Introduction

In this chapter, characteristics of *Listeria* and its species are discussed briefly. This is followed by brief discussion on ecology, pathogenesis and virulence factors, clinical manifestation, diagnosis, treatment and preventing methods.

*Listeria* species has emerged as an important food borne pathogen in the last two decades or so. The number of reports on *Listeria* isolation began to increase at the end of the 1970s and from 1983 onwards a series of epidemic outbreaks occurred in North America and Europe which clearly established listeriosis as an important food bone pathogen (Fleming et al., 1985; Bille, 1990 and Linnan, 1988). The disease in general exhibits neural, visceral and reproductive disorders particularly in various species of animals as well as humans who are immune compromised or those that are in contact with animals (Barbuddhe, 1996). Basically, it is an important foodborne disease in humans because it is associated with ingestion of contaminated food and water with pathogenic *Listeria* spp., while in ruminants it is caused by silage feeding (Low and Donachie, 1997). Besides, with a case fatality rate as high as 20-30% (Liu, 2006), this pathogen remains the most critical threat to food processing industries as it is responsible for thousands of foodborne listeriosis cases in several industrialized countries including India. Moreover, increasing industrialization, pollution, changing food habits, drug resistance, non-availability of suitable vaccine and capability of this bacterium to survive at refrigeration temperature suggests that listeriosis may be a single largest killer among man and animals (Liu, 2006).

1.1 Characteristics of the pathogen:

Taxonomically, the genus *Listeria* is placed in the Clostridium sub-branch, which also includes the genera *Staphylococcus, Streptococcus*, and *Lactobacillus* (Lorber, 1996). The genus *Listeria* (Group 19, Bergey’s Manual, 9th ed.) comprises of eight species, namely *L. monocytogenes, L. ivanovii, L. innocua, L. welshmeri, L. seeligeri, L. grayi, L.
marthii and L. rocourtiae of which, L. marthii (Graves et al., 2009) and L. rocourtiae (Leclercq et al., 2009) have been described recently. L. monocytogenes, L. innocua, L. ivanovii (subspecies and londoniensis), L. seeligeri, L. welshimeri. are genetically closely related to one another, whereas L. grayi stands apart. However, only two members of the genus viz: L. monocytogenes and L. ivanovii are mainly regarded as pathogenic species to humans and animals.

Members of the genus Listeria are gram-positive, non-spore-forming rods that are generally aerobic or facultative anaerobes. The organisms are 0.4 to 0.5 \( \mu \)m in size or more; some cells in older cultures may lose the ability to retain in Gram stain. Listeria organisms have one to five peritrichous flagella, which produce a “tumbling” motility in cultures grown at 20°C to 25°C and a characteristic “umbrella” below the surface of motility medium (Jemmi and Stephan, 2006). They grow best at neutral to slightly alkaline pH but die at pH below 5.5. Listeria species grow optimally between 30°C and 37°C but are capable of growth at the wide range of 1°C to 45°C. (Tienungoon et al., 2000).

Hemolysis is an important characteristic which would seem to be directly related to the pathogenicity of Listeria, since non-haemolytic Listeria species can be considered as non-pathogenic (Courtieu, 1991). The haemolytic species of Listeria namely L. monocytogenes and L. ivanovii produce a \( \beta \)-haemolysin called listeriolysin O (LLO) and ivanolysin O, respectively. (Hof and Hefner, 1988) while L. seeligeri shows mild hemolysis (OIE, 2008; Jallewar et al., 2007; Yadav et al., 2010). The confirmation of pathogenicity of L. monocytogenes isolates by Christie, Atkins, Munch-Petersen (CAMP) as its in vitro pathogenicity test has been stated by many workers (Buchanan et al., 1989; Kalorey et al., 2006; Bhanu Rekha et al., 2006).

Biochemical features of L. monocytogenes include catalase positivity, oxidase negativity, fermentative metabolism, methyl red positivity and Voges-Proskauer reaction positivity.

Strains of L. monocytogenes can be classified by several methods. A serotyping scheme based on both cellular O and flagellar H antigens defines 13 different serotypes; however, because only three of these (1/2a, 1/2b and 4b) account for about 95% of all humans cases of listeriosis, serotyping is of limited value for epidemiological studies (Liu et al., 2007). Other, more discriminating methods have been devised to further classify Listeria, including phage typing, multilocus enzyme electrophoretic analysis, subtyping based on patterns ofendonuclease restriction of chromosomal or ribosomal DNA, and
polymerase chain reaction (PCR) based typing. Studies illuminated the role of foods in outbreaks and sporadic cases of listeriosis have used multilocus enzyme electrophoretic analysis to compare clinical and food isolate of *L. monocytogenes* (Schuchat et al., 1991; Pinner et al., 1992).

1.2 Ecology:

*L. monocytogenes* is widely distributed widely in the environment. It is a well-known cause of disease among sheep and cattle, in which it causes septic abortion and “circling” disease (basilar meningoencephalities) and have been implicated in several food borne cases of listeriosis (Aureli et al., 2000). Many mammalian and avian species can harbor the organisms, and these have been readily isolated from soil, dust, fertilizer, vegetable, sewage, stream, water, plant, milk, animal’s manure and different foods stored at 4°C (Frederick et al., 1996). These organisms are widely disseminated in raw and decaying vegetables (Seeliger and Jones, 1986). These vegetables can become contaminated from the soil or from manures used as fertilizer. The bacterium has a wide reach and prospect to enter food production and processing environment (Recourt, 1996). Human can get listeriosis by eating food and vegetable contaminated with *L. monocytogenes* from soil and organic fertilizer. Thus *Listeria* goes from environment to animals and then again from animals to environment, in a cyclic manner. In addition, the organisms inhabit the gastrointestinal tract of 1% to 5% or more of asymptomatic humans (Kampelmacher et al., 1969; Schuchat et al., 1993). The organisms contain a surface protein, internalin that binds to E-cadherin of enterocytes. This interaction appears necessary for translocation from the gut and may be important in penetration in the blood-brain barrier and the fetoplacental barrier (Lecuit et al., 2001). Exposure appears to be extensive, but disease is largely dependent on the integrity of the immune system and possibly on inoculum size (Siegman-Igra et al., 2002).

1.3 Pathogenesis and virulence factors:

Listeriosis is one of the several diseases that are more common or more severe during pregnancy. Whether this immunologic alteration is systematic, local or both remain uncertain (Siegman-Igra et al., 2002).

Humoral immunity plays a limited role in establishing immunity to *Listeria*. Both immunoglobulin M (absent in newborn) and classical complement activity appear (low in
newborn) to be necessary for efficient opsonization of L. monocytogenes, suggesting possible mechanisms for the susceptibility of newborns to this infection (Bortolussi et al., 1986).

Experimental evidence supports the epidemiologic conclusion that Listeria organisms are transmitted by the food-borne route. Listeriosis can be induced in laboratory animals by oral feeding of pathogenic L. monocytogenes (Schlech et al., 1984). It has also been shown to invade and even multiply in enterocytes, supporting its role as an enteroinvasive pathogen.

L. monocytogenes is pathogenic to animals and human beings without showing any significant host specificity. Infection occurs in several steps: (a) entry of the bacterium into the host, (b) lysis of the phagosomal vacuole, (c) multiplication in the cytosol and (d) direct cell-to-cell spread using actin-based motility. Each step requires expression of specific virulence factors (Jemmi and Stephan, 2006). The major virulence genes are located in a cluster of genes on two different DNA loci and are mainly influenced by the positive regulatory factor A protein. L. monocytogenes has several groups of virulence factors (Table 1.1): (a) The internalines, encoded by different internaline genes (inl), which take part in the invasion of epithelial cells and seem to be jointly responsible for the tissue tropism of L. monocytogenes (Dramsi et al., 1997 and Schubert et al., 2002); (b) Listeriolysin O, encoded by the gene lctA, and phosphatidylinositol-specific phospholipase C encoded by the gene plcA, which take part in the lysis of the phagosomes of the host cell and thus make the intracellular growth of Listeria cells possible (Marquis et al., 1995; Sibelius et al., 1999); (c) act A-protein, which is involved in motility (Domann et al., 1992); (d) Enzymes such as lecithinase, zinc metal protease and serine protease (Gaillot et al., 2000; Ravenel et al., 1992); (e) A fibronectin-binding protein, fnbA, which seems to involve in intestinal and liver colonization processes (Dram et al., 2004).
Table 1.1: Showing properties of different gene of *L. monocytogenes*.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Encoded Protein</th>
<th>Activities</th>
</tr>
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<tbody>
<tr>
<td>hlyA</td>
<td>Listeriolysin O</td>
<td>Haemolysis</td>
</tr>
<tr>
<td>iap</td>
<td>Invasive associated protein</td>
<td>Invasion of cell</td>
</tr>
<tr>
<td>plcA</td>
<td>Phosphatidylinositol specific phospholipase C</td>
<td>Lysis of phagosome</td>
</tr>
<tr>
<td>ActA</td>
<td>Act A protein</td>
<td>Motility</td>
</tr>
<tr>
<td>plcB</td>
<td>Phospholipase C/lecithinase C</td>
<td>Intracellular mobility and cell-to-cell spread</td>
</tr>
<tr>
<td>prfA</td>
<td>Protein of 237 amino acid</td>
<td>Interrupt the deletion of a region downstream of <em>plcA</em> gene.</td>
</tr>
</tbody>
</table>

*L. monocytogenes* principally causes meningitis, encephalitis, abortion or septicemia. The disease affects pregnant women, newborn baby, persons with weakened immune system, suffering from cancer, diabetes, kidney disease, AIDS and those who are being treated with glucocorticosteroid medications (Lobert, 1996). Importance of *L. monocytogenes* further increases because of high fatality rate in cases affected with listeriosis.

The virulence potential of *L. monocytogenes* has been long established in diverse studies (Lecuit et al. 2005). Swaminath et al., (1989) reported that if not all but a good number of environmental isolates of *L. monocytogenes* are virulent and express virulence factors only when introduced into animal or human host.

### 1.4 Clinical manifestation:

Listeriosis during pregnancy can causes amnionitis, premature labor, premature rupture of membranes and stillbirth. Infant with no clinical infection have been born to women treated for listeriosis during pregnancy, suggesting that listeriosis does not invariably affect the fetus and that treatment of maternal cases may prevent adverse pregnancy outcomes.

#### 1.4.1 Listeriosis in neonates:

In contrast to the mild clinical syndrome that usually occurs in pregnant women, listeriosis can be a serious and often fatal illness in neonates (Siegman-Igra et al., 2002).
Usually two clinical forms of neonatal listeriosis are recognized: early-onset and late-onset disease. Early-onset disease generally occurs in infants infected in utero from bacteremic mothers, whereas infection in late-onset disease may result from passage through an infected birth canal or nosocomial transmission. Early-onset disease usually presents as sepsis rather than meningitis. It often occurs in preterm infants, perhaps causing prematurity. Classically, widely disseminated microabscesses are noted, to which the term granulomatosis infantispticum has been applied. No findings are specific for neonatal sepsis caused by *Listeria*, but popular skin lesions may occur. Late-onset disease tends to occur in full-term infants of uncomplicated pregnancies. These infants are usually healthy at birth. In late neonatal listeriosis, the clinical syndrome is more likely to be meningitis than sepsis. The morality rate is lower than for early-onset disease.

1.4.2 Nonperinatal listeriosis:

*Listeria* also causes nonperinatal listeriosis, which is generally an infection of patients immunocompromised by underlying illness or immunosuppressive therapy, accounting for 74% of reported cases (Siegman-Igra et al., 2002), but some patients who develop listeriosis have no apparent immunocompromising conditions. No symptoms or findings are specific for *L. monocytogenes* bacteremia. Central nervous system findings such as tremors, seizures, ataxia and fluctuating consciousness seem to be characteristic of *Listeria* meningitis, however, unlike other common bacterial pathogens that cause pyogenic meningitis, *Listeria* more commonly causes brain abscess, especially subcortical brain abscess with bacteremia (Mylonakis et al. 1998). As noted, *Listeria* causes other infections of the central nervous system such as meningoencephalitis, cerebritis, brainstem abscesses and spinal cord abscesses. *Listeria* endocarditis resembles other forms of subacute endocarditis, and usually occurs in patients who previously had valvular disease. A variety of focal infections with *L. monocytogenes* have been reported, including endophthalmitis, septic arthritis, osteomyelitis, liver abscesses, cholecystitis, hepatitis, peritonitis, pleuropulmonary infection and arterial infections (Siegman-Igra et al., 2002). *L. monocytogenes* like other food-borne pathogens may cause a gastrointestinal syndrome characterized by fever, nausea, diarrhea and abdominal pain (Aureli et al., 2000). Diarrheal illness that occurs in healthy adults within 2 days of exposure to heavily contaminated foods and the same strain of *L. monocytogenes*, confirms this association (Aureli et al., 2000).
1.4.3 Animal listeriosis:

Cases of Listeriosis arise mainly from the ingestion of contaminated food. It has been reported to cause encephalitis, abortion, mastitis, repeat breeding and endometriosis in animals (Low and Donachie, 1997; Malik et al., 2002; Rawool et al., 2007a).

1.5 Diagnosis:

The listeriosis may be suspected in case of (a) neonatal sepsis or meningitis; (b) meningitis or parenchymal brain infection in patients with compromised cell-mediated immunity or over 50 years of age; (c) subcortical brain abscess; (d) fever during pregnancy; (e) blood or spinal fluid with diphtheroids or gram-positive bacilli; or (f) food borne outbreak with negative routine cultures. A confirmed diagnosis depends on isolation of the organism from blood or cerebrospinal fluid, etc. A more specific serodiagnostic test for listerial infection in humans and animals was developed using purified listeriolyisin-O (LLO) as the test antigen. LLO has been identified as a candidate antigen for a serological assay (Low and Donachie, 1991; Barbuddie et al. 2002; Bhanu Rekha et al., 2006). However, serologic assays for antibodies to listeriolyisin O (Berche et al., 1990) may be useful in some epidemiologic investigations and have been used to suggest the diagnosis in culture-negative central nervous system infection (Gaillard et al., 1992).

1.6 Treatment:

The optimal antibiotic therapy of human listeriosis has not been defined by controlled clinical trials. The organism is usually sensitive to a wide range of antibiotics. Ampicillin, amoxicillin, tetracyclines, Chloramphenicol, β-lactam antibiotics, alongwith aminoglycosides, trimethoprim and sulphamethaxazole are recommended. However Ampicillin is the drug of choice in cases of encephalitis. (Malik et al., 2002)

For penicillin-allergic patients, trimethoprim-sulfamethoxazole may be useful; this combination demonstrates bactericidal activity against Listeria. (IIof, 1991). Other drugs including erythromycin, tetracycline and rifampin have been suggested. Information is inadequate to judge the use of the newer macrolide antibiotics in listeriosis. Optimal duration of therapy also remains uncertain. A prudent treatment course is 2 weeks for listeriosis in pregnancy; 2 to 3 weeks for neonatal listeriosis; 2 to 4 weeks for adults with bacteremia and longer for complicated infections such as meningitis, parenchymal central nervous system infections or endocarditis.
1.7 Prevention:

As *L. monocytogenes* is commonly found in the environment, avoiding exposure to the organism is difficult. However, several dietary measures can be taken to minimize risk for persons at increased risk including those who are pregnant or immunocompromised. Such measures include thorough cooking of foods of animal origin, avoiding foods made from unpasteurized milk, and avoiding cross contamination when handling raw foods of animal origin and foods that are ready to eat without further cooking. Pregnant women and immunosuppressed persons may also avoid the foods that have been epidemiologically linked with listeriosis.

Keeping in view the importance of *Listeria* as an opportunistic and emerging pathogen and scarcity of literature on its epidemiology in this part of the world, the present study was proposed with attempt to isolate and identify *Listeria* from various sources and characterization of isolates for pathogenic potential, with an expectation to generate sufficient useful data in the areas selected.