Review Article

Listeria monocytogenes: An Emerging Food Borne Pathogen

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Abstract

L. monocytogenes is a psychrophilic bacteria recognized as a pathogen of great importance of food. Infection with L. monocytogenes is a widespread zoonosis, affecting mainly cattle, sheep and goat herds. Listeria species are ubiquitous bacteria widely distributed in the natural environment. L. monocytogenes has been called an "emerging food-borne pathogen" because only recently we have recognized that it can be transmitted through food. It causes listeriosis, a serious infectious disease which occurs as a consequence of consumption of food contaminated with this pathogenic bacterium. It is a psychrophilic bacteria recognized as a pathogen of great importance of food. It is accepted that listeriosis in humans is a disease that is transmitted mainly through food. The series of outbreaks of the 1980s showed that L. monocytogenes causes very serious invasion and often life-threatening disease, constituting an economic burden for both public health services and food industry.

Keywords
L. monocytogenes, Listeria spp., Zoonosis, Foodborne

Introduction

India is the world’s largest producer of dairy products by volume and has the world’s largest dairy herd. The country accounts for more than 13% of world's total milk production and is also the world’s largest consumer of dairy products, consuming almost all of its own milk production (Singh, 2011).

There are many organisms secreted through milk, one of them is Listeria monocytogenes which causes significant public health problem. L. monocytogenes is the most important species in the genus Listeria creating human health hazard and having a worldwide distribution with an extensive host range which includes mammal, poultry, fish, crustacean and ticks. The name of the intracellular organism emphasizes the relationship between infection and the development of monocyctosis in the host, which is now considered to be an inconsistent feature of listeriosis in all animal species (OIE Manual, 2014).

L. monocytogenes as an important food-borne pathogen became evident in 1980s,
after several food-borne outbreaks proved to be caused by it (Schlech et al., 1983; Fleming et al., 1985; Linnan et al., 1988). After that, several reports have been recorded of food-borne listeriosis, both epidemics and sporadic cases, almost all kinds of foods (Goulet et al., 1995; Salvat et al., 1995; Loncarevic et al., 1997; Miettinen et al., 1999; Lyytikäinen et al., 2000). The first proof that milk products could be responsible for listeriosis outbreaks was corroborated by Fleming et al. (1985) which involved 49 cases, seven of them in the foetus or in infants and 42 in immune-compromised adults.

*L. monocytogenes* is known to be secreted in milk by both infected and healthy animals (Wagner et al., 2000). Human listeriosis is a food-borne disease, and it has been estimated that 99% of all human listeriosis cases are caused by the consumption of contaminated food products (Mead et al., 1999). Although listeriosis is not common in humans, it is a clinically significant disease because of its high mortality and severity (Atil et al., 2011).

*L. monocytogenes* has been called an "emerging food-borne pathogen" because only recently we have recognized that it can be transmitted through food. *Listeria monocytogenes* is a ubiquitous bacterium. It causes listeriosis, a serious infectious disease which occurs as a consequence of consumption of food contaminated with this pathogenic bacterium. Listeriosis is a significant public health problem (Rocourt and Catimel, 1985). The first communications/reports of the presence of *Listeria* in food associated with dairy products, where cow milk was mentioned as carrier of the fatal listeriosis (Farber and Peterkin, 1991).

According to many communications, consumption of milk and dairy products contaminated with *L. monocytogenes* can lead to individual cases of listeriosis or true outbreak of this disease. Of all dairy products, soft cheeses and non-pasteurized milk are most common causes of listeriosis. In the process of production of milk and dairy products, it most commonly occurs as a consequence of post-pasteurization contamination. Listeriosis is serious disease of humans, occurring sporadically or in the form of epidemic, with mortality rate of over 25% (USDA, 1999).

*L. monocytogenes* is a psychrophilic bacteria recognized as a pathogen of great importance of food. It is accepted that listeriosis in humans is a disease that is transmitted mainly through food. The series of outbreaks of the 1980s showed that *L. monocytogenes* causes very serious invasion and often life-threatening disease, constituting an economic burden for both public health services and food industry. Infection with *L. monocytogenes* is a widespread zoonosis, affecting mainly cattle, sheep, and goat herds. Listeria species are ubiquitous bacteria widely distributed in the natural environment. This specific character of the bacteria inevitably results in contamination of numerous food products. *Listeria* spp. is widely distributed in environment and is the causal agent of listeriosis, a disease that can be serious and is fatal among elderly, very young and immune-compromised persons, with an approximate 20% case-fatality rate (Rocourt and Catimel 1985), that may increase up to 75% in high risk groups, such as pregnant women, neonates, and immune-compromised adults. The incidence of listeriosis in developed countries is about 0.2 to 0.8 cases per 100,000 persons annually (Gellin et al., 1991; McLauchlin 1996; Kela and Holmström 2001; Lukinmaa et al., 2003). The incidence is not high, but as the mortality is about 20% (Gellin et al., 1931), the disease is a public health
concern. Listeriosis usually manifests in the elderly, in foetuses or newborns and in individuals with underlying diseases.

According to Bergey’s Manual of Systematic Bacteriology (1994) Listeria genus includes: L. monocytogenes, L. ivanovii, L. innocua, L. seeligeri L. welshimeri and L. gray. Among these L. monocytogenes is pathogenic for humans and animals, and L. ivanovii is mainly pathogenic for animals, primarily sheep. Other species are considered to be non-pathogenic.

All Listeria species are small, regular rods, 0.5 μm in diameter and 1–5 μm in length that do not form spores or capsule. They produce catalase but not oxidase. It is a Gram positive, facultative anaerobic bacterium with both psychrotropic and mesophilic features. Listeria species are found in the intracellular state within monocytes and neutrophils (Gray and Killinger, 1966). L. monocytogenes is motile due to one to five peritrichous flagella, which may be lost as the bacteria enter the human cell. Movement is still possible because the bacteria polymerize acting into long acting tails that propel the bacteria through the cytoplasm (Salyers and Whitt, 2002). Filaments, ranging in size of 6-20μm, may develop in old cultures.

Members of the Listeria genus are not forming spores and capsules, distributed individually and in form of short chains, sometimes in form of the letters ‘V’ and ‘Y’.

In direct smear they can be coccoid, and therefore mistaken with streptococci (Todar, 2009). In old cultures they form longer rods, with long filaments, and also Gram-negative units can occur. Listeria is mobile at the temperature of 20–25°C (they create peritrichous flagella) and immobile at 37°C. They are catalase positive, oxidase negative, Esulin hydrolysis positive. L. monocytogenes during its exponential phase of multiplication creates a toxin called listeriolyisin O (hemolysin), which leads to in-vitro hemolysis on blood agar. L. monocytogenes has a higher resistance to external environment.

L. monocytogenes is a psychrotroph with resistance to high temperatures. It has wide temperature range for growth. It multiplies at temperatures ranging from 1.5°C to 45°C. Optimal temperature for growth is 30–37°C. The organism reproduces/multiplies best at pH 7. It is resistant to acidic (pH< 5) and alkaline (pH 9.6) environment.

L. monocytogenes can grow well at the NaCl concentration of more than 10%. In one study it was reported that the organism survived for one year period in concentration of 16% NaCl, at pH 6.0 (Seeliger and Jones, 1984). In another study Conner et al. (1986) stated that the resistance of L. monocytogenes to common salt intensifies at lower temperatures. It can survive 100 days in concentration of 10.5–30% NaCl, at 4°C. The organism is resistant to UV radiation, gamma rays and x rays which greatly contribute to wide spreading of this bacterium (Bougle and Stahl, 1994).

L. monocytogenes is resistant to most of sanitation preparations/chemicals. Preparations that have proven to be the best in destruction of L. monocytogenes are iodoforms peracetic and peroctanoic acid, quarternary ammonium compounds and chlorine solutions (Tompkin et al., 1999; Schafer et al., 2000; Eifert and Sanglay, 2002).

The public health importance of listeriosis is not always recognized, particularly since
listeriosis is a relatively rare disease compared with other common food-borne illnesses such as salmonellosis or botulism. However, because of its high case fatality rate, listeriosis ranks among the most frequent causes of death due to food-borne illness second after salmonellosis (Rossmanith et al., 2006). It is responsible for the highest hospitalisation rates (91%) amongst known food-borne pathogens and has also been linked to sporadic episodes and large outbreaks of human illnesses worldwide (Jemmi and Keusch, 1994). Epidemiological data from different countries show that the majority of human outbreaks are associated with three L. monocytogenes serotypes (1/2a, 1/2b and 4b) among a total thirteen recognized serotypes.

The contamination of food by L. monocytogenes occurs along the food chain from farm-to-fork (Farber and Peterkin, 1991). Cross-contamination, which can occur within the environment of food-processing equipment, is considered to be a possible source of Listeria contamination in processed food. L. monocytogenes is able to attach and survive on various working contact surfaces. One reason may be its ability to form biofilms (Wong et al., 2012).

L. monocytogenes is associated with septicaemia, meningoencephalitis and abortion in humans and animals, primarily affecting pregnant, new-born, and immune-compromised individuals (Choi and Hong, 2003; Rossmanith et al., 2006).

Several outbreaks of listeriosis were proven to be associated with the consumption of milk and causing great concern in the dairy industry due to the number of cases and the nearly 30% overall mortality rate of these outbreaks (Amagiani et al., 2004).

Historical background

Early reports suggest that Listeria monocytogenes may have been isolated from tissue sections of patients in Germany in 1891, from rabbit liver in Sweden in 1911, and from spinal fluid of meningitis patients in 1917 and again in 1920 (Reed, 1958; McCarthy, 1990).

However, it was not until 1926 that the microorganism was fully described, when Murray et al. (1926) isolated a small, Gram-positive rod shaped bacterium that had caused an epizootic outbreak in 1924 among rabbits and guinea pigs. They named the organism Bacterium monocytogenes. This was a year after listeriosis in sheep was recognized in Germany as a disease syndrome, though the causative agent had not been isolated.

At approximately the same time, Pirie (1927) isolated and described the same organism from gerbils in South Africa. He named the bacterium Listerella hepatolytica, and subsequently recommended in 1940 that the name be changed to Listeria monocytogenes (Reed, 1958; McCarthy, 1990).

The first report of human Listeriosis was in 1929, and the first perinatal case was reported in 1936 (Gray and Killinger, 1966). The microorganism has been reported to cause disease in a wide range of wild and domestic animals, and has been isolated from numerous species of mammals, birds, amphibians, fish, crustaceans, insects and reptiles (Hird and Genigeorgis, 1990; McCarthy, 1990; Ryser and Marth, 1991). It is now widely recognized that human Listeriosis is largely attributable to food-borne transmission of the microorganism. It was not until several large, common-source outbreaks of Listeriosis occurred in North America and Europe during the 1980s that
the significance of foods as the primary route of transmission for human exposure to *L. monocytogenes* was recognized (Broome *et al.*, 1990; Bille, 1990). While the modes of transmission for *L. monocytogenes* can include vertical (mother to child), zoonotic (contact with animal to man), and nosocomial (hospital acquired), it is generally considered that most cases of human listeriosis involve food-borne transmission.

**Taxonomy**

*L. monocytogenes* was previously in the family Corynebacteriaceae (Stuart and Pease, 1972), but in the 8th edition of Bergey’s Manual of Determinative Bacteriology, *Listeria* along with *Erysipelothrix* and *Caryophanon* were grouped as uncertain affiliation. On the basis of DNA-DNA hybridization, Stuart and Welshimer (1974) suggested a new family *Listeriaceae* to accommodate genera *Listeria* and *Morraya*. Today, the genus *Listeria* belongs to the *Clostridium* subbranch together with *Staphylococcus*, *Streptococcus*, *Lactobacillus* and *Brochothrix*.

*Listeria* includes six species, of which one is divided into two subspecies: *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. grayii* and *L. ivanovii* subsp. *ivanovii* and *L. ivanovii* subsp. *londoniensis* (Boerline *et al.*, 1992). A seventh species, *Listeria murrayi*, was previously recognized in the *Listeria* genus; however, DNA-DNA hybridization analysis, multilocus enzyme electrophoresis, and rRNA restriction fragment length polymorphism analysis, proved that *L. murrayi* appeared to be subspecies within *L. grayii* (Boerlin *et al.*, 1991, 1992; Rocourt *et al.*, 1992).

Most recently, studies have proposed recognition of two novel species within the *Listeria genus*, including *Listeria marthii* and *Listeria rocourtiae* (Graves *et al.*, 2009). Only *L. monocytogenes* causes disease in both animals and humans. However, occasional human infection with *L. ivanovii* and *L. seeligeri* has been reported (Gilot and Content, 2002). *L. ivanovii* is known to cause spontaneous abortions in sheep.

**Genus description**

The *Listeria* species are regular Gram-positive non-sporing rods with a diameter of about 0.5 μm and a length of 0.5-2.0 μm. They are facultative anaerobes with no capsule. Moreover, they are catalase-positive, oxidase-negative and motile at 20-25°C due to peritrichous flagella but non-motile at 37°C (Seeliger and Jones, 1986). *Listeria* produces flagella at room temperature and exhibit a tumbling motion when examined in broth and a swarming motility can be observed in semi-soft agar at 30°C (Roberts *et al.*, 2009), but flagella are not produced at 37°C (Peel *et al.*, 1988).

**Morphology**

Ritz *et al.*, (2001) studied the morphology of *L. monocytogenes* through electron microscope. They described the observations as the average length and diameter of the organism is 4 micrometer and 0.4 micrometer and the cell surface are smooth.

**Growth and Survival characteristics**

The growth and survival of *L. monocytogenes* is influenced by a variety of factors. In food these include temperature, pH, water activity, salt and the presence of preservatives (Table 1). The temperature range for growth of *L. monocytogenes* is between -1.5 and 45°C, with the optimal temperature being 30–37°C. Temperatures
above 50°C are lethal to L. monocytogenes. Freezing can also lead to a reduction in L. monocytogenes numbers (Lado and Yousef 2007).

As L. monocytogenes can grow at temperatures as low as 0°C, it has the potential to grow, although slowly, in food during refrigerated storage. L. monocytogenes will grow in a broad pH range of 4.0–9.6 (Lado and Yousef 2007). Although growth at pH <4.0 has not been documented, L. monocytogenes appears to be relatively tolerant to acidic conditions.

L. monocytogenes becomes more sensitive to acidic conditions at higher temperatures (Lado and Yousef 2007). Like most bacterial species, L. monocytogenes grows optimally at a water activity (aw) of 0.97. However, L. monocytogenes also has the ability to grow at aw of 0.90 (Lado and Yousef 2007). Johnson et al. (1988) demonstrated that L. monocytogenes can survive for extended periods of time at aw value of 0.81. L. monocytogenes is reasonably tolerant to salt and has been reported to grow in 13–14% sodium chloride (Farber et al., 1992). Survival in the presence of salt is influenced by the storage temperature. Studies have indicated that in concentrated salt solutions, the survival rate of L. monocytogenes is higher when the temperature is lower (Lado and Yousef 2007). L. monocytogenes can grow under both aerobic and anaerobic conditions, although it grows better in an anaerobic environment (Lado and Yousef 2007).

The effect of preservatives on the growth of L. monocytogenes is influenced by the combined effects of temperature, pH, salt content and water activity. For example, sorbates and parabens are more effective at preventing growth of L. monocytogenes at lower storage temperatures and pH. Also, adding sodium chloride or lowering the temperature enhances the ability of lactate to prevent L. monocytogenes growth. At decreased temperatures (such as refrigeration storage) sodium diacetate, sodium propionate and sodium benzoate are more effective at preventing growth of L. monocytogenes (Lado and Yousef 2007). The organism can withstand repeated freeze-thaw cycles and high salt concentrations (0-10% NaCl) (Lovett, 1990).

Doyle (1988) reported that the organism can survive for 5 days in 20 - 30% NaCl. Survival for more than 100 days has been observed in 10.5 - 30% NaCl at 4°C (Lovett, 1989). The organism can grow over a wide pH range (5.2 - 9.6); and it has been shown to be quite resistant to alkaline pH. The organism is also capable of surviving a low water activity (<0.92) (Garland, 1995).

Serological association of Listeria species

Serology is a classic tool for epidemiological studies. It is the analysis of the properties and effects of serums to detect the presence of antibodies against a microorganism. Listeria species strains are stereotyped based on the cellular (O) and flagella (H) antigens (Graves et al., 1999; Seeliger and Hone, 1979). The serology description of Listeria is described by Seegliar and Donker based on 15 ‘O’ antigens and 5 ‘H’ antigens (Lovett, 1990). Table 2 provides a list of the serovars of Listeria species. Table 3 shows the various serotypes of the Listeria species. Over 90% of Listeria monocytogenes isolates can be serotyped with commercially available sera.

Sera contain antibodies that have been obtained from an animal which has been immunized either by antigen injection or infection with microorganisms containing the antigen. Most L. monocytogenes isolates obtained from patients and the environment are type 1 or 4 (Hitchin’s, 2003). All non-
pathogenic species, except L. welshimeri, share one or more somatic antigens with L. monocytogenes.

It is due to this reason that the FDA suggests that stereotyping alone without the completion of identification procedures is inadequate for identification of L. monocytogenes. L. monocytogenes is the only species that causes infections in humans while L. ivanovii is sporadically associated with abortions in animals.

Greater than 95% of human infections caused by strains of L. monocytogenes belong to the serotype ½ a, ½ b, and 4b (Graves et al., 1999).

Biochemical characteristics of L. monocytogenes and other Listeria spp.

All the isolates were subjected to standard biochemical tests such as Gram staining, Catalase test, motility at 25°C and 37°C, and acid production from mannitol, rhamnose, and xylose. For further confirmation of Listeria isolates, other biochemical reactions like β-haemolytic activity, and Christine-Atkins-Munch-Petersen (CAMP) were measured according to Bergey’s Manual of Systematic Bacteriology (Seeliger and Jones, 1986). L. ivanovii can be differentiated biochemically from L. Monocytogenes and other Listeria species by its production of a wide, clear or double zone of haemolysis on sheep or horse blood agar, a positive Christie–Atkins–Munch-Petersen (CAMP) reaction with Rhodococcus equi but not with haemolytic Staphylococcus aureus, and fermentation of D-xylose but not L-rhamnose and D-mannitol (Rocourt and Catimel, 1985). The species are distinguished by the haemolysis test, CAMP test and sugar fermentation (Seeliger and Jones 1986).

Listeriosis worldwide and in India

Listeriosis, a zoonotic bacterial disease has emerged as a major food borne disease during the past two decades, since it has a high case fatality rate (approximately 20 to 30%) (Doyle, 1988). Listeriosis, as an important cause of severe illness account to 3.8% of food-borne hospitalization and 27.6% of food-borne deaths in the united states (Adak et al., 2002). The severity of the disease includes meningitis, septicaemia and abortion. It also has a long incubation time and a predilection for individuals who have an underlying condition, which leads to impairment of (T-cell mediated) immunity.

In 1981, the first documented and confirmed food-borne outbreak occurred at Nova Scotia, Canada. The outbreak was linked to a food produce, locally prepared coleslaw (Schlech et al., 1983). The route of transmission of L. monocytogenes by food was evident only in 1980’s after a series of outbreaks (Schelch et al., 1983). Nearly all cases of Listeriosis are food-borne (Schwartz et al., 1988; Mead et al., 1999; Adak et al., 2002). Recently the proportion of Listeriosis cases due to food-borne transmission has been estimated to be 99% (Mead et al., 1999).

Due to poor net working, reporting system on the disease outbreak and food-borne disease surveillance, very limited information is available on the prevalence of food-borne disease in India. However, despite, the poor information management system on the incidence and epidemiology of diseases in the Indian subcontinent, reports on sporadic cases of both animal and human Listeriosis could be traced which dates back to the 1930’s.

Animal Listeriosis in India traces back to 1935 in Hyderabad states in sheep as
Meningoencephalitis neonatal Srivasta second report infant on with on With caused and north Thomas the on Bhujwala on the sub continent incidenc India, the reported Human (Bhujwala other 1950). Subsequently there have been a few other reports on the disease in India (Bhujwala and Hingorani, 1975).

Human Listeriosis cases have also been reported in India. Though not phenomenal, the number of human Listeriosis case in India, have been in the rise with reports on sporadic cases and incidence in clinical samples. Probably, it is for this reason that Chugh (2008) has quoted L. monocytogenes to be a growing threat and Listeriosis emerging food-borne diseases in India.

A few of the significant clinical reports on the incidence of Listeriosis in the Indian sub-continent is detailed below.

Usha et al. (1966) were the first to discuss on human Listeriosis in India.

Bhujwala et al. (1973) reported a pilot study on the genetic Listeriosis in Delhi. Again, in 1975, Bhujwala and Hingorani, reported on the perinatal Listeriosis in India.

Thomas et al. studied neonatal Listeriosis in north India in 1981, in the same year Gogate and Deodhar, reported a case of meningitis caused by Listeria infection.

With a break for about 15 odd years, reports on Listeriosis, emerged yet again in 1977 with the report of Gupta et al. They reported on opportunistic Listeria infection of an infant Gupta et al., in the year 2003 made a report of a sporadic case of Listeriosis in the second trimester of pregnancy. Srivasta et al., in the year 2005 reported on neonatal Listeriosis.

Lately, Peer et al. (2010), reported Meningoencehalitis caused by L. monocytogenes in an immunocompetent previously healthy 20 month old female child. Though Listeriosis and L.monocytogenes may not be seen as potential clinical threat in India today, the probable risk that it might pose in the year to come, cannot be ignored. As against the global trend in Listeria and Listeriosis research, the contribution from the Indian subcontinent is still naive.

Mode of transmission

The most common transmission route of L. monocytogenes to humans is via the consumption of contaminated food. However, L. monocytogenes can be transmitted directly from mother to child (vertical transmission), from contact with animals and through hospital acquired infections (Bell and Kyriakides 2005). Healthy individuals can be asymptomatic carriers of L. monocytogenes, with 0.6–3.4% of healthy people with unknown exposure to Listeria being found to shed L. monocytogenes in their faeces. However, outbreak investigations have shown that Listeriosis patients do not always shed the organism in their faeces (FDA/USDA/CDC, 2003; Painter and Slutsker, 2007). Therefore the role of healthy carriers in the transmission of L. monocytogenes is unclear.

Pathogenesis

Infection with L. 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 micro abscesses and peri-vascular lymphocytic cuffs. L. monocytogenes has the ability to invade both phagocytic and non-phagocytic cells, to survive and replicate intracellularly and to transfer from cell-to-cell without exposure to humoral defence mechanisms. Specific surface proteins, internalins, facilitated both the adherence of organisms to host membranes and their subsequent uptake. Virulent strains also possess a cytolitic toxin, listerialysis, 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 (Chakraborty and Wehland, 1997).

Clinical Listeriosis

In susceptible people and animals L. monocytogenes can cause a life threatening, invasive disease (Vazquez-Boland et al., 2001; Silk et al., 2012). 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 (Wilesmith and Gitter, 1986; Unanue, 1997; Denny and McLauchlin, 2008; Dalton et al., 2011; Silk et al., 2012). About 20% of invasive Listeriosis cases are fatal (Vazquez-Boland et al., 2001; Silk et al., 2012).

a. Human Listeriosis

In humans, 99% of Listeriosis cases are food-borne (Mead et al., 1999). The clinical symptoms of invasive Listeriosis typically begin 20–30 days after the ingestion, even though incubation period can be up to 72 days (Vazquez-Boland et al., 2001).

Most cases are sporadic, leading to meningitis, encephalitis, sepsis, and abortion, and reported in people with another severe underlying disease (Denny and McLauchlin, 2008; Dalton et al., 2011; Silk et al., 2012). Physiological reduction in cell-mediated immunity in pregnant women may result in Listeriosis with influenza-like symptoms and miscarriages (Silver, 1998). 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 (Ooi and Lorber, 2005; Goulet et al., 2012). Cutaneous and eye infections have rarely been reported, mainly in farmers and veterinarians in direct contact with afterbirths and infected foetuses (McLauchlin and Low, 1994; Regan et al., 2005; Tay et al., 2008). About 1% of asymptomatic humans occasionally excrete L. monocytogenes in their faeces (Lamont and Postlethwaite, 1986; Grif et al., 2001, 2003).

In non susceptible people, food containing 1.9 x 10^5 colony forming units/g has been reported to cause gastroenteritis, although clearly higher infectious doses have also been reported (Vazquez-Boland et al., 2001; Ooi and Lorber, 2005). Symptoms of gastroenteritis typically begin 24 hours after ingestion of the bacterium (Vazquez-Boland et al., 2001; Ooi and Lorber, 2005). In the stomach, the bacterium is exposed to low gastric pH, which reduces the number of viable cells (Vazquez-Boland et al., 2001).
The surviving cells pass into the intestine, passively cross the intestinal wall, proliferate mainly in Peyers patches, and spread to neighbouring enterocytes basolaterally (Vazquez-Boland et al., 2001; Ooi and Lorber, 2005). The massive invasion of *L. monocytogenes* to epithelial cells is thought to cause the symptoms of gastroenteritis (Vazquez-Boland et al., 2001; Ooi and Lorber, 2005). Following passage through the intestinal barrier, the bacterium enters the liver and, less extensively, the mesenteric lymph nodes and spleen through the lymph and blood (Marco et al., 1992; Melton-Witt et al., 2012). Kupffer cells destroy most of the *L. monocytogenes* cells, and surviving cells start to proliferate and spread into hepatocytes (Cheers et al., 1978; Gregory and Liu, 2000).

In healthy humans, the immune system destroys *L. monocytogenes* in the liver (Cheers et al., 1978; Gregory and Liu, 2000). 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 (Cheers et al., 1978; Gregory and Liu, 2000; Vazquez-Boland et al., 2001).

Exceptionally, other *Listeria* spp. have been reported to cause human Listeriosis (Guillet et al., 2010). In these cases, symptoms have been similar to those of invasive Listeriosis cases caused by *L. monocytogenes* (Vazquez-Boland et al., 2001; Guillet et al., 2010). In most cases, the foetus or newborn is more likely than the mother to be affected by Listeriosis associated with pregnancy (Silver, 1998).

**b. Listeriosis in Animals**

Listeriosis has been detected in nearly all domestic animals (Gray and Killinger, 1966). 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 (Beauregard and Malkin, 1971; Wilesmith and Gitter, 1986; Low and Donachie, 1997; Chand and Sadana, 1999; Wesley et al., 2002).

In sheep and cows, subclinical mastitis and gastroenteritis caused by *L. monocytogenes* have also been reported (Jensen et al., 1996; Clark et al., 2004; Rawool et al., 2007). In monogastric animals, listeriosis is rare, and large epidemics with generalized listeriosis and acute deaths have been reported only in farmed chinchillas (Wesley, 2007).

In swine, the primary manifestation of Listeriosis is septicemia, whereas in horses, abortions and encephalitis is also typical (Wesley, 2007). Listeriosis of fowls is probably secondary to viral infections, and typically causes septicemia with accompanying cardiac lesions (Cummins et al., 1988; Wesley, 2007). The sensitivity of pregnant animals to Listeriosis has led to epidemics in which the only symptoms were abortions (Wilesmith and Gitter, 1986). Symptomless faecal carriage of *L. monocytogenes* has been reported in primates, other mammals, and birds (Lyautey et al., 2007; Hellström et al., 2008; Esteban et al., 2009).

**Listeria monocytogenes as a food borne pathogen**

The trait that makes *L. monocytogenes* difficult to control during food processing is that it can multiply over a wide range of temperatures, especially at refrigerator temperatures (Salyers and Whitt, 2002).

The classical way of enriching for *Listeria* in samples that contain other bacteria is to incubate the sample for prolonged periods of time in the refrigerator. Some studies
suggest that 1–10% of humans may be intestinal carriers of *L. monocytogenes* without exhibiting signs of illness. It is estimated by the Centres for Disease Control (CDC) that approximately 2,500 cases of Listeriosis are reported each year with 500 of the cases resulting in death (Mead *et al.*, 1999). The incidence of *L. monocytogenes* is relatively low, but the consequences of infection may be severe. Levels of *L. monocytogenes* have been recovered at greater than 10³ CFU/g. Foods containing 100 CFU/ g or greater should be considered adulterated (Robert and Greenwood, 2003).

*L. monocytogenes* has been found in at least 37 mammalian species, both domestic and feral, as well as at least 17 species of birds and possibly some species of fish and shellfish. Studies have identified sheep as a major reservoir of *Listeria* in nature (Rodriguez *et al.*, 1984). *L. monocytogenes* can be isolated from soil, silage, and other environmental sources. *L. monocytogenes* is quite hardy and resists the deleterious effects of freezing, drying, and heat remarkably well for a bacterium that does not form spores. *L. monocytogenes* are pathogenic to varying degrees based on the different serotypes (Anonymous, 1992). The production of sulfahydryl-activated hemolysin, listeriolysin O (α-listeriolysin) is associated with the pathogenic potential of *L. monocytogenes* (Geoffroy *et al.*, 1989; Gaillard *et al.*, 1987).

**Antibiogram of *L. monocytogenes* Isolates**

Yu Shu-Bing *et al.* (2004) studied the status of food contamination with *L. monocytogenes* and its resistance to antibiotics. *L. monocytogenes* strains were isolated using PCR technique. The sensitivity of *L. monocytogenes* was determined using Kirby-Bauer technique. Results showed that the contamination rate was 5.18% (16/309). The contamination rates of uncooked meat, aquatic products, cooked meat products, and fresh milk were 8.0, 8.0, 11.51 and 3.51%, respectively.

No *Listeria* was detected in milk products and vegetables. The detection rate of *L. monocytogenes* was 1.29% (4/309). Sensitivity tests of *L. monocytogenes* to 12 antibiotics including gentamicin, vancomycin, kanamycin B, norfloxacin, ofloxacin, erythromycin, chloramphenicol, tetracycline, cephalothin and cefazolin, were carried out. The study revealed that *L. monocytogenes* was resistant to enrofloxacin and nitrofurantoin.

Chen HuiYan *et al.* (2006) examined a total of 45 food samples were examined for the presence of *Listeria* spp., of which 7 were positive using the international standard method and 9 using a modified method. All strains were identified as *L. monocytogenes* by the API method. Antibiotic susceptibility testing revealed that all strains were susceptible to vancomycin, erythromycin, midecamycin, cefazolin, sulfamethoxazole, gentamicin, and ampicillin, but were all resistant to nalidixic acid, cefotaxime, cefixime, optochin and polymyxin B.

Forty-one isolates of *L. monocytogenes*, which were obtained from raw burger patties, were tested for their susceptibility against eleven antibiotics by using standard disc diffusion method Wong *et al.* (2012). In particular, 31.7% of the isolates were found to be not resistant to any of the antibiotic tested while the rest showed resistance to at least one antibiotic. The result showed that resistance to tetracycline was the most common (46.3%), followed by erythromycin (36.6%), amikacin (31.7%), and sulfamethoxazole-trimethoprim (17.1%).
Table 1 Limits for growth of *L. monocytogenes* when other conditions are near optimum (Lado and Yousef, 2007)

<table>
<thead>
<tr>
<th>Minimum</th>
<th>Minimum</th>
<th>Optimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature(°C)</td>
<td>-1.5</td>
<td>30–37</td>
<td>45</td>
</tr>
<tr>
<td>PH</td>
<td>4.0</td>
<td>6.0–8.0</td>
<td>9.6</td>
</tr>
<tr>
<td>Water activity</td>
<td>0.90</td>
<td>0.97</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2 Serovar of genus *Listeria*

<table>
<thead>
<tr>
<th>Serotype</th>
<th>‘O’ antigen</th>
<th>‘H’ antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>½ a</td>
<td>I, II, (III) a</td>
<td>A, B, C</td>
</tr>
<tr>
<td>½ b</td>
<td>I, II, (III) a</td>
<td>A B C</td>
</tr>
<tr>
<td>½ c</td>
<td>II, (III) a</td>
<td>B D</td>
</tr>
<tr>
<td>3 a</td>
<td>II, (III), IV</td>
<td>A</td>
</tr>
<tr>
<td>3 b</td>
<td>(III), IV a, (XII) a, (XIII) a</td>
<td>A B C</td>
</tr>
<tr>
<td>3 c</td>
<td>(III) a, IV a, (XII) a, (XIII) a</td>
<td>B D</td>
</tr>
<tr>
<td>4 a</td>
<td>(III)a, (V) a, VI, VII, VIII b, IX, X b, XI b, XV b</td>
<td>A BC</td>
</tr>
<tr>
<td>4 ab</td>
<td>(III) a, V a, VI a, VII a, IX a, X a</td>
<td>A BC</td>
</tr>
<tr>
<td>4 b</td>
<td>(III) a, V, VI, VII b, VIII b, IX b, X b c, XI b, XV b</td>
<td>A BC</td>
</tr>
<tr>
<td>4 c</td>
<td>(III) a, V,VI, VII b, VIII b, IX b, XI b, XV b</td>
<td>A BC</td>
</tr>
<tr>
<td>4 d</td>
<td>(III) a, V,VI, VII, VIII, IX b, X b, XI b, XV b</td>
<td>A BC</td>
</tr>
<tr>
<td>4 e</td>
<td>(III) a, V a, VI a, (VIII) a, (IX) a</td>
<td>A BC</td>
</tr>
<tr>
<td>5</td>
<td>(III), V,VI, VII b, VIII, IX b, X, XI b, XV b</td>
<td>A BC</td>
</tr>
<tr>
<td>6 a</td>
<td>(III) a, V, VI, VII, VIII b, IX, X b, XI b, XV</td>
<td>A B C</td>
</tr>
<tr>
<td>6 b</td>
<td>(III) a, V,VI, VII, VIII b, IX, X, XI, XV b</td>
<td>A BC</td>
</tr>
<tr>
<td>7</td>
<td>(III) a, XII a, XIII a</td>
<td>A B C</td>
</tr>
</tbody>
</table>

( ) means antigen not always present; a O antigen reported by Lovett (1990) and not Graves *et al.* (1999)
b O antigen reported by Graves *et al.* (1999) and not by Lovett (1990)
c Identified from outbreak in United Kingdom (McLauchlin *et al.*, 1991)

Table 3 Serotype of the *Listeria* spp. (Adapted from Hitchins, 2003)

<table>
<thead>
<tr>
<th>Listeria species</th>
<th>Serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>½ a, ½ b, ½ c, 3 a, 3 b, 3 c, 4 a, 4 ab, 4 b, 4 c, 4 d, 4 e, “7”</td>
</tr>
<tr>
<td><em>Listeria ivanovii</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Listeria innocua</em></td>
<td>4 ab, 6 ab, 6 b, Un</td>
</tr>
<tr>
<td><em>Listeria welshimeri</em></td>
<td>6 a, 6 b</td>
</tr>
<tr>
<td><em>Listeria seeligeri</em></td>
<td>1/2 b, 4 c, 4 d, 6 b, Un</td>
</tr>
</tbody>
</table>

Un: undefined
Table 4 Principle characteristics of *Listeria* species

<table>
<thead>
<tr>
<th>Test</th>
<th><em>Listeria</em> spp. Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>Positive</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>Short (0.4-0.5 μm x 0.5-2.0 μm) non spore forming rod with a few peritrichous flagella</td>
</tr>
<tr>
<td>Growth conditions</td>
<td>Aerobic and facultative anaerobic</td>
</tr>
<tr>
<td>Motility</td>
<td>Positive tumbling motility at 25°C, negative at 37°C</td>
</tr>
<tr>
<td>Catalase</td>
<td>Positive</td>
</tr>
<tr>
<td>Oxidase</td>
<td>Negative</td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>Positive</td>
</tr>
<tr>
<td>Indole</td>
<td>Negative</td>
</tr>
<tr>
<td>Urease</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Table 5 Differentiation of *Listeria* species

<table>
<thead>
<tr>
<th>Species</th>
<th>Beta haemolysis</th>
<th>Production of acid from</th>
<th>Christie, Atkins, Munch-Petersen (CAMP) reaction on sheep blood with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L-Rhamnose</td>
<td>D-Xylose</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>+</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td><em>L. innocua</em></td>
<td>_</td>
<td>V</td>
<td>_</td>
</tr>
<tr>
<td><em>L. ivanovii subsp. Ivanovii</em></td>
<td>+</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td><em>L. ivanovii subsp. Londoniensis</em></td>
<td>+</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td><em>L seeligeri</em></td>
<td>(+)</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td><em>L. welshimeri</em></td>
<td>_</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td><em>L. grayi subsp. Grayi</em></td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td><em>L. grayi subsp. Murrayi</em></td>
<td>_</td>
<td>+</td>
<td>_</td>
</tr>
</tbody>
</table>

V: Variable, (+): weak reaction; +: >90% positive reactions; -: no reaction.

A surveillance study carried out by Rahimi *et al.* (2012) to determine the prevalence of *Listeria* spp. in traditional dairy products in Chahar Mahal and Bakhtiari province, Iran. From February 2009 to February 2010, a total of 290 samples of various traditional dairy products were obtained from randomly selected retail stores located in 6 major cities of the province. Using cultural method, 21 samples (7.2%) were found positive for *Listeria* spp. The highest prevalence of *Listeria* was found in traditional ice-cream (16.7%), followed by cheese (15.0%), butter (7.5%), and kashk (2.2%) samples. The overall prevalence of *Listeria* was 7.2%, in which *L. innocua* was the most commonly recovered species (66.6%); the remaining isolates were identified as *L. monocytogenes*.
(23.8%), *L. murrayi* (4.8%) and *L. seeligeri* (4.8%).

All 5 *Listeria* strains identified as *L. monocytogenes* were also positive using polymerase chain reaction (PCR). Susceptibilities of the 21 strains to nine antimicrobial drugs were determined using the disk diffusion assay. All isolates were resistant to one or more antimicrobial agents. Six strains (28.6%) were resistant to a single and 5 strains (23.8%) showed resistance to two antimicrobial agents. Multi-drug resistance was established in 23.8% of *Listeria* strains. Resistance to nalidixic acid was the commonest finding (85.7%), followed by resistance to penicillin (47.6%), and tetracycline (33.3%).

Altuntas *et al.* (2012) aimed a study to determine the antibiotic sensitivity of *L. monocytogenes* strains isolated from animal derived foods. With disc diffusion assay, all fourteen *L. monocytogenes* strains were susceptible to the antibiotics, including penicillin G, vancomycin, tetracycline, chloramphenicol, rifampicin, erythromycin, gentamicin and trimethoprim. However, the percentages of fosfomycin and streptomycin resistances were 92.9% and 7.1%, respectively. Multiple resistances were not observed among the tested strains.

Enurah *et al.* (2013) studied the prevalence of *L. monocytogenes* in fresh raw milk and abattoir effluents in the six zones of Nigeria. Antibiotic resistant profile of the isolates was examined using the Bauer-Kirby disc diffusion assay. A total of 626 food and environmental samples were cultured on selective media out of which 54 (8.6%) were positive for *L. monocytogenes*. Chloramphenicol was the most effective antibiotic against the isolates with the least resistance (3.70%) while nalidixic acid proved to be least effective with resistance of 90.74%. The multiple-antibiotic resistant pattern of the isolates showed nalidixic acid/cloxacin (35.2%), nalidixic acid/colistin (31.5%) and cloxacin/colistin/ nalidixicacid (29.6%) to be most prominent. The least value was observed in chloramphenicol/nitrofurantoin/ cotrimoxazole with 5.6%. The moral values of the minimum inhibitory concentrations (MICs) of the antibiotics to the isolates ranged between 4.0 and >16.0 µg/ml. Chloramphenicol, nitrofurantoin and gentamycin recorded the highest MIC compared with other antibiotics. This study has demonstrated that a wide and rapidly expanding range of undesirable and, in some cases, multi-resistant determinants is currently present in *L. monocytogenes*.

**Reference**


Enurah, L.U., Aboaba, O.O., Nwachukwu, S.C.U., Nwosuh, C.I. 2013. Antibiotic resistant profiles of food (fresh raw milk) and environmental (abattoir


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