection: *Listeria monocytogenes* contamination usually occurs in very low numbers both in foods and in the processing environment so it is vital that any analysis performed includes one or more enrichment steps which inhibit other microflora and allow both the increase of *Listeria monocytogenes* in sufficient numbers to allow detection and the recovery of injured/stressed cells. The standard microbiological methods for identification of Listeria species are laborious and time consuming, requiring a minimum of five (5) days to recognize listeria spp and about 10 days to identify *Listeria monocytogenes* by confirmation test [38]. It is therefore necessary to use a chromogenic medium that will give results within 24 hrs. Three methods of analysis are most commonly used: the International Standard (ISO-11290) method which uses a two-step enrichment in Fraser broth, the United States Department of Agriculture (USDA) method which uses a two-step enrichment in University of Vermont media (UVM) and the One-broth Listeria method which has been approved for use by the Association Française de Normalisation (AFNOR) and takes considerably less incubation time and yields results in 2 days as opposed to the 4-5 days needed for the other two methods [39]. All these methods involve plating on Listeria selective agar (Traditional or chromogenic agars) and require confirmation of isolates as *Listeria monocytogenes* by biochemical or molecular tests. The use of real-time PCR (RTi-PCR), in combination with traditional culture, to detect the presence or absence of Listeria has also been explored in recent years [40]. By amplifying Listeria specific genes through PCR and quantifying them by the detection of a fluorescent probe attached to the DNA fragments, even low numbers of the bacteria can be detected within a few hours (After enrichment) as opposed to the several days it takes to complete traditional plating techniques. For best use, RTi-PCR should be combined with the traditional methods so that isolates can be obtained from the direct detection of *Listeria monocytogenes* in food as it lacks the required sensitivity, may be subject to inhibition by food ingredients and can detect the presence of DNA from live as well as dead cells [11]

Management Strategies

Treatment of *Listeria monocytogenes*: Treatment of human listeriosis can be a challenging task as *L. monocytogenes* may invade almost all cell types [41]. Furthermore, the treatment of human listeriosis is often ineffective because of long incubation period of *L. monocytogenes*, which makes the treatment period to vary according to the level of the infection. However, antibiotics have been used to treat human listeriosis successfully for a very long time [42]. *Listeria monocytogenes* is generally susceptible to the majority of antibiotics, but cephalosporin, fosfomycin and fluoroquinolones are not active against this pathogen [43]. The intrinsic resistance of *L. monocytogenes* against these antibiotics is because of lack or low affinity of enzyme catalysing the final step of cell wall synthesis [42]. The antibiotic of choice for treating human listeriosis is ampicillin or penicillin G in combination with an

aminoglycoside such as gentamicin. Trimethoprim in combination with a sulfonamide, such as sulfamethoxazoleco-trimoxazole, is considered second choice of therapy [44]. Furthermore, tetracycline, erythromycin and vancomycin have been used to treat human *listeriosis* [45]. However, evolution of bacteria towards resistance has been considerably accelerated in *L. monocytogenes* [46]. Highest resistance detected against penicillin, nalidixic acid and erythromycin, with all 78 (100 %) tested for Listeria species presenting resistance [47].

Prevention and Control: As *Listeria monocytogenes* is a ubiquitous organism, its complete elimination is an unrealistic aim. Control is a more practical approach. Such control can be achieved by attention to detail in hygiene strategies, monitoring occurrence of the organism or using novel control methods such as bacteriocins (Are ribosomallysynthesised peptides that are pore-forming agents, which act by disrupting the integrity of the target cell membrane) and bacteriophage (Are viruses that infect and can kill bacteria) and are logical candidates for bio -control of *Listeria monocytogenes* in food [48].

The potentially long incubation time for *L.monocytogenes* to cause disease can also make it difficult to trace the disease to a specific food and source of contamination [49]. It is therefore important to remove as many sources of contamination as possible from the food processing environment to reduce the possibility of food contamination [48].

Non-food contact surfaces, especially floors and drains, can be a reservoir of *Listeria monocytogenes* in the meat industry [26]. Care has to be taken to clean and sanitize these sites, because they may contaminate other sites in the food processing facility. Cooking is effective methods to eliminate *Listeria monocytogenes* from meat. The ideal processing method would improve the shelf life and safety of the meat product, not compromise organoleptic or nutritional value is convenient and economical to apply and not cause objections by consumers [50].

CONCLUSION AND RECOMMENDATION

Listeria monocytogenes are amongst major food-borne pathogen in the world that has commanded most research and surveillance attention from government agencies and food industry over the last few years. Furthermore, methods for isolation, detection, identification and subtyping for *L. monocytogenes* from food products have increased rapidly with WGS as the new gold standard for typing of this pathogen. Despite the extensive research and development on *L. monocytogenes*, outbreaks associated with this pathogen continue to be reported and are exacerbated by a high number of susceptible individuals in most countries. However, there are no data on the prevalence of *L. monocytogenes* from most African countries that are considered to have a significant population that is immunocompromised because of HIV, TB, malaria and other infectious diseases associated with poverty. Therefore, targeted surveillance programmes are necessary in those countries not only to determine prevalence but also for the development of regulations and microbiological Criteria. In addition, increases in antibiotic resistance amongst *L. monocytogenes* strains are in line with a worldwide pattern of an increasing prevalence of antibiotic resistance amongst food-borne pathogens. Alternative therapies such as bacteriophages, bacteriocins and essential oil have been explored and show promising results.

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