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Characterization and Diagnostics of *Listeria Monocytogenes*: A Human Pathogen

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Abstract

Listeria monocytogenes, Gram positive bacteria, rod-shaped, intracellular, opportunistic, invasive food borne bacterium, which is ubiquitous in nature. Soil, vegetation, sewage, water, and fecal materials are its primary source through which it reaches to our food system. It is one of the leading food borne bacteria which is pathogenic, causing Listeriosis in immunodeficient children, adult, pregnant women, central nervous system infection, bacteremia, and other clinical manifestation. Bacterium has arsenal of virulence factors Listeriolysin, phospholipases, internalins and Act A protein which help to enter, invade and infect the host cell, escape from autophagy and promote cell to cell spread. It can withstand diverse environmental parameters, that is, low temperatures, pH, osmotic and oxidative stress. Bacterium is deadly to humans and is food borne, causing economic losses and is a threat to food industry. Present review gives an overview of bacterial characteristics, etiology, isolation, distribution and pathogenicity of *L. monocytogenes*.

Keywords: Detection and diagnosis, Food borne bacteria, *Listeria monocytogenes*, Listeriosis, Virulence factors.

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Introduction

Listeria monocytogenes is a gram positive, opportunistic, intracellular food borne pathogenic bacterium causing severe life threatening infection in human and other mammals.[1,2] The bacterium is responsible for listeriosis disease which predominantly affects immunocompressesed individuals, pregnant women, unborn and new born[3,4] with fatality rate of 20-30%.[5-7] Listeria was initially reported in 1927 by E.G.D. Murray and J. Pirie independently. Murray isolated the pathogenic agent from rabbit and named Bacterium monocytogenes.[8] Afterward, Pirie isolated the same causal agent from the liver of gerbils and named *Listerella hepatolytica*.[9] Official generic name *L. monocytogenes* was proposed by Pirie[10] in honour of great pioneer antiseptic surgeon, Joseph Lister. The isolated pathogenic bacterium was *L. monocytogenes*. After many years of discovery, Listeria was a monospecific genera having only one members *L. monocytogenes*; many authors discovered and added new species from time to time. Chiara *et al*.[11] divided the Listeria into two groups (i) *Listeria* sensu strictu (ii) *Listeria* sensu lato. *Listeria* sensu strictu comprises *L. monocytogenes* and closely related species and *Listeria* sensu lato contains non-pathogenic more basal and dynamic evolutionary genomic level species Up to date count of the number of Listeria species are 20.[130][Table 1].

L. monocytogenes is extensively distributed in a wide range of agricultural environment such as soil, manure, and water.[26] It is found as a saprotroph in soil, but it can transform into a pathogenic form when enters into the animal or human cell.[27] One of the possible sources of contamination of *L. monocytogenes* in food products and food industry is due to the cross contamination. It has the ability to attach and form biofilm on working contact surfaces [Table 2].[28,29]

Growth Characteristics and Survival of the *L. Monocytogenes*

It is ubiquitous in nature and have the ability to grow in a wide range of temperature, varied habitats, shows intrinsic physiological resistance, can survive in external environmental stress, survive and grow in anaerobic condition.[45,47,48] It grows at 2–45°C,[49] growth is also reported at 0.4°C but the growth below 4°C is generally very slow as reported by Yousef and Lou.[50] Bacterium show optimum growth, multiplication and survival at 30–37°C and can grow up to 45°C optimum temperature is 37°C but can survive at a wide range of temperature.[51,52] Carpentier and Cerf[53] stated that *L. monocytogenes* is psychotropic and can grow under narrow range of temperature without proliferation. Motility has been showed below 30°C; activation of anti-repressor GmR inhibits MogR and permit transcription of flagella gene.[54] It is nonmotile and does not produce flagella at 37°C because repression of MogR flagellar genes transcriptions.[55,56] *L. monocytogenes* can grow in a wide range of pH ranging from 4.6 to 9.5.[53] Growth also depend on water activity (A_w) and growth diminishes at A_w lower than 0.92.[57] Bacterium is halotolerant, can grow and survive in a salt concentration and high CO_2 condition.[47,58]

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Isolation, Detection and Diagnosis of *L. monocytogenes*

Contamination of food stuff results into Listeriosis was 1st time reported by Schlech *et al*.[59] in Canada 1981, since then *L. monocytogenes* is recognized as food borne pathogenic bacterium.[60] It can grow and survive in eclectic range of environmental condition and contaminated food and food stuff which are manufactured, prepared and served, but it is a nonfastidious bacteria and growth occurs in a variety of media viz. minimal and defined media.[61] Standard

method for the isolation and detection of *L. monocytogenes* in food and food products is FDA –BAM and International Organization for Standardization (ISO) 11290 [Table 3].[62,63]

In both method 25 gm of food samples is added in selective broth to overcome the growth of competitor microorganism. Further plating of the aliquot on selective reference agar, purify the isolated colonies and then biochemical test can be done for species level identification.[62,63] Some other enrichment method for particular food sample such as eggs, poultry, meat, and environmental sample USDA and for dairy product AOAC/IDF method 993.12 protocol also used by researchers.[64,65] In conventional isolation methods PALCAM and Oxford agar is used as selective media but can not distinguish into pathogenic and nonpathogenic *Listeria* spp.[66] For isolation of pathogenic *Listeria* spp. viz. *L. monocytogenes*, chromogenic media is used which is based on virulence factor of *Listeria*. ALOA media detects *L. monocytogenes* and some strains of *Listeria ivanovii* by phosphatidylinositol specific phospholipase C (PI-PLC) activity and hydrolyze 1-α-phosphatidylinnositol, producing fatty acid which form halo around the colonies.[67]

Table 1: *Listeria* species

S. No.	Group	Listeria Species	References
1	*Listeria* sensu strictu	*Listeria grayi*	[12]
2		*Listeria innocua*	[13]
3		*Listeria innovii*	[14]
4		*Listeria monocytogenes*	[15]
5		*Listeria sellegiri*	[16]
6		*Listeria welshimeri*	[16]
7	*Listeria* sensu lato	*Listeria aquatica*	[17]
8		*Listeria booriae*	[18]
9		*Listeria cornellensis*	[17]
10		*Listeria fleishmani*	[19]
11		*Listeria floridensis*	[17]
12		*Listeria gardensis*	[17]
13		*Listeria marthii*	[20]
14		*Listeria newyorkensis*	[18]
15		*Listeria riparia*	[17]
16		*Listeria weishenstephanesis*	[21]
17		*Listeria goaensis*	[22]
18		*Listeria thialandensis*	[23]
19		*Listeria costaricensis*	[24]
20		*Listeria rocurtia*	[25]

Biochemical Characterization

First approach to identify the bacteria is entirely culture-based phenotypic and classical after enrichment of food samples. Identification of *L. monocytogenes* through biochemical test is useful technique to analyze the food samples in less than a week. Several identification kits are also available to identify the target microorganism [Table 4].[1,68]

Biochemical identification of bacteria is a laborious task and takes much time, 4 days to a week. Mishandling and improper working may create a great chance of inaccuracy. Therefore, molecular techniques are most frequently and commonly used.

Molecular Identification Techniques

Immuno Assay

Immuno assay test based on antibody binding naturally to specific antigen on bacterial surface, mainly Listeriolysin (LLO)

Table 2: Prevalence and source of *Listeria monocytogenes* as reported around the world

S. No.	Country	Source	% of Prevalence	References
1	Brazil	Meat	48.7	[30]
2	China	Meat.Poultry	8.91	[31]
3	China	Rte meat products	5.3	[32]
4	Egypt	Minced meat	14	[33]
		Fish filet	8	
		Sausage	6	
		Raw milk	6	
5	Europe	Vegetables	34	[34]
6	Ghana	Milk	5.5	[35]
7	India	Vegetables	10	[36]
8	India	Meat	9.2	[37]
9	India	Meat & Milk	3.9	[38]
10	India	Raw milk	6	[39]
		Lassi dahi	8	
11	Instambul	Cheese	4.8	[40]
12	Latvia	Fish	13	[41]
13	Northern Ireland	Processing environment	6.3	[42]
14	Poland	Ready to eat food	13.5	[43]
15	Turkey	Ready to eat food	8.5	[44]
16	U.S.A. (Newyork)	Soil sample	15	[46]

Table 3: Isolation method for *Listeria species*

S. No.	Isolation method	Enrichment step	Enrichment media	Isolation Media	
				Selective media	Differential media
1	FDA-BAM	One step	Buffered Listeria Enrichment Broth	1. PALCAM	1. ALOA
2	ISO11290	Two step	1. Half Fraser Broth 2. Full Fraser Broth	2. OXFORD	2. CHROM Agar 3. RAPID L' mono
3.	USDA	Two step	1. University of Vermont Medium 2. Fraser Broth	Modified Oxford Agar Media	

Table 4: Biochemical Profile of *Listeria monocytogenes*

S. No.	Test	Test Result	References
1	Catalase	+	[62,69,70]
2	Voges- Proskauer	+	
3	Methyl Red	+	
4	Hemolysin	+	
5	Esculin Hydrolysis	+	
6	Camp Test	+	
7	Oxidase	-	
8	Mannitol	-	
9	Rhamnose	+	
10	D-Xylose	-	
11	α -Methyl D Mannoside	+	
12	Sucrose	-	

+: Positive, –: Negative

toxin, flagella, protein p60 are the main antigenic structural part used for the detection. There are many techniques, that is, ELISA, ELFA, and Latex agglutination.[70] Serological investigation resulted in negotiating of 15 serotype based on O antigen but at least 13 serotype having both group specific O and H antigen. However, this method is less sensitive than other molecular techniques.[71] Most of the epidemic listeriosis were caused by 4b and sporadic infection causes were reported by 1/2a and 1/2b serotypes of *L. monocytogenes.*

Pulse Field Gel Electrophoresis (PFGE)

Most trusted and gold standard method for subtyping of *L. monocytogenes* in an epidemic outbreak, it have high discriminatory power and reproducibility.[72-74] PFGE is a gel electrophoresis technique in which current periodically changes in three direction in a gel matrix to larger DNA fragment.[72]

Multi Locus Sequence Typing (MLST)

MLST works on nucleotide sequence of gene of multi –virulence and housekeeping genes. General targets housekeeping genes are ABC transporter, superoxide dismutase, 1-lactate dehydrogenase, phosphoglucomutase, β-glucoside, histidine kinase, amino acid aminotransferase, multivirulent sequence of three genes viz. Prf A, inlB, inlC and associated virulent genes dal, lisR and clpP are target of known sequence of primer pairs.[75,76]

Ribotyping

This molecular technique is mostly and extremely used during epidemics. Phylogenetic relatedness and prokaryotes modern systemic is based on ribotypoing. Ribosomal genes presume to be constant during evolution and unchanged; hence, ribosomal gens coding for rRNA is important for evolution.[77-79]

Phage Typing

Bacteriophages are viruses which infect and are enemies of host specific bacteria. They disintegrate and lyse the specific bacterial cell. This property is adopted and applied to detect the *L. monocytogenes*.[80,81] This is based on phenomenon of lysis of *Listeria* spp. by a specific bacteriophage interaction.[82] This phenomenon used to differentiate Listeria spp. From diary and other food using 16 phages.[83,84]

Whole Genome Sequencing (WGS)

WGS provides unique resolution by sequencing the whole genome of the organism, other method target only small regions of specific DNA fragment of genome. In outbreak investigation, it is found that isolation of *L. monocytogenes* in surveillance has better and superior capacity than PFGE, MLST and serotyping.[85,86]

LOOP Mediated Isothermal Amplification (LAMP)

For investigation of *L. monocytogenes*, LAMP is a highly specific, efficient and fast method at isothermic temperature (60–65°C). LAMP is an isothermic DNA amplification technique which utilizes two inner and two outer primers for identification of 6 definite regions of specific target genes.[87,88]

MALDI –TOF- MS

One of the fast and rapid techniques for the identification of foodborne bacteria from food isolate by comparing the biomolecules in stored database.[89,90] Detection of microorganism based on this biomolecules spectra profile is mostly obtained from bacterial cell lysates, bacterial extract and ribosomes.[91]

Virulence Factors

To colonize, compete with microbiota, *L. monocytogenes* subvert host immune system and invade the macrophages, dendritic and epithelial cells to cause disease.[92] Essential determinants of bacterial associated molecule virulence factors are vital for this process. Subsequently, we present different virulence involve during the infection cycle of *L. monocytogenes*.

Internalins

Internalins A and B are surface proteins encoded by InlA and InlB genes, in which InlA specifically characteristics gene in *L. monocytogenes*.[93] Pathogen's host cell contain E cadherin and Met acts as receptor of internalins. Internalins are 22 amino acids leucine rich repeats having single peptides at N-terminus.[94]

LLO

LLO is the only member of CDC produce by intracellular pathogen.[95-97] LLO is essential to escape from primary and secondary vacuolar structure of cell after internalization of bacterium, it also obstruct phagosomal maturation (Henry *et al*., 2006; Shaughnessy *et al*., 2006).[98,99] LLO stimulate pore formation and the demolition of host protein, but the mechanism is unknown.[100-102] It causes dephosphorylation of H_3 and deacetylation of H_4 of histone affecting the expression 146 genes (Hamon *et al*., 2007).[103] LLo also leads to cytoplasmic elevation result into activation of multiple signalling pathway of host cell.[104,105]

Phosholipase- Phosphatidylinositol- specific phospholipase (PI-PLC or PLC A), non specific phosphadylcholine phospholipase C (PC-PLC or PLC B) are two phospholipase secreted by *L. monocytogenes* encoded by Plc A and Plc B gene (Smith *et al*., 1995).[106] PI-PLC and PC-PLC help to manage pre autophagosomal maturation,[107] along with LLO both phospholipase help to escape from vacuolar membrane during

cell to cell spread.[108] In absence or deficiency of LLO PLC B help to mediate vacuolar escape, cell to cell spread and function as sphingomyelinase important for bacterium dispersed in host cell. PLC B is an important virulence factor in murine cerebral listeriosis. Plc A lead to the activation of host protein kinase C which help to escape the *L. monocytogenes* from macrophage like cell's phagosome.[109,110]

Actin Assembly Inducing Protein

Act A protein is bacterial surface protein of *L. monocytogenes* show intracellular motility in host cell cytoplasm due to the aid actin filament toward one pole. This is done by the Act A protein, encoded by act A gene.[111] Act A expression has ability to obstruct the autophagy by macrophage but not strictly needed.[112] Act A is necessary for polymerization of actin filament to propel the bacterium cytoplasm and facilitate cell to cell spread, Act A is the first bacterial surface protein found to function as actin nucleation and mimic eukaryotic WASP family protein.[113,114]

Other virulence factors which are responsible for pathogenesis are invasion associated protein encoded by iap gene, surface protein p104, murine hydrolase enzyme (p60) help to invade the epithelial cell of intestine.[115]

L. monocytogenes: A Human Pathogen

L. monocytogenes and *L. ivanovii*, both are pathogenic to human beings and animals because of their specific virulence factors. *L. ivanovii* rarely infect human population, it generally infect animals especially ruminants while *L. monocytogenes* severely infects humans and animals.[116,117] The first link of *L. monocytogenes* as a foodborne pathogen, its outbreak, study, research and survey has been done in 1981 after first infection caused by bacterium since 1920's.[118] *L. monocytogenes* infects and cause disease in a wide variety of host species and host cell primary passage of infection is consumption/exposure of contaminated food and food products, bacterium cross the intestinal epithelium and enters the bloodstream.[119]

Diseases, General symptoms and etiology – listeriosis is one of the major severe foodborne diseases caused by *L. monocytogenes*. It causes human listeriosis due consumption of *L. monocytogenes* contaminated food stuff. Symptoms in human are mild to severe illness and non – specific, in immunocompetent individuals it can cause febrile gastroenteritis.[120] Immuno compromised people of all ages with acute, serious diseased condition endurance severe septicemia and central nervous system infection; can finally generate fatal condition.[121] In pregnant women, listeriosis may show flu like symptoms and infection, can cause pre term delivery, miscarriage and meningitis in new born babies.[122-125] Brown *et al*.[126] reported a case of Listeria pericarditis in right atrial mural thrombus. Listeria pericarditis reported a highly mortality rate of about 60% which is a very serious concern to cardiology.[127] Having intracellular pathogenic essence an effective antibacterial therapeutics is arduous for *L. monocytogenes*. Fluoroquinolones, Vancomycin, nanovaccine[128] are effective measures. Ampicillin and Penicillin contemplate in the treatment of listeriosis.[129]

Conclusion

Bacterial food contamination is a major threat to food system, food industry and worldwide public health issue. *L. monocytogenes* is one of the most deadliest sporadic food borne bacteria with a high mortality and morbidity can persist in a wide range of unfavorable environmental condition i.e. temperature, pH, chemical and physiological stress. Vegetables, fruits, sea food, dairy, meat, and ready to eat products frequently contaminated through the intact food surface expedite colonization and biofilm formation in food system environment. In India, prevalence and outbreak of *L. monocytogenes* is less studied. New techniques such as WGS enable to trace the pathogen entrance course and spread patterns in an epidemic. Therefore, isolation, detection, identification, and subtyping of *L. monocytogenes* in food system are very important for establishment of risk assessment, investigation and management of food system.

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