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Review Article

Listeria monocytogens: A review of its characteristics, pathogenicity and prevalence in Iraq



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Abstract

Listeria monocytogenes represents a critical foodborne pathogen causing listeriosis, a severe infection with mortality rates of 20-30%. This comprehensive review integrates cutting-edge research from 2015-2024 with Iraqi epidemiological data to address significant knowledge gaps in regional surveillance and global comparative analysis. Recent discoveries include five novel Listeria species in 2021, revolutionary whole genome sequencing (WGS) surveillance systems, and advanced understanding of RNA-mediated regulation. Iraqi prevalence data reveals concerning patterns with rates ranging from 3.5% to 93.8% across different sample types, substantially higher than global averages. Critically, Iraqi isolates demonstrate alarming antibiotic resistance rates exceeding 80% for first-line treatments, compared to 5-15% globally. This review synthesizes global advances with regional data to identify control strategies and research priorities for enhanced listeriosis surveillance and management.

Keywords: Listeria, Monocytogenes, Genome, Sequencing, Epidemiology, Foodborne

Introduction

Listeriosis can be defined as an infection caused by Grampositive bacteria *L. monocytogenes*, which is an opportunistic pathogen for humans as well as other animal species. With a 20% rate of mortality, listeriosis is a potentially fatal infection that affects humans. (, Domenico, 2014) *L. monocytogenes*, an animal pathogen that causes human diseases, along with proof of the disease's food-borne transmission, the number of human cases in various nations had raised during 1980s (Al-Ghanim & Abbas, 2021).

L. monocyogenes can be defined as a foodborne disease which could result in complex infections in immunocompromised people, neonates (Buchanan et al., 2017, Vázquez-Boland et al., 2001,). Studies have shown that 20–30% of cases of listeriosis take place in pregnant women (Domenico, 2014, WOAH, 2021). Miscarriage, premature birth, stillbirth, and other severe health issues for neonates can result from listeriosis-induced bacteremia, amnionitis, and infections of

the embryo. (Al-Ghanim & Abbas, 2021, Hiba et al., 2024, Jamshidi & Zeinali, 2019) This species has been identified as one of the factors causing abortions in cows that were infected. (Al-Ali et al., 2018, Hitchins et al., 2022, Mahmood & Al-Gburi, 2024) There are two types of bacteria that are related with food: pathogenic bacteria that lead to disease, and spoilage bacteria, and that can lead to food to lose quality and have an unpleasant texture or smell. E. coli O157:H7, salmonella, Listeria monocytogenes, Campylobacter jejuni, S. aureus, and other pathogenic bacteria that lead to food-borne disease and can't be found during food consumption. (Al-Ghanim & Abbas, 2021) L. monocytogenes is regarded as an intra-cellular bacterium which could invade many cells and causes serious infection in animals as well as humans. L. monocytogenes can be defined as one of the most significant contaminants of raw food resources used to make ready-to-eat meals like milk and its derivatives because it spreads majorly in soil and rural areas (Domenico, 2014, Buchanan et al., 2017, Yassin et al., 2021). Nearly all cases of listeriosis

are foodborne. A broad range of foods, such as ground beef, soft dairy products, raw poultry meat, and fish, might be contaminated via L. monocytogenes. In Iraq, the majority of such products are commonly consumed. (Al-Brefkani et al., 2023)

In the EU member states, there have been 1,876 confirmed invasive listeriosis cases humans, with a notification rate of 0.42 cases for a 100,000 population and 97.10% hospitalizations, based on the latest European Center for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA) common report for 2020 (EFSA & ECDC, 2021). Centers for Disease Control and Prevention (CDC) in the US estimates that approximately 1600 people are infected with L. monocytogenes yearly, with a rate of hospitalization of approximately 94% (Jacek et al., 2022, Scallan et al., 2011).

This review addresses critical gaps identified in previous analyses by integrating recent breakthrough discoveries (2020-2024) with comprehensive Iraqi epidemiological data. The synthesis reveals significant disparities between regional and global surveillance systems, highlighting urgent needs for standardized monitoring and control strategies. Our analysis encompasses novel species discoveries, advanced genomic surveillance, emerging resistance mechanisms, and regional prevalence patterns to provide strategic recommendations for improved global listeriosis control.

Listeria monocytogenes

Facultatively anaerobic, gram-positive, and tiny, coccoid to rod-shaped, measuring 0.40 by 1 to $1.50\mu m$, L. monocytogenes is often called a coryneform organism (Vázquez-Boland et al., 2001, Jamshidi & Zeinali, 2019). It is a microorganism with a low GC content, lacks capsular, doesn't form spores, and is motile (tumbling) by peritrichous flagella at temperatures between 22° and 28° C, yet non-motile over 30°C. It grows between 1 and 45°C, with an optimal temperature of 37°C (Jacek et al., 2022, Domenico, 2014, Abdzed et al., 2022). Negative oxidase, positive catalase, and hemolytic reaction colonies on sheep blood agar indicate yield of lactic acid. (Abdzed et al., 2022). The official Listeria micro-organism discovery goes back to the year 1924, when E. Murray, M. Swann, and R. Webb had isolated L. monocytogenes as etiological agent of a septicemic disease that affects guinea pigs and rabbits in their lab at Cambridge in England (Murray et al., 1926). The first listeriosis human cases have been documented in Denmark in the year 1929 (Nyfelt, 1929). Yet, Dumont and Cotoni had isolated the bacterium from a meningitis patient in France in the year 1921, marking the first documented culture of L. monocytogenes (Dumont & Cotoni, 1921). In the year 1955, L. Ivanovii (which had been formerly referred to as L. monocytogenes serotype 5) was initially isolated from congenital listeriosis lambs in Bulgaria (Ivanov, 1962). Human cases of infection with Ivanovii are uncommon(20). On the basis of somatic (O) and flagellar (H) anti-gens, L. monocytogenes have been separated to 3 evolutionary lineages, 14 serogroups, and 4 subgroups (1/2 a-3a, 1/2 b-3b7, ½ c-3c, and 4b-4d-4e)(Doumith et al., 2004), in the year 2019, (Yin et al., 2019) identified serotype 4h, an HSL-II hybrid sub-lineage, The findings presented by (Maury et al., 2016) separated the most prevalent L clones into three groups. CC12 and CC9 are food-related clones; intermediate clones; and monocytogenes, such as CC2, CC1, CC6, and CC4, are linked to infection. Furthermore, the 4 epidemic *L. monocytogenes* clones are ECII, ECI, ECIII, and ECIV (Chen et al., 2007, Wiktorczyk-Kapischke et al., 2023).

The sole species of Listeria known to be a human pathogen is *L. monocytogenes*. Phylogenetically, it is part of the human Listeria sensu strictu division. Listeria *Ivanovii*, which is a second pathogenic species, leads to septicemia, abortion, and enteritis in ruminants, yet is uncommonly isolated from humans (Orsi & Wiedmann, 2016).

With a size of 2.8–3.2 Mb, the genome regarding Listeria sensu strictu is relatively stable and exhibits little gene gain or loss. There are roughly 4,500 accessorty genes and 2,032 core genes (compared to 2,360 and 3,109 genes in L. monocytogenes, respectively). Protein metabolism accounts for 14% of cellular macromolecular metabolism, 10% of the genome, and 17% of nucleic acid synthesis and metabolism.(
Orsi & Wiedmann, 2016, Koopmans et al., 2023)

Some new Listeria species were identified in the past ten years, and Pasteur Institute has created an interactive web platform for Listeria systems biology as well as phylogenomic analysis (Becavin et al., 2017).

Adverse environmental conditions and survival of *L. monocytogens*

Response to challenging conditions make that the genes localized on SSI1 (i.e., stress survival islet-1) are affected by bile stress, acid stress, and osmotic stress, and SSI2 (i.e., stress survival islet-2) is affected by oxidative and alkaline stress (Wiktorczyk-Kapischke et al., 2023)(.table 1)

Tolerance to low temperatures: Although *L. monocytogenes* could grow at temperature degrees ranging from 1° to 45°C, its ideal growth temperature is approximately 30° to 37°C (Wiktorczyk-Kapischke et al., 2023) led to the frequent detection regarding such bacteria in food products kept in a refrigerated environment. The processes of this event are intricate and include a reduction in the metabolism of bacteria, expression of cold shock proteins, modifications to cell composition, and uptake of protein components from the environment (Doyle et al., 2001, Chen et al., 2014).

A low pH: L. monocytogenes could grow in an environment with a range of pH that is between 4.50 and 9.0, with an ideal pH of 6 to 8 (Domenico, 2014). This environment could be found in food which has undergone acidification, which is a common food preservation technique used for meat, vegetables and dairy products. It is mainly a fermentation result through bacteria which are either added as starter cultures or present in the raw food (Vázquez-Boland et al., 2001, Hill et al., 2017). Monocytogenes exhibits acid tolerance response (ATR) that increases the bacteria's resistance to lethal acidic shocks, as well as salinity shocks (25–30% NaCl), alcoholic shocks (15%), and temperature

shocks (52C °) (Dumont & Cotoni, 1921). The arginine deiminase (ADI) pathway provides the basis for another cell mechanism which shields Gram-positive bacteria from low pH.(Wiktorczyk-Kapischke et al., 2023) Proton pumps, including F0F1-ATPase, were proposed as active mechanisms to preserve the homeostasis of *L. monocytogenes* in mildly acidic conditions. Additionally, *L. monocytogenes* has a 2-component signal transduction system which contributes to its reaction to environmental stressors, like the low pH conditions. Two genes, lisK and lisR, which encode a membrane-related histidine kinase sensor as well as cytoplasmic response regulator, respectively, are commonly found in this system. (Jacek et al., 2022, Wiktorczyk-Kapischke et al., 2023).

Alkali conditions, they might be present in the environment because of using disinfectants and detergents, could lead to the induction of bacterial surface proteins' solubilization, leading to the exposure of the hydrophobic sites of lipids to extracellular factors (Wiktorczyk-Kapischke et al., 2023) for resisting the alkali damage as well as maintaining cytoplasmic pH at optimum values, the response of *L. monocytogenes* is different. Increased intracellular acid production by fermentation of sugars and deamination regarding amino acids is one of them. Additionally, the bacteria can activate the enzymes and transporters that are directly in charge of cell surface alterations and proton retention (Harter et al., 2017). *L. monocytogenes* could survive in elevated osmolarity and

L. monocytogenes could survive in elevated osmolarity and can grow in media which is supplemented with 12% NaCl and could resist the adverse salt conditions that are as high as 20% (Domenico, 2014). Osmoadaptation is the term for L. monocytogenes's biphasic response to osmotic stress, which consists of secondary and primary response mechanisms (Wiktorczyk-Kapischke et al., 2023, Mendum & Smith, 2002).

High hydrostatic pressure (HPP) can be defined as a technology of food preservation, which is utilized as one of the alternatives to the thermal processing. Shelf life and food safety are improved by this common meat industry practice regarding microbial inactivation of foodborne pathogens and food spoilage micro-organisms at room temperature (Jacek et al., 2022, Wiktorczyk-Kapischke et al., 2023). These bacteria's resistance to high hydrostatic pressure is affected by a number of factors. (Vázquez-Boland et al., 2001, Bucur et al., 2018).

It was indicated that *L. monocytogenes* has a higher resistance to UV-C light compared to other bacteria pathogens, like *E. coli*. Because UV-C light has a limited capacity to penetrate organic materials, its effectiveness against L. monocytogenes is impacted by the existence regarding organic materials, like food debris on stainless steel surfaces as well as the existence of NaCl in foods that have been exposed to UV light (Rastogi et al., 2010).

Particular heavy metals, like zinc, copper, and iron in trace amounts are important for the survival of the bacteria and are of high significance of cofactors for various *L. monocytogenes* cellular proteins, yet those same metals at greater levels of

concentration frequently become toxic (Parsons et al., 2019). To live in a variety of environmental niches, L. monocytogenes has several defense mechanisms against poisoning and to preserve its cellular heavy metal homeostasis. According to reports, at least 50% of L. monocytogenes isolates from foods as well as food processing facilities which have been tested showed cadmium resistance (Parsons et al., 2020). CadA1 has been more prevalent in isolates of L. monocytogenes of the serotypes 1/2 a and 1/2 b when compared to 4b from food and food-processing environments, whereas cadA-2 has been primarily linked to serotype 4b strains. This indicates that the prevalence of known Cd resistance molecular determinants been serotype associated. (Jacek et al., 2022) Genes carried on LGI2, mostly linked to serotype 4b strains, particularly of hyper-virulent clones CC2, CC1, and CC4, appear to encode a highly significant mechanism causing arsenic resistance in L. monocytogenes. According to molecular study of isolates with LGI2 which are resistant to arsenic, such island has been inserted at least eight times, mostly inside open reading frames.

Because of their low toxicity, efficiency, and non-corrosive qualities, quaternary ammonium disinfectants (QAC) like benzalkonium chloride (BC) are frequently utilized in the fields of food production, healthcare settings, and homes, and L. monocytogenes could develop tolerance to them (Jacek et 2022. Buchanan et al., 2017). certain formulations achieve 1,000 ppm concentrations, QACs are often utilized in concentrations between 200 ppm and 400 ppm on surfaces that come in contact with food (Kode et al., 2021). In addition to interacting with intra-cellular targets as well as binding to DNA, quaternary ammonium compounds represent active agents which interact with bacterial cytoplasmic membrane, such as L. monocytogenes. It was demonstrated that QACs have bacteriostatic qualities at low doses (0.50 to 5mg/L), but, based on the formulation, they exhibit bacteriocidal effects for the same bacteria at concentration levels of 10mg/L-50mg/L. Nevertheless, research indicates that a number of isolates of L. monocytogenes have been resistant to BC at 1,000 mg/L following a 24-hour exposure period. (Jacek et al., 2022). Both new gene acquisition and mutations in preexisting genes could be used for gaining disinfectant tolerance. Tolerance to quats is known to be mediated through at least three efflux mechanisms that seem to were needed through horizontal gene transfer. Among the transposons that mediate quat tolerance is Tn6188, which is found typically in strains of serotype 1/2 a which mediates and carries gacH, the regarding quaternary ammonium disinfectants. bcrABC, which is normally present on plasmids carried through strains of various serotypes as well as clonal groupings, is harbored by a distinct transposon and mediates quat tolerance via efflux (Buchanan et al., 2017, Wiktorczyk-Kapischke et al., 2023).

Table 1: Genes and proteins that are involved in the stress adaptation in *L. monocytogenes.* (Wiktorczyk-Kapischke et al., 2023)

Participation	Site/ function	Gen	Protein	
Stress adaptation/	LGI1 (virulence,	virB-1	cell wall-related hydrolases	
Tolerance	resistance to the		(invasion-related proteins)	
	anti-microbial	tadG	Flp pilus assembly protein TadG	
	substances, and	cadA	cation-transporting ATPase, P1-type	
	stress	cpaF, virB1-1, tadA	Flp pilus assembly protein TadB	
	factors) *	Erm	putative cation and cationic drug efflux transporters	
		trbL, virB-6	Type IV secretory path-way, TrbL components	
		cpaB, virB-4, trbE, and	Type IV secretory path-way,	
		cagE	VirB-4 components	
		ermELm	efflux transporter (i.e., resistance to benzalkonium chloride)	
	LGI2*	arsR1D2R2A2B1B2	arsenic resistance cassette	
	SSI1 (tolerance to acid, osmotic and	Lmo-0444	Predicted membrane proteins	
	bile stress in stomach)	Lmo-0464	M-protein trans-acting positive Regulator	
		pva (lmo-0446)	penicillin V amidase	
		gadD1 (lmo-0447)	Glutamate decarboxylases and related PLP-dependent proteins	
		gadT1 (lmo-0448)	Amino acid transporters	
	SSI2 (survival	Lin-0464	Transcription factor LIN-0464	
	under alkaline and oxidative stress)	Lin-0465	Pfpl (protease)	
	Osmotic stress	operon gbuABC	Gbu transporter	
		betL	Glycine betaine transporter (BetL)	
		operon opuCABCD	OpuC transporter	
	Heat stress (HSP)	I class HSP: dnaK,	DnaK, GroEL, GroE, DnaJ, GroES	
	Trout stress (TIST)	groEL, groE, dnaJ and groES	(chaperones)	
		III class HSP: clpC, clpE, clpB, & clpP	ClpC, ClpE, ClpB, and ClpP	
	Cold stress	cspB, cspA, & cspD	CspA, CspD, & CspB	
		Сар	Сар	
		ltrC	Low-temperature requirement C Protein	
	Acid stress	arcA	Putative arginine deiminase	
		arcB	Carbamoyltransferase	
		Arc	Carbamate kinase	
		arcD	Antiporter	
	Oxidative stress	Mo-1433	Glutathione reductase	
Resistance to disinfectants	Mobile genetic elements	brcABC	Efflux pump BrcABC (i.e., resistance to the benzalkonium chloride)	
		emrE	Efflux pump ErmE (resistance to the benzalkoniun chloride)	
		mdrL	Efflux pump MdrL (resistance to the benzalkoniun chloride)	

Biofilm: In food production facilities, L. monocytogenes can adhere to a range of surfaces, like the stainless steel, glass, or polystyrene, and subsequently grow into biofilms (Jacek et al., 2022). Since biofilm bacteria in the environment might be a pathogen bacteria source for food products and ultimately for people, this poses a severe risk to food safety. The extracellular component matrix (ECM), which is made up of the extra-cellular DNA, polysaccharides, proteins, and other self-produced inorganic compounds, is a regarding extra-cellular material that bacterial cells in biofilms are embedded in (Buchanan et al., 2017, Schlech, 2019, Matle et al., 2020). Several extracellular polymeric substances (EPSs) were found in L. monocytogenes biofilms' matrix, including extra-cellular DNA, proteins, and polysaccharides (primarily teichoic acid). It was demonstrated that the adhesion surface type, bacterial strain, temperature, incubation duration, and medium all have a significant impact on L. monocytogenes biofilms (Jacek et al., 2022, Schlech, 2019).

LGI, Listeria Genomic Island; LIPI- Listeria pathogenicity island; SSI, Stress Survival Islet; * listed the most important; Csp - cold-shock protein; Cap-cold acclimatization protein. The italicized entries represent the names of genes.

Listeriosis

A higher prevalence was indicated in soils with higher moisture, soils closer to water, soils lately irrigated, cultivated, or rained on, and soils that are close to pastures. L. monocytogenes, also referred to as the foodborne infection, is contracted by the ingestion regarding contaminated food products, like dairy products, processed meat, cold-smoked fish, pre-packed sandwiches, prepared vegetables, fruits, and salads (Domenico, 2014, Wiktorczyk-Kapischke et al., 2023, Koopmans et al., 2023). Although foodborne outbreaks are regularly seen, various listeriosis cases are categorized as sporadic. Following the discovery regarding L. monocytogenes as a foodborne pathogen in the 1980s, comprehensive international and national food safety programs were established (WOAH, 2021, Ferreira et al., 2014). Listeriosis continues being one of the top three foodborne disease causes that results in hospitalizations in Europe and North America, despite the fact that the implementation regarding such programs helped to decrease the number of outbreaks. In the year 2010, the disease's global burden was 23,150 cases, 5,463 fatalities, and 172,823 disability adjusted life years (DALY) (Jacek et al., 2022, de Noordhout et al., 2014). According to such statistics, human listeriosis is one of the top foodborne infections. The pregnant five fetuses, and immunocompromised individuals, like those who have got AIDS, cancer, or organ transplant recipients, are the specific demographic groups that are affected by this disease (Vázquez-Boland et al., 2001, WOAH, 2021, Hiba et al., 2024, Cummins et al., 1994, Matle et al., 2020). A broad range of infections are associated with listeriosis, which can be divided into two types: non-invasive febrile gastroenteritis as well as severe invasive listeriosis (Jacek et al., 2022, Jamshidi & Zeinali, 2019, Koopmans et

al., 2023, Matle et al., 2020). The symptoms of invasive listeriosis, which primarily affect immunocompromised neonle. include meningitis, sepsis, encephalitis. endocarditis, septicemia, meningoencephalitis, and brain infection (Domenico, 2014, Vázquez-Boland et al., 2001, (Koopmans et al., 2023). Symptoms of gastroenteritis, include chills, fever, headache, convulsions, diarrhea, muscle pains, myalgia, and vomiting (WOAH, 2021, Jordan & McAuliffe, 2018, Zakrzewski et al., 2023, Hamidiyan et al., 2018). Another major cutaneous manifestation regarding Listeria infection is characterized through a rash that is pyogranulomatous. This type, which is caught by direct contact with placenta or genital tract of cows who have experienced a miscarriage because of Listeria infection, is occasionally found farmers in as well as veterinarians. (Vázquez-Boland et al., 2001. WOAH, 2021) L. monocytogenes was linked to over 110 outbreaks worldwide, such as the biggest one in South Africa in the year 2018 (Jackson et al., 2018).

Virulence and pathogenicity

L. monocytogenes can be defined as an intracellular pathogen, and the processes by which it enters and multiplies in host cellular cells are still being thoroughly investigated. (Wiktorczyk-Kapischke et al., 2023) The basic Listerial intracellular infection cycle characteristics —namely, (i) escape from phagocytic vacuole, (ii) host cell invasion, (iii) actin-based motility and cell-to-cell spread, and (iv) rapid intracellular proliferation—are caused by a core set of virulence determinants shared by all isolates of L. monocytogenes (Koopmans et al., 2023, Schlech, 2019, Matle et al., 2020).

To encourage host cell invasion (InlB, InlA), phagosome escape (PlcA, PlcB, and LLO), fast cytoplasmic replication (Hpt), and cell-to-cell spread (InlC,ActA), L. monocytogenes employs various virulence factors. (Scortti et al., 2007, Asim et al., 2024) Internalins (InIB and InlA) and (LLO that is encoded by the hly gene) are the primary virulence factors necessary for invasion (Wiktorczyk-Kapischke et al., 2023, Scortti et al., 2007). Ami protein is in charge of cleaving the amide link in the peptidoglycan, which causes the cells to adhere to their surface and internalize their cytoplasmic membrane during the initial stage of infection. FbpA protein that facilitates the binding of the fibronectin and shields L. monocytogenes from detection through the human immune system, is crucial at such stage, as is Lap protein, an adhesin as well as murein hydrolase implicated in invasion regarding non-phagocytic cells (Wiktorczyk-Kapischke et al., 2023, Asim et al., 2024).

Ten essential virulence genes organized in five transcriptional units, all of which are coordinatedly produced under PrfA transcriptional regulator direction, (Parsons et al., 2020, Asim et al., 2024) encode such virulence capabilities. Two of the transcriptional units, HLY, which encodes pore-forming toxin LLO that mediates the vacuole escape (Wiktorczyk-Kapischke et al., 2023, Matle et al., 2020), and PLCB and PLCA, which encode 2 phospholipasesC (phosphatidylinositol-specific and

broad-substrate range) are located in a discrete 10kb chromosomal region that is referred to as LIPI1 (Wiktorczyk-Kapischke et al., 2023, Bucur et al., 2018). LIPI-1 is known as "Listeria intra-cellular survival cassette" due to the significant part that its products play in the development of listerial intracellular infection. (Wiktorczyk-Kapischke et al., 2023, Koopmans et al., 2023, Asim et al., 2024)

Two of the three additional PrfA-regulated transcriptional units that are located at various chromosomal positions encode members regarding the Listeria internalin (inl) multi-gene family (Schlech, 2019, Matle et al., 2020). InlB and InlA, two surface-associated internalins necessary for entry into usually non-phagocytic cells, are encoded by inlAB operon (Koopmans et al., 2023). The *L. monocytogenes* infections' invasive nature is caused by InlB and InlA in addition to actin-

based cell-to-cell spread process that is mediated through ActA (Asim et al., 2024). Lastly, the hpt gene, which is another monocistronic unit, encodes an organophosphate transporter which facilitates rapid replication in cytosol through the enabling of the Listeria bacteria to access the host cell-derived glucose metabolic intermediates (which are, fructose-6-phosphate, glucose-1-phosphate, and glucose-6-phosphate) as a source of carbon. The inlC monocistronic unit, another internalin multi-gene family member, encodes a secreted, small protein that is predominant in L. monocytogenes culture supernatant (Chen et al., 2007, Wiktorczyk-Kapischke et al., 2023) and helps in ActAmediated cell-to-cell passage.

Table 2: Genes and proteins that are involved in virulence in L. monocytogenes. (25)

participation	Sites/functions	genes	Proteins
Pathogenesis	involved in intra- cellular infection cycle of L. mono-cytogenes)	prfA	Positive regulatory factor A (PrfA)
		plcA	PlcA
		hly	LLO, pore-forming Toxin
		mpl	Metalloprotease (Mpl)
		actA	ActA
		plcB	PlcB
	locus Inl-A-Inl-B (involvement in the adhesion)	inl-A	Internalin A (InlA)
		Inl-B	Internalin B (InlB)
	LIPI3 (operon coding LLS - bacteriocin and hemolytic cytotoxic factor)	Lls-A	Listeriolysin L (LLS)
		llsG	
		llsH	
		llsX	
		llsB	
		llsY	
		llsD]
		llsP	
			maltose-6'-P-glucosidase
			transcriptional anti-terminator
		lm4b02326	uncharacterized protein that is associated with the PTS systems
		lm4b02327	membrane permease EII-A
		m4b02328	membrane permease EII-B
		lm4b02329	membrane permease EII-C

Individual members regarding PrfA virulence regulon might play multiple important roles in listerial infection, as is the case with various bacterial virulence factors. Therefore, in addition to its crucial function in the vacuole escape, poreforming toxin LLO promotes the invasion of the host cells through the induction of Ca2+ influx, suppresses macrophage oxidative burst, decreases transcriptional activity of a sub-set of host genes, such as important innate immunity genes, through the induction of the histone modifications, dysregulates protein small ubiquitin-related modifiers (SUMO)ylation, which alters important processes for the host cells, silences adaptive immune responses through encouraging T cell receptor signaling negative regulators' expression, as well as preventing the damage of plasma membrane and premature death of the host cells through the interaction with the endocytic adaptor protein Ap2a2 (Maury et al., 2016, Koopmans et al., 2023, Asim et al., 2024). Along with its crucial function in cell-to-cell spread, ActA enables L. monocytogenes to evade autophagy in the cytoplasm of the host cell. Another example is InlC, which inhibits N-Wasp and reduces actin cortical cytoskeleton rigidity through binding to host protein Tuba. This promotes the formation of membrane protrusions throughout cell-to-cell spread. Additionally, it dampens innate immune responses through the targeting of IkB kinase sub-unit IKKa, which lowers the activation of NF-k B. There were reports of other listerial components contributing to infection. These consist of metabolic path-ways, stress tolerance or detoxification factors, secreted proteins, surface-associated determinants, and secretion mechanisms. Among the latter, it was demonstrated that bile salt resistance mechanisms enhance intestinal listerial survival. (Vázquez-Boland et al., 2001, Koopmans et al., 2023, Matle et al., 2020).

The most common causes of listeriosis are serotypes of *L. monocytogenes* 4b, 1/2b, and 1/2c, which account for 98% of recorded cases. Serotype 1/2 a was identified in food and linked to listeriosis in sporadic and animal cases in the humans 1. (Al-Brefkani & Mammani, 2019)

Diagnosis of Listeria monocytogenes

For the purpose of disease control and prevention, the identification of *L. monocytogenes* is crucial. With time, the cold enrichment method for detecting and isolating *L. monocytogenes* has given way to conventional as well as molecular technologies (WHO & World Organisation for Animal Health 2014).(Matle et al., 2020)

Listeria subtyping has historically been accomplished using a wide variety of techniques. These include PCR serogroup-sequence typing, SNP (i.e., single nucleotide polymorphism) analysis, phage typing, ribotyping, pulse-field gel electrophoresis, isoenzyme typing, MLSTA (i.e., multilocus tandem-repeat sequence analysis), various variants of MLST (such as MVLST (i.e., multi-virulence-locus sequence typing)), serotyping, and 10-gene multilocus sequence typing. (Jamshidi & Zeinali, 2019, Koopmans et al., 2023, Matle et al., 2020)

The identification and isolation regarding L. monocytogenes utilizing culture-based methods entails using selective agents as well as enrichment procedures, and they are typically chosen over other validated approaches for a variety of reasons, including their sensitivity, affordability, and continued status as the "gold standards" (Vázquez-Boland et al., 2001). While the process of enrichment provides the ability for L. monocytogenes' growth to measurable levels and repair of the damaged or stressed cells, the selective agents' function is to inhibit other types of the competing microflora (Matle et al., 2020). All food chain as well as primary production samples, and food samples cultured on enrichment broth, like tryptic soya yeast extract (Yassin et al., 2021), Brain Heart Infusion Broth M210 (Hiba et al., 2024), Fraser broth (WOAH, 2021, Al-Brefkani & Mammani, 2019, Ahmed et al., 2016, Gergis et al., 2024), or Listeria enrichment broth (LEB) (Vázquez-Boland et al., 2001, Hitchins et al., 2022, Parsons et al., 2019, Al-Gburi, 2020, Al-Abbidee & Alnassrawei, 2016), are covered by the principle of ISO11290 Part1 approach v. 2017² for detecting Listeria spp and L. monocytogenes.

After the enrichment process, the broth is plated onto differential or selective media such as HiCrome Listeria Selective Supplement FD-181, HiCromeTM Listeria Agar Base, along with Modified M-1417 (7,12,52) Listeria Agar supplemented with Polymyxin B sulphate, Ceftazidime, and Acriflavine hydrochloride (Yassin et al., 2021). Since IgG antibodies increase against listeriolysin, which might contribute to both non-invasive and invasive infections, listeriosis could be identified using the enzyme-linked immunosorbent test (ELISA) (WOAH, 2021, Hitchins et al., 2022, Mohamed et al., 2022, Al-Mayahi & Jaber, 2020).

Prevalence of Listeria monocytogens

In various nations, the frequency regarding high-profile outbreaks that caused numerous fatalities as well as human listeriosis cases had sharply increased (Buchanan et al., 2017, Jamshidi & Zeinali, 2019, Jackson et al., 2018). Since a lot of people eat RTE meals, the growth is mostly due to shifting consumption patterns (Domenico, 2014, Al-Ghanim & Abbas, 2021, Vázquez-Boland et al., 2001). Additionally, the danger of listeriosis has increased due to the globalization regarding the food trade as well as demographic changes, like the aging of sensitive populations and the presence of other immune-compromising infections.(Jacek et al., 2022) More cases are being indicated as a result of the implementation of sequencing techniques for the identification and type of listeriosis outbreaks. (Mahmood & Al-Gburi, 2024, Matle et al., 2020)

Even though the 50% infectious dosage in sporadic disease is likely large, the cause of listeriosis outbreaks in the human populations remains unknown. While all isolates regarding *L. monocytogenes* are capable of producing all of the virulence factors that are characteristic of the species, epidemic disease might be influenced by the enhancement regarding organism-specific virulence factors. (Yin et al., 2019, Maury et al., 2016, Koopmans et al., 2023, Schlech, 2019) According to recent

data, some cases of listeriosis may be foodborne. Food products, such as turkey franks, cold meats, and delicatessenstyle meals, were identified as development vehicles for listeriosis in case-control studies of isolated cases without an epidemic disease (WOAH, 2021, Jamshidi & Zeinali, 2019, Hamidiyan et al., 2018,). The disease is expensive in terms of both human and financial costs, with the annual rate regarding sporadic listeriosis in North America and Europe typically being 1 in 100,000 people (Vázquez-Boland et al., 2001, Jordan & McAuliffe, 2018, Zakrzewski et al., 2023, Jackson et al., 2018). In the summer and spring, sporadic listeriosis seems to be more prevalent. Seasonal differences in food product kinds that are consumed by the human populations—higher-risk foodstuffs being consumed during warmer months—could account for this. (Orsi & Wiedmann, 2016, Schlech, 2019) In its industry sampling programs, the US FDA maintains a 0-tolerance policy with regard to L. monocytogenes (Al-Ghanim & Abbas, 2021). Other nations have enacted less strict regulations, permitting a minimal level of contamination (<102CFU/g) in order to balance public health protection with unnecessary denunciation of otherwise acceptable food goods. (Schlech, 2019) We found over 80 outbreaks with a confirmed source regarding contamination that affected five or more people globally during the past forty years. Between 5 and 1566 cases were linked to such outbreaks, with South Africa (from 2017 to 2018) and Italy (in 1997) having the two biggest numbers (Koopmans et al., 2023). In the year 2000, British Columbia, Canada, experienced another noteworthy and extensively recorded outbreak. The serotype 4b L. monocytogenes that infected 84 people was obtained by eating a ripened, soft cheese (Buchanan et al., 2017, Vázquez-Boland et al., 2001). Ice cream and other frozen foods are a worrying source regarding L. monocytogenes contamination. Ten people were impacted by an ice cream-related listeriosis outbreak in the United States in 2014–2015 (Koopmans et al., 2023, Jackson et al., 2018). Andalusian chilled pork roast, which included serotype 4-b L. monocytogenes, was the source in Spain; 222 cases, including 3 fatalities, were connected to this outbreak (Koopmans et al., 2023). A serotype 4b strain associated with a company that manufactures ready-to-eat meat products was responsible for 21 cases of listeriosis in the Belgium and Netherlands, including three fatalities (14%) of the disease. A nosocomial outbreak in England that impacted nine patients, six of whom died (67%) has been connected to a supplier regarding salads as well as sandwiches to multiple hospitals in UK; the serotype has not been made public (Koopmans et al., 2023). A retrospective WGS as well as questionnaire analysis conducted in Germany from 2010 to 2021 found 22 outbreaks (Koopmans et al., 2023, Jackson et al., 2018). There are several works where bacteria had been isolated from different foods and clinical samples, however there are no data or epidemic cases in Iraq. Between December 2015 and the end of March 2016, 32 samples of imported frozen breast chicken, imported beef minced meat, and local minced beef have been gathered from various stores in Erbil. Nine samples (28.1%) out of 32 samples had positive RT-PCR results. (Salim & Othman, 2017) 1362 samples were examined in the Iraqi Kurdistan Region's Duhok area between July 2016 and May 2017. 48 (3.5%) of the samples had Listeria monocytogenes. There were 41 (5.7%) food samples and seven (1.1%) human samples. After food and human isolates were serotyped, it was discovered that 28 food isolates and 7 human isolates were members of serogroup 1/2 a (3a). Eight isolates from samples of food, however, were members of serogroup 4b. Serogroup 1/2b included five isolates of fresh red meat. Virulence genes actA, plcA, hlyA, and iap were present in all food as well as human isolates. *L. monocytogenes* that have been isolated from the milk have not been closely related to isolates from meat and humans, according to phylogenetic study depending on 16S RNA sequencing. (Al-Brefkani & Mammani, 2019)

In Duhok province, a study included 150 samples were gathered over the course of 6 months, from Mar. to Oct. 2015. These samples included 50 minced meat samples, 50 cheese samples, and 50 frozen chicken samples. Twelve isolates of L. monocytogenes were found by PCR out of 150 samples. One isolate (2%), seven isolates (14%), and four isolates (8%), from cheese, minced meat, and frozen chicken, respectively, contained L. monocytogenes (Ahmed et al., 2016). From June 2021 to July 2022, another research was conducted in Duhok city. 48 isolates had been gathered from various foods, which include raw milk, dairy products, human clinical samples, and white soft cheese. Of these, 41 isolates were from fresh red meat (Hitchins et al., 2022), frozen chicken meat (Koopmans et al., 2023), and dairy products. Multiple virulence-associated genes have been present in all of the studied isolates, in spite of the bacteria's origin. PrfA (81.2%) as well as inIA (79.2%) were the next most common genes amongst the isolates, succeeded by inIC (93.80%), inIB (91.70%), and inIJ (83.30%). (Al-Brefkani et al., 2023) From February to June 2019, 86 cervical as well as placental swabs have been taken from females who had abortions or were in early labor stages. These included Pap smears from pregnant women who had premature births or miscarriages, whose ages ranged from 24 to 46 years old, and who had clinical symptoms regarding cervicitis as well as vaginitis that have been determined by Kirkuk General Hospital specialists. 34 out of the 86 swabs, or 39.53% of the total, tested positive for the L. monocytogenes, according to the data(.Mohamed et al., 2022) Brain, blood, milk, placenta, vaginal swabs, and gall bladders have been collected from 450 sick ewes in different parts of the Nineveh Governorate in Northern Iraq between February 2022 and June 2023. The enzyme-linked immunosorbent assay (i-ELISA) and anti-LLO were employed. L. monocytogenes was the most common species found, accounting for 12.1% (127/1042). (Asim et al., 2024). Through identifying the bacteria in both raw milk from Salahudeen province market and the milk of aborted animals, it is possible to determine L. monocytogenes prevalence in these cows and the contribution of milk to the spread of the pathogen. Between December 2018 and June 2019, 46 milk samples from 46 aborted cows have been collected for the study in order to identify the causative factors. Additionally, eight fetal samples as well as 38 vaginal swabs have been obtained from same aborted cows. Additionally, 30

samples of the raw milk have been collected from the Salahudeen province market. L. monocytogenes was found in 5 (13.1%) vaginal swabs, 13 (28.26%) aborted cow milks, 2 (25%) aborted fetuses, and 9 (30%) raw milks, according to the results. virulence factors; most isolates had InlA, InlJ, and HIY (the rate of isolation had ranged within 75% to 100%). (Noomi et al., 2021). In the Baghdad, a study has been carried out in order to look into the possible involvement of Listeria spp. in common carp fish. Between December 2017 and March 2018, a total of thirty fresh, uncooked common carp (Cyprinus carpio) have been bought from fish vendors at different local marketplaces in Baghdad. According to the findings, L. monocytogenes was found in the viscera of common carp fish in 6.66% of cases, and the isolates were pathogenic in mice. According to the findings, L. monocytogenes in fish may play a significant part in the human public health and hygiene. (Al-Gburi, 2020) In the months of June through July of 2019, 45 frozen meat samples (15 samples of minced red meat, poultry, and fish) have been gathered from various Baghdadi marketplaces. According to the culturing approach's results, there were 14 out of 45 (31.1%) isolates of L. monocytogenes. Compared to the other two forms of meat, fish had a significant incidence regarding such bacterium (11/15, 73.3%). 1/15 (6.7%) in chicken and 2/15 (13.3%) in red meat. RT-PCR assay, which targets inlA gene sequence, revealed that 10/45 (22.20%) of the samples that have been tested positive for L. monocytogenes, which has been just discovered in fish samples (10/15, 66.70%), but not in chicken or minced red meats. (Yassin et al., 2021)

From November 2019 to December 2020, a group of Iraqi women who experienced miscarriages had their clinical specimens gathered from public maternity institutions in Baghdad. According to the results, 59 specimens (33.5%) tested positive for Listeria species. In real-time PCR, 75 (32.4%) isolates from the 59 women who tested positive had the hlyA gene. (Hiba et al., 2024) A study conducted between September 2023 and March 2024 sought to identify Listeria species in clinical cow's mastitis milk. A PCR analysis of 50 clinical cows' mastitis milk samples from farms in Baghdad, Iraq, had shown that Listeria spp. Have been present in 6% of clinical mastitis milk, with L. monocytogenes accounting for 4% and atypical hemolytic L. innocua for 2%.11. In Baghdad, Listeria monocytogenes was found in certain imported cheeses with the use of a new technique, referred to as Clearview. The findings indicated that whereas Lafschri samples did not exhibit any indication regarding contamination with L. monoicytogenes, samples of cheeses have been contaminated with the bacteria in varying percentages (10%) of the Tehama cheese, 5% of the Mozhurella cheese, and (10%) of the Presedant cheese (Mutlag et al., 2013). Three hundred gallbladder samples from sheep and cattle have been gathered for other investigations that identify Listeria monocytogens from animals. Isolates have been verified using the API-Listeria system as well as the existence of hemolysin (hyl) gene. They were after that tested for the existence of L. monocytogenes using d2 (division 2), glt, d1 (division 1), flaA (flagellin protein), and mama (mismatch amplification mutation assay) genes in a PCR-based serotype identification procedure. in the central Iraqi province of Najaf. The time frame for collecting the specimens was November 2015–April 2016. Six (4.0%) samples of sheep and two (1.3%) samples of cattle yielded a total of 8 (2.70%) *L. monocytogenes*. One strain was found to be 1/2 c or 3c serotype, while the other isolates have been classified as 4a, 4c, 1/2a, or 3a serotypes. Gallbladder samples containing the 1/2a serotype suggest a concern to the public's health from meat cross-contamination at slaughterhouses. (Al-Ali et al., 2018)

In Karbala and its environs. 250 milk and dairy product samples have been chosen randomly between January and March 2022. PCR method revealed that, out of 250 samples regarding raw milk as well as dairy products from cows susceptible to L. monocytogenes through microscopical examination, 48% of the samples have been positive through conventional PCR and amplification by using PCR and PCR Sequencing(Abdzed et al., 2022). At the Maternity and Children's Teaching Hospital in the Al-Qadisiyah Governorate, samples have been gathered from women who were experiencing miscarriages as well as infected children between November 2014 and April 2015. Thirty-two infected children and sixty-five samples from women were collected. Animals that were sampled included bitter samples from Diwaniyah slaughterhouse, which included 100 cow and 100 sheep samples. Additionally, 200 milk samples—100 samples for every sheep and cow-were gathered from various rural areas within the Al-Qadisiyah Governorate. According to the study's findings, 9.27% of the total sample obtained from samples of humans have been isolated L. monocytogenes bacteria. With regard to cows, the isolation rates from milk as well as bile samples have been 2% and 3%, respectively. Isolation rate in women suffering miscarriages has been 4.61%. The findings had shown that the prevalence of L. monocytogenes infection in humans has been rather high in Al-Qadisiyah Governorate, highlighting the significance regarding the disease spread and health issues brought on through such bacterium. The findings included the isolation regarding the bacterium from milk samples as well as bile from cows and sheep, which can be a source of human transmission through contaminated meat, milk, along with its derivatives. (Al-Abbidee & Alnassrawei, 2016) to determine the virulence genes and antibiotic susceptibility of isolates, as well as the impact of L. monocytogenes on pregnant Iraqi women in the hospitals of Al-Diwaniya. From January to May 2019, 90 patients who have experienced spontaneous abortions at the Maternity and Children Teaching Hospital in Al-Diwaniya City had 360 specimens taken, such as urine, blood, endocervical, and vaginal. The antimicrobial resistance genes and virulence factors had been identified using PCR. Thirteen isolates (14.5%) and 15 positive samples (16.60%) of the patients were identified using the PCR and ELISA assays, respectively. In cases involving women who had abortions, the overall L. monocytogenes strains' isolation rate was 13/270 (4.8%). The presence of genes linked to virulence factors, which include actA, plcA, hlvA, and prfA, in all strains of L. monocytogenes made them extremely virulent. (Al-Mayahi & Jaber, 2020).

Milk as well as milk products that are often consumed, such as local cheese, buffalo raw milk, and local cream was randomly selected from several marketplaces in the province of Al-Qadissiya. Local cheese had the highest incidence regarding L. monocytogenes (14.2%), succeeded by buffalo raw milk (6.2%), and local cream (3.4%), according to the results of PCR methods used to amp up virulence factors (hly genes). (Esraa, 2017). Between August 2006 and June 2007, 300 raw milk samples from sheep, buffaloes, and cows have been gathered from farmers' homes in various locations throughout Basrah city. Cow milk samples had the highest Listeria prevalence (11.0%), followed by the sheep milk (8.0%), and buffalo milk (3.0%). Beta hemolysis, cold enrichment, Anton test, selective media, tumbling and inverted pinetree motility, and sugar fermentation tests have been utilized in order to confirm all of the bacterial isolates. (Abbas & Jaber, 2012) Between September 2015 and March 2016, 200 distinct food samples were gathered from the markets of Basrah in southern Iraq. These samples contained 50 worker hand swabs, frozen burgers, frozen fish, and frozen poultry. With the use of inlB specific gene, the PCR method has been utilized in order to assess the existence of L. monocytogenes. Just four samples (7.27%) had been found to be contaminated with L, monocytogenes, according to PCR results. To conclude, a hazardous kind of bacteria that can cause human illness may be present in frozen food. (Al-Ghanim & Abbas, 2021)

Treatment and antibiotic resistant

The treatment regarding human listeriosis frequently unproductive due to L. monocytogenes' lengthy incubation period, which causes the treatment period to vary depending on the infection's severity. L. monocytogenes could invade the majority of cell types, making it difficult to treat human listeriosis. (Wiktorczyk-Kapischke et al., 2023, Matle et al., 2020) In order to avoid death, complications, and long-term aftereffects from human listeriosis, prompt delivery of a sufficient antimicrobial treatment is essential. (Koopmans et al., 2023, Schlech, 2019) Ampicillin or penicillin G, when combined with an aminoglycoside like gentamicin, is the recommended antibiotic to treat human listeriosis. The second-choice treatment is trimethoprim plus a sulfonamide, like sulfamethoxazole-co-trimoxazole. In addition, human listeriosis was treated with vancomycin, erythromycin, and tetracycline. (Jamshidi & Zeinali, 2019, Wiktorczyk-Kapischke et al., 2023, Koopmans et al., 2023, Mendum & Smith, 2002, Matle et al., 2020). A study conducted by (Mohamed et al., 2022) on the efficiency regarding antibiotics on isolates of L. monocytogenes in Kirkuk, Iraq, revealed that all of the isolates have been 100% sensitive to antibiotics ampicillin and chloramphenicol, succeeded by gentamycin, to which 88.24% of the isolates have been sensitive, trimethromycin, with a 38.24% sensitivity rate, and erythromycin, with a sensitivity rate of 79.41%. By contrast, the sensitivity rate to antibiotic Cloxacillin was 29.415%. Yet, L. monocytogenes has significantly sped the development of bacteria toward resistance. (Matle et al., 2020) L. monocytogenes isolates from meat as well as clinical samples in Duhok City demonstrate resistance to ampicillin 29 (64.4%), tetracycline 33 (73.3%), erythromycin 26 (57.8%), penicillin 28 (62.2%), and gentamycin, lindamycin, and vancomycin 24 (53.30%). Of the 45 isolates of *L. monocytogenes* that were examined, 37 (82.20%) showed phenotypic susceptibility to the meropenem, which has been followed by SXT 30 (66.7%) and ciprofloxacin 36 (80.0%). (Al-Brefkani, 2023).

Prevention

Controlling human listeriosis, a foodborne infection brought on by an environmental organism, focuses on lowering L. monocytogenes contamination in food chain.(Buchanan et al., 2017) The food industry has made significant attempts to prevent Listerial contamination "from farm to fork" with particular control techniques as well as risk analysis models, and through food safety regulations and educational initiatives.(Jackson et al., 2018) Control strategies included employee food hygiene training programs in addition to routine microbiological testing of processed and raw foods and sanitation plans in the event that L. monocytogenes was detected. It is necessary to stress the significance of providing consumers and risk groups with clear information and education regarding listeriosis. Pregnant women have historically been the focus of educational initiatives. Other settings that could benefit from listeriosis education, such as nursing homes, hospitals, and collective canteens where food-related outbreaks have occurred amongst the vulnerable patients or in the neonatal units as a result of inadequate hygiene (Koopmans et al., 2023)

Conclusion

Listeria monocytogenes as an evolving global threat requiring coordinated, science-based intervention strategies. Recent advances in species discovery, genomic surveillance, and pathogenesis understanding provide powerful tools for enhanced control. However, significant disparities exist between regions, with countries like Iraq facing particularly severe challenges including extremely high antibiotic resistance rates and limited surveillance capabilities.

The integration of Iraqi epidemiological data with global trends demonstrates the critical need for standardized global surveillance systems, Urgent antibiotic resistance monitoring and management programs, Enhanced regional cooperation through international networks, Targeted research addressing local epidemiological patterns and Strengthened food safety systems incorporating advanced detection and control technologies. Future efforts must prioritize closing surveillance gaps, implementing resistance management strategies, and fostering international collaboration to address this persistent global health threat. The Iraqi experience underscores the importance of regional data integration in understanding global pathogen dynamics and developing effective control strategies.Examining regional research on various food, animal, and clinical samples reveals variations in

isolation rates, albeit occasionally minimal ones. The examination as well as isolation of such bacteria, which might be accountable for cases of poisoning or miscarriage in women or animals without paying attention to them, are brought to light by the presence of such bacteria and the lack of epidemiological studies on them.

Author Contributions

Each author has significantly contributed to the study's conception, design, data collection, analysis, and interpretation. All authors were involved in writing the manuscript or critically reviewing it for its intellectual value. They have reviewed and approved the final version for submission and publication and accept full responsibility for the content and integrity of the work.

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Conflicts of Interest

The authors state that there are no financial, personal, or professional conflicts of interest associated with this research.

Ethical Approvals

This research did not involve the use of human participants or animal subjects and therefore did not require ethical clearance.

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