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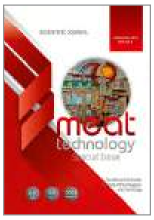

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Review Paper

The presence of virulence-related genes among *Listeria Monocytogenes* strains and their correlation with pathogenic potential

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ABSTRACT

Listeria monocytogenes remains a significant public health concern due to its high mortality rate in susceptible individuals. Contaminated food, particularly ready-to-eat products, are the primary route of transmission to humans. Previous studies have shown that *L. monocytogenes* exhibits heterogeneous virulence, with strains ranging from hypervirulent to hypovirulent. *L. monocytogenes* has been found to have pathogenicity islands and other virulence factors scattered across the bacterial genome (e.g. *inlAB* locus). Further, the *inlA-inlB* locus and LIPI-1 are conserved in almost all *L. monocytogenes* isolates, emphasizing their important role for pathogenicity. Literature data showed that using genetic virulence profiles to predict virulence potential offers useful information for risk assessment in the food sector, although it also has drawbacks. Also, the use of whole-genome sequencing as the gold standard approach, enhances quantitative microbiological risk assessment and improve listeriosis control.

1. Introduction

It is well known that the intracellular pathogen *Listeria monocytogenes* is the cause of listeriosis in humans and animals such as sheep, goats, cattle, pigs and fowl (*Borovic et al.*, 2014; *Headley et al.*, 2014; *OIE,* 2014; *Nilsson & Karlsson*, 1959; *Biester & Schwarte*, 1940; *Ramos et al.*, 1988). In ruminants, several predisposing factors contribute to the occurrence of listeriosis (*Burgess & Lohmann,* 2006; *Amene & Firesbhat,* 2016) including immune status, seasonal variations, production-related stress and management practices such as inadequate housing, overcrowding and insanitary conditions. This bacterium is widespread in farm environments, food processing areas and food products (*Lakicevic et al.*, 2022). Although *L. monocytogenes* evolves slowly, it exhibits a high degree of diversity (*Ragon et al.*, 2008; *Orsi et al.*, 2011). It is a member of a genus that currently includes 28 known species, 22 of which have been reported since 2010 (*Orsi et al.*, 2024). As noted by *Dreyer et al.* (2016), ruminants are exposed to a wide range of genetically diverse strains during their time on farms. Nonetheless, a particular hypervirulent genotype, ST1, is more frequently associated with rhombencephalitis than with other illnesses. Also, *L. ivanovii* has pathogenic potential to infect ruminants, causing abortions, stillbirth and sepsis. These are the two (*L. monocytogenes* and *L. ivanovii*)

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species that share many virulence factors, contributing to their ability to cause disease (*Orsi & Wiedmann,* 2016).

L. monocytogenes infection depends on various virulence factors involved in pathogenesis, the effectiveness of the host's immune system and the number of ingested bacteria (FAO/WHO, 2004). Since variations in virulence among strains of *L. monocytogenes* can influence infection and clinical outcome, assessing the virulence potential of isolates is crucial for public health (*Smith et al.*, 2019). Important virulence factors are encoded by the PrfA- regulated *inlAB* locus (involvement in adhesion) and the pathogenicity islands LIPI-1, LIPI-3, and LIPI-4 (*Gelbíčová et al.*, 2015; *Maury et al.*, 2016; *Quereda et al.*, 2018). Significantly, all *L. monocytogenes* isolates have a conserved *inlAB* locus and the LIPI-1 island underlying their critical role in pathogenicity.

2. Internalins

Internalin A (*inlA*), an 80 kDa surface-invasion protein, is one of *L. monocytogenes*' primary virulence factors and was first reported by *Gaillard et al.* (1991). In the initial stage of intestinal barrier invasion, *inlA* mediates the pathogen's adherence and internalization into enterocytes via its specific receptor, E-cadherin (*Drolia & Bhunia,* 2019). Of the four divergent lineages, only hypovirulent lineage II strains harbor a truncated form of *inlA*, which could explain the low occurrence of these strains in human outbreaks (*Maury et al.*, 2016; *Van Stelten et al.*, 2010). There have been fewer documented listeriosis outbreaks in Japan compared to other countries, which may suggest that circulating strains of *L. monocytogenes* in Japan possess more mutations in *inlA* or in other virulence factors, potentially reducing their ability to invade cells (*Yamazaki et al.,* 2025). Internalin B (*inlB*), another a surface protein, also plays a pivotal role in initiating infection via its cellular receptor, c-Met, but unlike *inlA*, it does not possess the LPXTG motif (*Buchrieser*, 2007). *Quereda et al.* (2019), using the lineage I strain F2365, showed that *inlB* increases *L. monocytogenes* infection of the liver and spleen. Besides *inlA* and *inlB*, the literature also describes other members of the *inl* family, with *inlC*, *inlF* and *inlP* being extensively characterized (*Bierne et al.,* 2007; *Disson & Lecuit*, 2013; *Faralla et al.,* 2018; *Ghosh et al.*, 2018).

3. Listeriolysin

Listeriolysin (LLO), a pore-forming cytolysin encoded by *hly*A, enables bacteria to escape the phagosome and enter the cytosol of the infected cell where they multiply (*Birmingham et al.*, 2008; *Nguyen et al.*, 2019). To prevent lysis of the host plasma membrane, the activity of LLO must be regulated through multiple regulatory mechanisms. Recently, *Agbavor et al.* (2024) demonstrated that chaperone PrsA2 controls the secretion, stability, and folding of LLO throughout the bacterial infection. According to *Wang et al.* (2015), the flavonoid fisetin is an effective antagonist of LLO-mediated hemolysis. Furthermore, *Li et al.* (2020) concluded that morin, an edible flavonoid, prevents the oligomerization of LLO and effectively reduces the inflammation caused by *Listeria* infection. *Wang et al.* (2022) demonstrated that kaempferol reduces *L. monocytogenes* infection by inhibiting LLO pore formation and the inflammatory response.

4. Listeria pathogenicity island (LIPI-1)

The highly conserved *Listeria* pathogenicity island (LIPI-1) harbors the most significant virulence-associated genes of *L. monocytogenes*, including the previously mentioned *hly*, *actA*, *plcA, plcB* and *mlp* genes, which are positively regulated by transcriptional regulator PrfA (*Wiktorczyk-Kapischke et al., 2023*; *Sibanda & Buys, 2022*; *Osek & Wieczorek*, 2022; *Quereda et al.*, 2021*)*. LIPI-1 is present in both pathogenic *L. monocytogenes* and *L. ivanovii* but it is absent in the avirulent *L. innocua*. However, LIPI-1 was discovered in atypical hemolytic *L. innocua* strains (*Johnson et al.,* 2004), which exhibited virulence potential in both the mouse model (*Bolger et al.*, 2014) and zebrafish larvae model (*Kaszoni-Rückerl et al.*, 2020) although these strains were less virulent compared to *L. monocytogenes.* The study of *Gradovska et al.* (2023) highlighted that *L. innocua* possesses diverse virulence potentials for cattle, underscoring its significance in the dairy production chain and cattle breeding. The authors also emphasize that unrecognized *L. monocytogenes* contamination and outbreak events could be indicated by the presence of *L. innocua*. Also, some isolates of *L. seeligeri* exhibit hemolytic activity and carry LIPI-1 (*Müller et al.,* 2010; *den Bakker et al.,* 2010). A previously healthy adult presenting with acute purulent meningitis is one of the rare human cases of *L. seeligeri* that have been reported (*Rocourt et al.*, 1986).

L. ivanovii harbors a similar chromosomal island called LIPI-2 (22 kbp), which can cause listeriosis in ruminants, particularly sheep (*Vázquez-Boland et al.*, 2001). This region includes the genes *i-inlB2*, *i-inlL*, *i-inlK*, *i-inlB1*, *i-inlJ*, *i-inlI*, *i-inlH*, *i-inlG*, *smcL*, *i-inlF*, *i-inlE* and *surF3, which mainly code for listerial proteins of the internalin family (Sergeant et al.*, 1991; *Guillet et al.,* 2010).

4. Listeria pathogenicity island (LIPI-3)

Listeria pathogenicity island (LIPI-3), primarily found in lineage I isolates and typically absent from lineage II isolates, encodes listeriolysin S (LLS), a bacteriocin that alter the host intestinal microbiota (*Quereda et al.,* 2017). Oxidative stress induces the expression of LLS, which enhances its ability to evade phagosomes and increases pathogenicity. Although it is representative of clinical isolates, LIPI-3 is also found in food strains, emphasizing their possible risk to humans (*de Melo Tavares et al.,* 2020).

4. Listeria pathogenicity island (LIPI-4)

Listeria pathogenicity island (LIPI-4), a six virulence gene cluster (6 kbp), is linked to central nervous system infections and to maternal neonatal infections (*Maury et al.*, 2016). It was reported that LIPI-4 is most prevalent in hypervirulent CC4 and CC87 strains (*Kim et al.*, 2018). However, literature data also show that some *L. innocua* strains carry LIPI-4 (*Disson et al.*, 2021; *Moura et al.*, 2019). *Lee et al.* (2023) reported that LIPI-4 was highly conserved across examined strains of *L. innocua* and strains that had been previously studied (*Moura et al.*, 2019). Nevertheless, LIPI-4 of *L. innocua* and its counterpart in *L. monocytogenes* have been shown to be much less similar, with sequence identities ranging from 83.7% to 84.0%, indicating that these two species possess distinct variants of LIPI-4 (*Lee et al.*, 2023).

5. Conclusion

Understanding the intraspecies variability of virulence genes in *L. monocytogenes* is essential for risk assessment, outbreak investigation and the development of targeted prevention and control strategies. Whole genome sequencing supports risk assessment and surveillance, helping to identify potentially high-risk strains in food production and processing environments. Despite extensive understanding of intraspecies pathogenicity, all strains are still classified as pathogenic for regulatory purposes.

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