

A review on *Listeria monocytogenes* in food: prevalence, pathogenicity, survivability and antibiotic resistance

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Abstract

The ubiquitous of *Listeria monocytogenes* in the environment and in the niche of foods can be related with the ability of the pathogen to survive in adverse conditions, in example, low temperature and pH, high concentrations of salt and bile, oxidative stress and carbon starvation. Besides, most of the human listeriosis are food-borne and *L. monocytogenes* consists of a spectrum of strains with varying virulence and pathogenicity. Moreover, the virulent regulators play a role in facilitating better adaption or persistence of *L. monocytogenes* in the food environment. This review included prevalence, pathogenicity, survivability and antibiotic resistance of *L. monocytogenes* in various categories of foods such as meat, seafood, dairy products and vegetables. The recent updates on listeriosis or foodborne cases associated with *L. monocytogenes* should be studied in order to have a better understanding of their persistence in the food chain and its public health impact especially among the high-risk groups: elderly, infant, pregnant woman and immunocompromised individuals.

1. Introduction

Listeria monocytogenes is a facultative saprotroph which is present broadly in dirt, plants, ground water and vegetation. It has been related with numerous foodborne diseases to the consumption contaminated foods particularly ready-to-eat products, dairy products, meat and poultry products (Letchumanan *et al.*, 2018). Initially, there were only three genetic lineage that represent the phylogeny of *L. monocytogenes*. Later, lineage IV was discovered that showed some differences in terms of phylogeny compared to other lineages (Lomonaco *et al.*, 2015). *Listeria* is a psychrotropic organism which is likely to endure well and to develop gradually at chill temperatures - clarified by the evidence of vast increment of *L. monocytogenes* within beef carcasses through the production chain until ground beef products (29%) (Khen *et al.*, 2014). Besides, *L. monocytogenes* is a halotolerant pathogen with the ability to survive and endure low pH and high salt environment as Meloni (2015) reported in the prevalence of 45% *L. monocytogenes* in Mediterranean-style dry fermented sausages.

There are thirteen isolates of *L. monocytogenes* that separated according to somatic (O) and flagellar (H),

including 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7. (Allerberger, 2003). Li *et al.* (2018) reported that 1/2a, 1/2b, 1/2c and 4b are the strains that frequently isolated from the food in China. In Europe, listeriosis incidence had been increasing for the last few years which may be related to multi-component foods such as sandwiches and mixed salad that supports the growth of *Listeria* (Lomonaco *et al.*, 2015). Besides, there are studies that showed recombination is possible in the *Listeria* genome for example between *L. monocytogenes* / *L. innocua* and this condition may cause certain strains to survive in various environments including adverse conditions (Orsi *et al.*, 2008; den Bakker *et al.*, 2008). According to a meta-analysis review from Iran by Ranjbar and Halaji (2008), the prevalence of *L. monocytogenes* from human origin was higher (10%) compared with animal and food resources (7% and 4% respectively). However, international comparison on the prevalence of *L. monocytogenes* is difficult due to multifactorial nature of *L. monocytogenes* (Ranjbar and Halaji, 2018).

There are certain groups of individuals that have a higher risk to be infected by listeriosis. In the high-risk group, pregnant women are at the highest risk of contracting listeriosis, which can potentially lead to

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miscarriage, stillbirth, preterm birth, and congenital neonatal infections (Vázquez-boland *et al.*, 2017). Nayak *et al.* (2018) reported that a three-year-old child developed infection due to *L. monocytogenes* and was treated with IV ceftriaxone. Unfortunately, the child died due to the transmission of transposon from other bacteria that mutually-induce the resistance of *L. monocytogenes* towards empirically administered antibiotics. Based on the results from the relative risk of listeriosis study, the incidence rate of listeriosis increased with age (especially after age of 45 years old) (WHO and FAO, 2004). The incidence rate was reported by Poulliot *et al.* (2012) to be 4.7 and 53.8 for population of 45-49 years old and 85 years old respectively.

Over the years, *L. monocytogenes* remained a 'highlighted' bacterium in the food industrial sector due to its high incidence and mortality rates. Hence, it is necessary to have updated information on the risk profile of *L. monocytogenes* in order to manage the risk of *L. monocytogenes* in food safety. This review is aimed to discuss the prevalence and antibiotic profile of *L. monocytogenes* in various food matrices recently and the related foodborne outbreaks in Asia countries as continuous updated case-study and review about the spreading of *L. monocytogenes* is important for tracking the source of contamination.

2. Virulence and pathogenicity

For *L. monocytogenes* to infect the host cell successfully, the bacterium not only have to adhere, invade, multiply and spread to neighboring cell but, also require some regulators to monitor the virulence and pathogenicity of bacterium. Table 1 summarizes the required virulence regulators by *L. monocytogenes* (Camejo *et al.*, 2011).

Human can be infected by *L. monocytogenes* due to human gastrointestinal cells have receptors that facilitates adhesion and internalization of internalin A and B of *L. monocytogenes* (Buchanan *et al.*, 2017). In addition, *L. monocytogenes* has different mechanisms that aid in the adaptation of adverse conditions in the gastrointestinal tract of human. For example, the acidic condition of stomach is mediated by the glutamate decarboxylate system and arginine deiminase system of the bacteria and thus, the pH of bacterial cytoplasm is maintained (Ryan *et al.*, 2009). However, sometimes Premature Stop Codons (PMSC) that happened in internalin A of *L. monocytogenes* strains (serotype 1/2 a, 1/2b and 1/2c that frequently isolated from food sample and environmental resources (about 50%) are not pathogenic and thus will not cause listeriosis (Marquis *et al.*, 2015).

3. Foodborne outbreaks

The prevalence of *L. monocytogenes* in retail raw foods covered most of the common place capitals in China were contemplated with testing of 1036 samples of vegetables, mushrooms, crude meat, seafood and quick-frozen items by which the absolute pervasiveness of *L. monocytogenes* was 20.0% (Wu *et al.*, 2015). There was no national clinical listeriosis reconnaissance in China until 2013, however, numerous Chinese medical journals have reported listeriosis reports. One past deliberate survey was directed to delineate the clinical and epidemiologic qualities of listeriosis in China through writing recovery from 1964 to 2010, and the outcomes demonstrated that 52% of clinical cases were pregnancy-related patients; the most normal clinical appearance was septicemia (46%), trailed by CNS contamination (31%) and central disease or

Table 1. *Listeria monocytogenes* virulence regulators and their functions.

Virulence Regulators	Characteristics	Functions	References
<i>PrfA</i>	<ul style="list-style-type: none"> Composed of 10 virulence genes 	<ul style="list-style-type: none"> Control the expression of the main virulence determinants of bacterium 	
σ^B	<ul style="list-style-type: none"> Always joint with <i>prfA</i> (subset of <i>prfA</i>) 	<ul style="list-style-type: none"> Regulates wide set of genes that aid the bacterium in stress tolerance, carbohydrate metabolism, motility and cell envelope process 	
<i>VirR</i>	<ul style="list-style-type: none"> <i>VirR</i> regulon were increased during infection 	<ul style="list-style-type: none"> Involved in <i>Listeria</i> pathogenesis Activate <i>mprF</i> gene that provide resistance to defensins during invasion into host cell 	(Camejo <i>et al.</i> , 2012)
<i>MogR</i> , <i>DegU</i> , <i>GmaR</i>	<ul style="list-style-type: none"> After initiation, flagellar production will be decreased 	<ul style="list-style-type: none"> Control flagellar production Aid the colonization of bacterium into host cell 	
<i>Hfq</i>	<ul style="list-style-type: none"> RNA binding protein 	<ul style="list-style-type: none"> Regulates stress response and virulence of bacterium 	

Table 2. Summary of some reported food-borne listeriosis in some Asia Countries

Country	Case Description	Sources
Vietnam	<ul style="list-style-type: none"> • Three adults were presented with <i>L. monocytogenes meningitis</i> • Three of them experienced fever, headache and vomiting • Consumption of contaminated raw milk and soft cheese might be one of the reason for the outbreak 	(Chau et al., 2010)
China	<ul style="list-style-type: none"> • Eighty-two cases caused by contaminated egg products during preparation and packaging • Four pregnant woman infected through consumption of roast meat, dairy products, seafood and preserved vegetables. 	(Feng et al., 2013)
Japan	<ul style="list-style-type: none"> • Eighty six persons had been infected with <i>L. monocytogenes</i> • Forty-four percent of patients developed gastroenteritis • Outbreak was caused by cheese 	(Makino et al., 2005)
Thailand	<ul style="list-style-type: none"> • An immunocompromised patient had brain abscess due to <i>L. monocytogenes</i> • Most probably due to exposure to contaminated shrimp product in company 	(Treebupachatsakul et al., 2006)
Turkey	<ul style="list-style-type: none"> • Fifty-four case patients were identified • Eight died and three pregnant women had fetal deaths • Turkey deli-meat was the source of multistate outbreak 	(Gottlieb et al., 2006)

gastroenteritis (23%), and the general case-casualty rate was 26% (Feng et al., 2013). In the United States, the Centers for Disease Control and Prevention (CDC) assesses that listeriosis is in charge of around 1,600 cases and 260 deaths for each year (Morrison et al., 2018). The general EU reported rate of listeriosis was 0.46 cases per 100,000 population with a case-casualty rate of 17.7%. In Asia, there were few listeriosis outbreak reported in some Asia countries as shown in Table 2.

4. Meat

Alewy et al. (2016) reported that among different types of red meat from the study in Dharma Governorate, prevalence of *L. monocytogenes* is the highest from cattle (26%), trailed by goat meat (25%) and sheep meat (17.9%) and therefore, red meat was proved to be the vehicles of transmission of *L. monocytogenes* to human. In country with four seasons, there was a statistically significant effect by the seasonal variation on the occurrence of *L. monocytogenes* as shown in study in Southern Italy that the number of meat derived products (raw pork sausage and entrails lamb rolls) were higher in spring and autumn by which the pathogen was detected in January, March and October but none for the samples in February, June, July, August and November (D'Ostuni et al., 2016). It is noteworthy that both of the study in Malaysia and Egypt, the isolation rate of *L. monocytogenes* from beef burger were high which was 4.0% and 22.9%, respectively, in fact, the added spice could not prevent the growth of *L. monocytogenes* on raw meat effectively (Wong et al., 2012; Reda et al., 2016). In Thailand, a study showed that improper application of hygienic or good manufacturing practices had caused the contamination of *L. monocytogenes* in samples of fresh frozen chicken meat and RTE chicken products (2.5% and 0.2%) respectively as there was no

contamination detected in the primary production stages. However, *L. monocytogenes* was detected in the slaughterhouse and RTE processing plants (Kanarat and Sukhapesna, 2011). The oxygen level, temperature and time of storage could be the factors that contributed to the growth of *L. monocytogenes*. Cell counts of *L. monocytogenes* in beef was higher (5-7 log CFU/g) when stored at 22°C compared with 7°C. Besides, the growth rate of *L. monocytogenes* was the lowest when stored in anaerobic condition (Al-qadiri et al., 2015).

Over the last couple of decades, the expansive usage of antibiotics in human and animals, has significantly added to the development and spread of antimicrobial resistance foodborne pathogens including *L. monocytogenes* (Wilson et al., 2018). For example, a study done in Shanghai, China showed that isolates of *L. monocytogenes* (5.83%) from some of the meat products such as duck, chicken, pork and beef are resistant towards tetracycline (Su et al., 2016). Previously, penicillin, ampicillin and aminoglycoside are an effective treatment for listeriosis. However, in a study done in Turkey showed that some strains of *L. monocytogenes* (6.1%) isolated from raw meat showed resistant to ampicillin and the isolates are said to harbor the virulence gene of *hlyA*, *actA*, *inlA*, *inlB*, *inlC*, *inlJ*, *prfA*, *plcA* and *iap* (Arslan et al., 2018). In southern China, a study showed that secondary contamination is the major reason that cause the wider resistance range of antibiotics in isolates (10.2%) from pork and its products in the market (Li et al., 2016).

5. Seafood

Smoked fish, especially cool smoked salmon, which is mainstream in the food industry of Ireland and Scotland, ought to be of worry because it is always

processed as a ready-to-eat (RTE) food. Cold smoking process tend not to have sufficient microbial inactivation steps that could decontaminate *L. monocytogenes* from the crude material (Rotariu *et al.*, 2013). Over 21 months of sampling period, there were 4.8% and 10% of smoked salmon contamination with *L. monocytogenes* from two food processing facility respectively in Ireland (Leong *et al.*, 2015). Besides, there was a study that demonstrated raw fish samples and the fish samples from the open-air market can lead to zoonotic potential of listeriosis as there were 72.1% of *L. monocytogenes* belonged to serovars 1/2a, 4b and 1/2b recovered from the samples. Furthermore, 14% of *L. monocytogenes* isolates possessed more than one antimicrobial resistance genes that resulted significant resistance to tetracycline (27.9%), trailed by ampicillin (20.9%), cephalothin, penicillin and streptomycin (16.3%) (Jamali *et al.*, 2015).

The transcriptomic analysis revealed that *L. monocytogenes* able to survive in salmon by upregulating the cobalamin biosynthesis pathway in order to utilize ethanolamine as a nitrogen source and 1,2-propanediol as a carbon source from salmon and thus facilitate adaption of the bacterium in vacuum-packed cold-smoked salmon (Tang *et al.*, 2015). Besides, salmon is believed to be a common or native source for isolation of T4 pulsotype in Ireland and T4 is a strain that harbored Tn6188 transposon gene which related to stress resistance, like resist to commonly used sanitizer (BC sanitizer) which is an alcohol based sanitizer in food processing facility and thus support the occurrence and persistence of *L. monocytogenes* in seafood processing facilities and smoked salmon (Leong *et al.*, 2015). Moreover, the good adaptability of *L. monocytogenes* to cold condition also offer an advantage for the survivability of *L. monocytogenes* in fish product as demonstrated by Liu *et al.* (2016), *L. monocytogenes* are able to multiply critically (1.5 to 7.0 log CFU/g) during refrigerated storage while in 12 weeks of frozen storage (-18°C), less than 1-log unit of reductions were observed in tuna samples.

6. Dairy products

In 2015, although the rate of listeria contamination on the ice-cream products were low which is 8 cells/g (unable to support the growth and development of *L. monocytogenes*), listeriosis outbreak still could happen in a condition that most purchasers of the items won't turn out to be sick (Pouillot *et al.*, 2016). In Europe, the first reported listeriosis outbreak was associated with fresh cheese (Linnan *et al.*, 1988) and from an EU baseline survey (EFSA, 2013), there are 0.47% sample of cheese at retail tested positive for *L. monocytogenes*. Furthermore, there was another study in Turkey

demonstrated that the risk of *L. monocytogenes* contamination in dairy products was still low, by which only two cheese samples positive for *L. monocytogenes* (2.35%) but none from the raw milk (0%) (Aygun and Pehlivanlar, 2006). The survival of *L. monocytogenes* can be varied according to different types of cheese, for example the growth of the pathogen is quite impossible in the hard cheese compared with soft or semi-soft cheeses due to lower pH and moisture content (Gérard *et al.*, 2018). For example, a study showed that 41.17% isolates of *L. monocytogenes* harbored the serotypes, 1/2a, 1/2b and 4b, from a Brazilian soft cheese (Da Silva *et al.*, 1998). However, in Chile, no contamination of *Listeria* spp. in hard cheese (Cordano and Rocourt, 2001). The dairy farm environment can be the source for the survival and dissemination of *L. monocytogenes* from the livestock in the food chain due to poor silage quality and insufficient hygiene. A total of 38 isolates of *L. monocytogenes* from four dairy farms in east Tennessee, USA. The most worrying part is that 19 isolates from 38 harbored high frequency of antimicrobial resistance gene floR (66%) and followed by others such as penA (37%), strA(34%), tetA(32%) and sullI(16%) which resulted most isolates resistant to ampicillin (92%), rifampicin and rifamycin (84%), tetracycline (45%) and penicillin (40%) (Srinivasan *et al.*, 2005).

Tolerance response is correlated with the survivability of *L. monocytogenes* in cheese and dairy environment such as acid response, sigma regulators mechanisms, osmotolerance, oxidative stress, heat and cold tolerance and cross-protection (due to sublethal processing) (Melo *et al.*, 2015). In fact, a study showed that sub-lethal stresses such as exposed to adverse condition in food chain may facilitate survival of *L. monocytogenes* through the stimulation of antibiotic resistance responses in *L. monocytogenes* strain. For example, the antibiotic resistance of dairy isolates increased when salt concentration increased to 12% at pH 5 and temperature declined to 10°C from 37°C (Al-Nabulsi *et al.*, 2015).

7. Vegetables

Agroecosystem is known to be a commonplace where *L. monocytogenes* is known to be present particularly in the vegetables and soil. Soni *et al.* (2014) demonstrated 10% of vegetables (brinjal, cauliflower, dolichos-bean, tomato, chappan-kadu and chilli) and 5% soil samples from a farm tested positive for *L. monocytogenes*. RTE vegetables can be vehicles for transmission of *L. monocytogenes* as Sant'Ana *et al.* (2012) reported that 3.1% of the samples in Brazil were positive with *L. monocytogenes* belonged to the serotype 1/2b and 4b. Moreover, most isolates are able to survive

when being exposed to 125 ppm of chlorine-based disinfectant for 3 mins. In Malaysia, “ulam” which is a local salad dish that was commonly eaten raw has been reported to be contaminated by *L. monocytogenes*. Ponniah *et al.* (2010) reported that Japanese parsley (31.3%) and yardlong bean (27.2%) were frequently detected for positive *L. monocytogenes*.

In Brazil, De Vasconcelos Byrne *et al.* (2016) reported that one isolate of *L. monocytogenes* from RTE vegetable show resistance to penicillin while in Nigeria, Ieren *et al.* (2013) reported that about 64.3% of *L. monocytogenes* isolated from salad vegetables exhibit resistance to more than 4 antibiotics. The fresh produce growing environment and the formation of biofilm are believed to be the factors that support the survivability of *L. monocytogenes* in fresh produce. *L. monocytogenes* is always cycled in the food chain that involved contaminated soil, vegetables, human and animals (Zhu *et al.*, 2017). For example, fertilizers used that contained the bacterium can caused the bacterium to transform from saprophytic to pathogenic when it enters human or animal, further spreading the pathogens to consumers (Rahman and Noor, 2012). A study demonstrated that after 48 hours of incubation, biofilms of *L. monocytogenes* on green leaf lettuce cannot be removed by any types of sanitizers such as ozone (2 mg/L), chlorine (100 mg/L) and organic acid (0.50 g/100 g ascorbic acid). As a result, the biofilm embedded with bacterial cells was clearly visible in the SEM images (Ölmez *et al.*, 2010). Besides, the temperature of storage or display at retail could play a role in the survivability of *L. monocytogenes* in vegetable, for example Tang *et al.* (2018) reported that the positive samples from wetmarket was lower (7%) due to the psychrotrophic properties of *L. monocytogenes*.

8. Conclusion

L. monocytogenes is a pathogen that is ubiquitously present in the environment and a variety of food. Its survivability and persistence in the food matrixes and processing environment make it an important pathogen as one of the causal agents of foodborne poisoning. Serotype 1/2a strains of *L. monocytogenes* are frequently isolated from the food matrices or the environment while serotype 4b strains are frequently associated with real episodes of human listeriosis. To make things worse, emergence of multiple antibiotic resistance strains poses challenges to the clinical treatment for invasive infection among high-risk groups. The data on the evolution of the multiple antibiotic resistance should be constantly up to date in order to mitigate the risk.

Conflict of Interest

Authors declare no conflict of interest.

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