



Influence of agronomic practices and pre-harvest conditions on the attachment and development of *Listeria monocytogenes* in vegetables

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Abstract

Interest in fresh vegetables is on the increase due to their protective effects against several diseases. *Listeria monocytogenes* is a human pathogen easily found in vegetables. The purpose of this review article is to analyse the influence of the agricultural practices applied in pre-harvest, the environmental biotic and abiotic factors characterising the cultivation field, as well as the handling procedures at harvest that might greatly influence the presence and the levels of *L. monocytogenes* in fresh produce. This review article describes the routes of *L. monocytogenes* infections in relation to the agricultural practices commonly applied during vegetable cultivation. It also analyses the influence of the different cultivation systems as well as the main environmental factors and compares the effects of manual and mechanical harvest retrieving data from literature. Even though post-harvest sanitising is a common practice, fresh produce is still responsible for foodborne diseases. In the last years, the number of cases of human listeriosis is on the increase, and the consumption of fresh vegetables is being more frequently associated with these events. While still relatively rare, human listeriosis is one of the most serious food-borne diseases and continues to be one of the more lethal foodborne pathogens associated with vegetables. Seed decontamination represents an efficient operation to reduce microbial plant internalisation and diffusion. Since *L. monocytogenes* persists in soil for long periods, the hydroponic systems have been found to reduce its contamination of vegetables.

Keywords Agricultural practices · Contamination routes · Food safety · Fresh produce · *Listeria monocytogenes* · Vegetables

Introduction

Fruits and vegetables are important components of a healthy diet (FAO 2004). Fresh produce provide a variety of nutrients (vitamins, carbohydrates, and proteins) and numerous health-related benefits to the human body due to the phytochemicals with healthy properties (Tango et al. 2018). Thus, increasing the consumption of vegetables is a policy common to several countries. Indeed, the request of vegetables is on the increase with the direct consequence that, over the past 25 years, the world value of trade in vegetables exceeded that of cereals (Chakraborty and Chattopadhyay 2018). This phenomenon is due to the growing awareness of consumers towards the ravages of unhealthy foods (Lynch et al. 2009; Settanni et al.

2013; Tomasi et al. 2015; Khan et al. 2017) and the direct role of vegetables in reducing the risk of cancers of the organs as well as gastrointestinal tract (Soerjomataram et al. 2010), cardiovascular, coronary heart, metabolic, and degenerative diseases (Karam et al. 2016). It acts as protective foods (Chakraborty and Chattopadhyay 2018).

Nowadays, consumers have a great choice of vegetables that are available in several forms: fresh unprocessed, minimally processed, canned, frozen, cooked, or precooked. Different plant tissues can be consumed fresh or subjected to transformation. The edible parts of the most common vegetables available in retail markets (Table 1) are characterised by substantial differences regarding the structure, nutritional characteristics, mode of consumption, and hygienic characteristics/risks and shelf life. Due to the modern frenetic lifestyle, the demand for healthy foods characterised by high convenience of use has determined the success of fresh-cut vegetables (Miceli and Miceli 2014). In general, vegetables eaten raw are gaining more and more popularity (Ssemanda et al. 2018). Ready-to-eat (RTE) production represents the

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Table 1 Classification of vegetables based on the edible tissues

Edible tissues	Vegetables
Root	Carrot, turnip, radish
Tuber	Potato, tapioca
Bulb	Onion, garlic, leek
Leaf	Cabbage, spinach, chard, lettuce, red chicory, etc.
Bud	Soy, asparagus
Flower	Cauliflower, broccoli, artichoke
Fruit	Tomato, pepper, aubergine, courgette, cucumber, melons, squash, etc.
Seed	Pea, bean, fava bean

sector of vegetable typology that is increasing more rapidly (Putnik et al. 2011). The consumption of RTE vegetables has also increased in developing countries (Mir et al. 2018).

Among the different typologies of processed vegetables, those treated minimally are characterised by the highest retention of the bioactive components of fresh vegetables, particularly vitamins and phytochemicals, such as polyphenols, flavonoids, sterols, carotenoids, chlorophylls, and anthocyanins, which possess antioxidant properties (Andersen and Jordheim 2006; Siriamornpun et al. 2012). RTE vegetables can be purchased in several forms: “snacks,” portioned vegetables to be consumed *on the go*, “party trays,” several portioned vegetables to be consumed at the table, “meals,” or lunch boxes. Furthermore, several food distribution chains in France and the UK include “salad bars,” shelves with several portioned vegetables for self-service.

A very short shelf life characterises fresh-cut vegetables due to their high perishability caused by the high moisture content (Orsat et al. 2006) and, especially, because the wounding associated with processing leads to several physical and physiological changes that affect their quality (Saltveit 1997; Miceli et al. 2013, 2014, 2015a, b). Thus, the alteration of produce quality due to minimal processes cannot be avoided (Ansah et al. 2018) because the microorganisms enter through wounded tissues (Hussein et al. 2018), such as a tear in the waxy plant cuticle (Shenoy et al. 2017). The rate of microbial growth in fresh-cut vegetables increases with the reduction of leaf size due to cutting (Francis et al. 2012). As a matter of fact, fresh-cut vegetables deteriorate faster than intact produce. Furthermore, disrupted cells release their content (excellent source of energy and nutrients to support microbial proliferation) with the direct consequence that several microorganisms can easily grow (Alfonzo et al. 2018; Miceli et al., 2019). However, due to the different nutritional availabilities caused by the cutting operation, microbial alterations of fresh entire vegetables can differ from those of fresh-cut produce (Barth et al. 2009).

All steps along the crop cultivation cycle for the production of raw produce are indicated as “pre-harvest” (Wos 2016), and the microbial contamination of fresh vegetables occurs mainly

at this stage. This contamination is due to the direct contact with faeces and soil particles, to air, dust, insects, and the water used for irrigation, fertigation, or for reconstituting fungicides and insecticides, to inadequately composted manure as well as to animal and human handling (Beuchat 1996; Buck et al. 2003; Islam et al. 2004; Olaimat and Holley 2012; Rajwar et al. 2016). The pre-harvest microbial contamination plays a defining role on the microbiological quality of unprocessed and minimally processed vegetables. Even though post-harvest cleaning is applied to vegetables, the sanitisation is a difficult process because the complete elimination of pathogens cannot be pursued by the conventional methods of sanitation applied on plant surfaces (Olaimat and Holley 2012). Currently, the only way growers can reduce the possibility of contaminating their produce with foodborne pathogens is to utilise preventative measures on-farm (Kader 2006). The microbiological quality of vegetables is also affected by harvest, during transport, and through the application of process operations and storage (Martínez-Sánchez et al. 2006).

Although spoilage bacteria, yeasts, and moulds dominate the microflora of fruits and vegetables, pathogenic bacteria, parasites, and viruses capable of causing human infections are occasionally present on these produce (Beuchat 2002; Cheong et al. 2009). The presence of pathogenic bacteria on fresh-cut products that are consumed raw can cause food-borne outbreaks (Stuart et al. 2006; Settanni et al. 2012; Buchanan et al. 2017). This review focuses on the main factors at the pre-harvest and harvest stages (Fig. 1) affecting the hygienic quality of vegetables, in terms of the presence of *Listeria monocytogenes*, and analyses the risks related to their consumption.

Food-borne pathogens vectored by fresh vegetables

The increasing demand for fresh vegetables generated a big challenge: to produce high volumes of vegetables with high (organoleptic and nutritional) quality characterised by highly hygienic standards. The bacterial pathogens frequently found associated with vegetables are *Aeromonas hydrophila*, *Bacillus cereus*, *Clostridium* spp., *Escherichia coli*

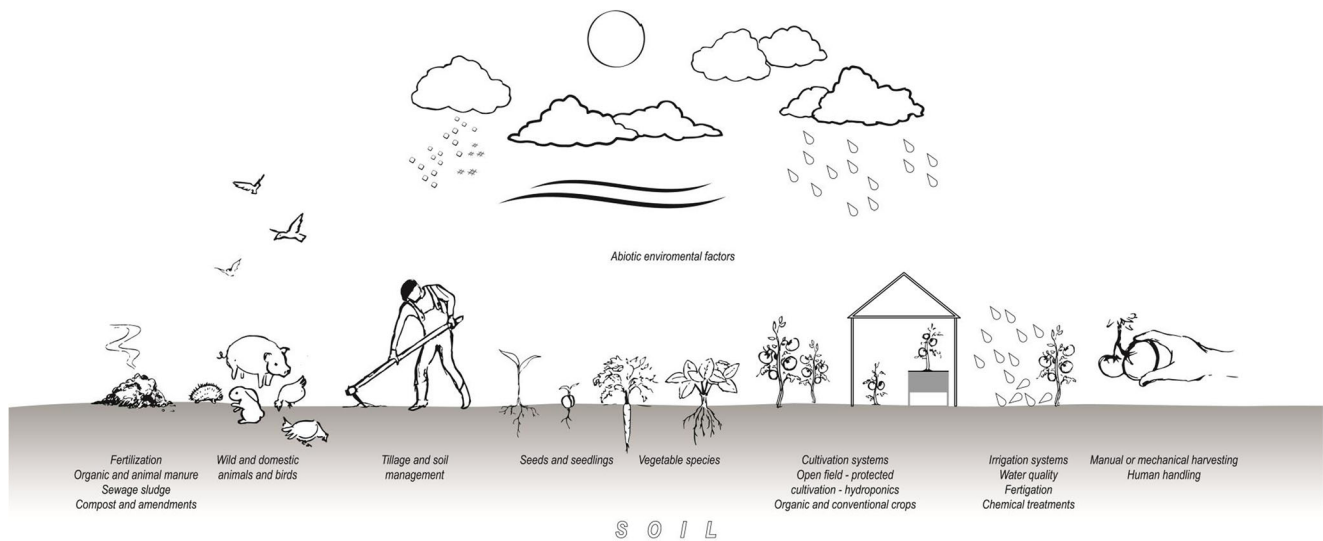


Fig. 1 Factors influencing the presence of *L. monocytogenes* in vegetables

O157:H7, *L. monocytogenes*, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, *Campylobacter* spp., and *Yersinia enterocolitica* (Beuchat 2002) with *L. monocytogenes*, *Salmonella enterica*, *B. cereus*, and *E. coli* being the most common species (Little et al. 2007; Valero et al. 2007; Cordano and Jacquet 2009; Taban and Halkman 2011; Faour-Klingbeil et al. 2016; Tango et al. 2018). Regarding the last pathogen, in 2011, the contamination of different vegetable products by the serovar O124 caused the death of several persons of different ages in Northern Europe (Moulson 2011), and this event generated a big alert in the entire continent.

Several bacterial mechanisms contribute to the attachment (Gorski et al. 2004) of human enteric pathogens to vegetables, such as the presence or absence of fimbriae (Fratamico et al. 1996), extracellular polymeric substances (Junkins and Doyle 1992) and biofilm formation (Whipps et al. 2008; Zhu et al. 2017), cell surface hydrophobicity (Dickson and Koohmaraie 1989), divalent cationic bridges (Hassan and Frank 2003), and bacterial surface charge (Tromp et al. 2010). Furthermore, internalisation of pathogens in plants plays a crucial role (Whipps et al. 2008; Macarasin et al. 2017). For this purpose, it is considered that bacterial flagellum is a critical factor (Gorski et al. 2003). However, in case of *L. monocytogenes*, not all strains need this cell appendix for plant colonisation, but the fitness of colonisation is improved by flagellum-functional motility (Gorski et al. 2009). Olaimat and Holley (2012) suggested to deeply investigate the route followed by pathogens into the vascular system to reduce the risk of foodborne pathogen development.

L. monocytogenes is often found in leafy vegetables (Aparecida et al. 2010; Zhu et al. 2017). In general, it is found with a prevalence around 1% in fresh-cut products (Tango et al. 2018) right up to almost 4% in farmers' market fresh

produce (Rodriguez-Romo and Yousef 2006). When RTE salads are mixed with other ingredients (such as ham, chicken, salmon, or pasta), the contamination by *L. monocytogenes* may reach 10% of products (Söderqvist 2017). Although until 20 years ago *L. monocytogenes* was among the emerging pathogens (Farber 2000), nowadays, it is recognised as a danger to humans due to the high fatality rate exceeding 30% (Drevets and Bronze 2008).

Incidence of listeriosis caused by fresh vegetables

The genus *Listeria* includes 18 species (Orsi and Wiedmann 2016), with *Listeria grayi*, *Listeria innocua*, *Listeria ivanovii*, *L. monocytogenes*, *Listeria seeligeri*, and *Listeria welshimeri* being those mostly characterised (Rocourt and Cossart 1997) and *Listeria costaricensis* being the species more recently described (Núñez-Montero et al. 2018). Among these species, only *L. monocytogenes* is recognised as a human and animal pathogen, capable of causing severe infections (Settanni and Corsetti 2007). In particular, it can attack the central nervous system causing listeriosis determining meningoencephalitis and brain abscesses (Join-Lambert et al. 2005). Listeriosis is a disease of immunocompromised individuals, newborns, pregnant women, and the elderly (Schuchat et al. 1991; Vazquez-Boland et al. 2001). The common symptoms of listeriosis are fever, muscle pain, and serious gastrointestinal problems, but infection could also hit the nerve system (headache, confusion, loss of balance) (Kljujev et al. 2018).

L. monocytogenes is ubiquitous in the environment; it is generally found in soil and can be associated with vegetation in decay (Beuchat 1996). Contrary to other pathogens typically associated with the enteric tract of animals, such as *Campylobacter*, *Salmonella*, and *E. coli*, it survives in soil for long periods (Olaimat and Holley 2012); Nicholson et al.

(2005) registered a *L. monocytogenes* survival in soil in the range of 45–100 days. Actually, this species prefers high moisture-containing soils (McLaughlin et al. 2011; Falardeau et al. 2018), but physical and chemical properties of soil influence its survival (Locatelli et al. 2013). Falardeau et al. (2018) reported that *L. monocytogenes* survival in soil was correlated with a neutral pH. *L. monocytogenes* is a very good endophytic coloniser of vegetable plant roots (Kljujev et al. 2018). For these reasons, *L. monocytogenes* is generally found associated with root crops (Lianou and Sofos 2007). However, until a few years ago, experts of food hygiene and food microbiologists were not acknowledging that *L. monocytogenes* could represent a problem for the microbial safety of vegetables.

Indeed, *L. monocytogenes* can contaminate several vegetables (Beuchat 2002), even at low (refrigeration) temperatures (Schuchat et al. 1991); it is known that *L. monocytogenes* grows at temperatures above 3.8 °C (Rodriguez-Romo and Yousef 2006). However, this character is dependent on the interaction between pH and temperature (De Roever 1998). In general, *L. monocytogenes* can grow in the range of pH 4.3–9.4 (Te Giffel and Zwietering 1999). Storage temperatures at retail and domestic level, duration of storage, and portion sizes are the factors that mostly affect the risk of listeriosis due to the consumption of vegetables (Tromp et al. 2010; Ding et al. 2013; Sant’Ana et al. 2014).

The ingestion of contaminated milk and meat-derived products and vegetables is considered to be the primary source of infection for both sporadic and epidemic human listeriosis (Schlech et al. 1983). Also, *L. innocua* frequently occurs in food and it can be considered as a bacterium indicator for the presence of *L. monocytogenes*; for this reason, *L. innocua* is often used as a surrogate species of *L. monocytogenes* for experimental trials carried out in safety conditions (Oliveira et al. 2010). Both species have been isolated from soil and vegetables, indicating that the pathogen is almost ubiquitous in nature (Schuchat et al. 1991). A recent work carried out on 602 samples of pre-cut and uncut vegetables purchased in retail markets in Italy showed an overall prevalence of 1% *Listeria* spp. represented by *L. innocua* and *L. seeligeri* (Santarelli et al. 2018).

The number of human listeriosis confirmed cases increased in the last years with 2536, 2242, and 1720 cases registered in the European economic area in 2016, 2014, and 2012, respectively, even though *Listeria* is rarely above the food safety limits set by the EU for RTE foods (EFSA 2017). The EU countries with the highest number of listeriosis notified in 2016 were Germany, France, Spain, the UK, and Italy. However, this disease has a low incidence in Europe, since only 1437 listeriosis infections were directly acquired in EU countries out of the total of 2536 cases registered in the EU in 2016. As matter of fact, listeriosis is characterised by a consistent social and public health impact as being the foodborne

disease with the highest hospitalisation and mortality rate (99.1 and 15.6%, respectively) (Scientific Report of EFSA and ECDC 2015). At the EU level, the proportion of listeriosis cases among elderly people has steadily increased from 52.9% in 2008 to 61.9% in 2016. The case fatality was 18.9 and 26.1% in the age group over 64 and 84 years, respectively, in 2016 (EFSA 2017).

In 2016, 13 EU member states provided data from investigations of *L. monocytogenes* on 1772 units of RTE fruits and vegetables tested using the detection method, and half of these data were reported by Italy. *L. monocytogenes* was detected in 2.0% of the tested samples of RTE salads in Europe (EFSA 2017), showing a consistent presence of this pathogen, despite the control measures being applied (Gil et al. 2015). Table 2 reports the outbreaks caused by the presence of *L. monocytogenes* in fresh produce from 2010 until present.

Environmental factors

The conditions at the growing location are major components that influence the microbial safety of raw products (Brackett 1999). The geographical and climatic conditions in which vegetables are cultivated and harvested may have a wide range of variations (Gil et al. 2015). However, different environmental (abiotic and biotic) factors, especially climate conditions, may contribute to *L. monocytogenes* proliferation on fresh produce. For these reasons, among the farm management practices considered for limiting pre-harvest pathogen contamination, also meteorological factors should be considered (Pang et al. 2017).

Indeed, the interactions between environmental conditions and the native microbiota of vegetables are particularly

Table 2 Food-borne outbreaks caused by *L. monocytogenes* in fresh vegetables from 2010 to present

Product	Cases	Deaths	Year	Country	Reference
RTE celery	10	5	2010	USA	(Gaul et al. 2013)
Cantaloupe	146	33	2011	USA	(CDC 2016)
Romaine lettuce	99	15	2011	USA	(Shrivastava 2011)
Vegetables	3	1	2013	Germany	(Ricci et al. 2018)
Mung bean sprouts	5	2	2014	USA	(Garner & Kathariou 2016)
RTE salad	32	4	2014	Switzerland	(CDC 2016b, Stephan et al. 2015)
Packaged salad	19	1	2016	USA	(CDC 2016b)
Frozen vegetables	9	3	2016	USA	(CDC 2016a)
Frozen vegetables	0	0	2018	Italy	This work

complex (Devleeschauwer et al. 2017). Pang et al. (2017) found that the amount and frequency of rain precipitation and the speed of the wind can affect the presence of *L. monocytogenes* in farms with mixed production. This evidence suggests that run-off and soil particles or dust driven by the wind might be responsible for the diffusion of this pathogen. Rain events are associated with an increased prevalence of foodborne pathogens in production environments. Weller et al. (2015) found that the likelihood of *Listeria* species and *L. monocytogenes* isolation from spinach field soils was highest during the 24 h immediately following rain and irrigation events. That study showed the possibility of limiting the contamination of fresh vegetables with *L. monocytogenes* by simply avoiding irrigation before harvest. Among biotic factors, wild and domestic animals, including mammals, birds, reptiles, and insects are direct sources of pathogenic bacteria in agricultural environments (De Roever 1998), because their faeces might contain several pathogens of intestinal origin (Ackers et al. 1998). Birds can be a particularly important contamination source because of their ability to transmit bacteria over long distances. For example, it has been reported that gulls feeding at sewage works are an important vector for subsequently introducing *L. monocytogenes* and enteric bacteria into the agricultural environment (Fenlon 1985).

Seed germination and seedlings

Among the effects of farm management practices on the microbial contamination of vegetables, planting procedures are reported to be responsible for the transfer of *L. monocytogenes* (Park et al. 2012). Human pathogen bacteria, including *L. monocytogenes*, could exist on the edges of damaged seeds during germination, and this determines their entrance into the plants (Gorski et al. 2004). However, this aptitude is considered a strain-dependent character (Gorski et al. 2009). Minute tears in the root tissue during germination facilitate this process (Erickson et al. 2014). Jablasone et al. (2005) found that *L. monocytogenes* became established and persisted in 9-day-old seedlings obtained from inoculated seeds of cress, radish, spinach, lettuce, mustard, carrots, and tomatoes. In that condition, *L. monocytogenes* was not found inside seedling tissues but persisted on plant surface during all the cultivation period. Similarly, Milillo et al. (2008) found that if *Arabidopsis thaliana* (a well characterised plant model) seeds were exposed to *L. monocytogenes*, between 4.23 and 4.57 log CFU/cm² were recovered from the leaves 7 days post-germination, suggesting that contaminated seeds can produce contaminated plants. This might represent a relevant issue if contamination occurs during vegetable seedling production in nurseries, from which contaminated plants can be transferred to large cultivation areas.

Based on the previous results, Settanni et al. (2012) also focused the attention on the transfer of *L. monocytogenes* from contaminated soil to aromatic plants sown on contaminated soil. To this end, basil and rocket were followed because they represent different growth habitus, rosette, or caulescent plants, respectively. The soils inoculated with *L. monocytogenes* were monitored for its presence during the cultivation and showed a reduction of the initial inoculums. At harvest, both plant and soil were analysed by culture-independent analysis that confirmed the contamination of the soil but did not find *L. monocytogenes* on plant leaves. Similar results were reported for sweet pepper by Füstös et al. (2017). The authors performed pepper seed bacterization using *L. monocytogenes* and *E. coli*, but the culture-dependent and -independent techniques applied on 6–7-week-old plants did not evidence the presence of stable or transient colonies of the inoculated bacteria, concluding that human bacterial pathogens are not able to internalise germinating seeds of sweet pepper.

Seeds are also considered the main source of contamination for sprouts. It is very difficult to wash off or inactivate pathogens from sprout (Saroj et al. 2006). The biofilms that can be formed on cotyledons, hypocotyls, and roots are structures that protect *L. monocytogenes*, making it resistant to antimicrobial compounds (Fett 2000; Lisa et al. 2004). Thus, due to the difficulty in guaranteeing pathogen-free raw sprouts, in 1999 the U.S. government issued a warning regarding the hazard of eating raw sprouts (NACMCF 1999).

Although *L. monocytogenes* can also internalise vegetables during post-harvest washing (dump tank washing and immersion-type hydrocooling) as registered by Macarisin et al. (2014) with cantaloupes, seed decontamination is an efficient operation to reduce microbial plant internalisation. For this purpose, a promising strategy to decontaminate seeds during storage and at germination is represented by plasma technology, which is also reported to enhance seed germination and growth of plants and beneficial microorganisms (Ito et al. 2018).

Cultivation system

The farm is a dynamic environment where pathogens and agricultural practices/human interventions, as well as environmental factors and animal/insects, interact continuously (Kumar and Thakur 2018). Vegetables are cultivated using various agricultural inputs and technologies and on farms or in protected culture of varying sizes (Gil et al. 2015).

From the microbiological perspective, protected cultivation is assumed to be safer than open field; this is related to the limitation of some risk factors linked with sources of pre-harvest contamination (Orozco et al. 2008a, b). Greenhouse cultivation is often associated with hydroponics. Hydroponic cultivation systems are largely adopted for vegetables. These methods allow the growing of plants out of the soil (with or

without a solid substrate) using nutrient solutions made with water fortified with essential nutrients. Plant roots may be directly dipped in the nutrient solution or plants may grow on mineral (perlite, rockwool, etc.) or organic (coconut fibre, peat, etc.) medium. Nutrient solution may be the vector of microorganism and spread contaminations among plants. Nevertheless, even if some studies detected colonies of *Salmonella* spp. and pathogenic *E. coli* in this type of crop management system (Orozco et al. 2008a, b), *L. monocytogenes* was not detected in hydroponically grown tomato (López-Gálvez et al. 2014) and bell peppers and the materials associated with their production (Avila-Vega et al. 2014). On the contrary, Koseki et al. (2011) observed that spinach leaves grown in a hydroponic cultivation system can be contaminated by *L. monocytogenes* through the root system if the nutrient solution was added with a cell density of 10^6 CFU/ml. To better investigate pathogen plant internalisation, Settanni et al. (2013) tested several species, including *L. monocytogenes*, in hydroponic systems that facilitate the contact of bacteria and plant roots using radish as vegetable plant model. The food pathogenic bacteria were inoculated in the nutrient solution during the growth period. At harvest, only *Citrobacter freundii*, *Enterobacter* spp., and *Klebsiella oxytoca* internalised, while *L. monocytogenes* and the other bacteria tested were not found in radishes. On the contrary, Shenoy et al. (2017) found viable cells of *L. monocytogenes* in pith, cortex, xylem, phloem, and epidermis of lettuce plants cultivated under greenhouse conditions. Considering that the extent of internalisation also depends on the type and age of the plant (Alegbeye et al. 2018), these studies suggest that the contamination could be related to vegetable species.

Organic and conventional agriculture differ greatly in the amounts of organic matter inputs during cultivation, which may determine the increase of contamination risks. Oliveira et al. (2010) evaluated the microbiological quality of fresh lettuce from organic and conventional productions, monitoring 18 farms, but none of the sampled objects of study hosted *L. monocytogenes*. The absence of this microorganism in lettuce was attributed not only to its absence in soil and water but also to the background microbiota present on the vegetables that might exert an inhibitory effect on *L. monocytogenes*.

Kuan et al. (2017) stated that one particular type of farming practices would not affect the microbiological profiles of fresh produce, because no trend was shown that either organically or conventionally grown cabbage, carrot, calamondin, cherry tomato, Bird's eye chilli, cucumber, eggplant, winged bean, lettuce, sweet potato, tomato, and white radish posed significant microbiological risks. Thus, those authors argued that to guarantee safe fresh produce with a good hygienic quality, appropriate post-harvest handling practices should be followed for any kind of vegetable, regardless of farming methods.

Kljujev et al. (2018) compared the presence of *Listeria* spp. and *L. monocytogenes* in different vegetables (tomato, sweet

peppers, cabbage, hot pepper, cucumber, potato, carrot, and parsley) grown under field and greenhouse conditions showing that 25.58% of samples were infected by *Listeria* spp., but only one carrot sample cultivated in open field was positive for *L. monocytogenes*.

Tillage or cover crops can modify soil microbial biomass and composition, but no apparent effect of tillage or cover crop on the presence of *Listeria* on fruits of muskmelon produced with different soil management and tillage was registered (Krzton-Presson et al. 2016). Nevertheless, there is evidence that *L. innocua* can overwinter and persist for more than 10 months in the soil; thus human pathogens cannot be eliminated by leaving a field fallow over the winter (Krzton-Presson et al. 2016).

Mulching is one of the techniques used for the cultivation of vegetables that stands out for improving crop productivity and quality. Plastic mulching, especially, increases soil moisture and temperature, thus changing the biological characteristics, enhancing soil microbial biomass, cycling nutrients, and providing a more stable soil microenvironment (Li et al. 2004). Differences in cultivation methods (plastic mulch vs bare soil) may affect the microflora counts at harvest and its behaviour during post-harvest storage of products. Mulching can give cleaner products as it separates the soil from plants, but it has been registered that it determines a higher microbial count than plants from bare soil at harvest (Agüero et al. 2008; Ponce et al. 2008). These conditions could be favourable also for foodborne pathogens, although, so far, there is no direct evidence of the effect of plastic mulching on *L. monocytogenes*.

Influence of irrigation water and irrigation system

The demand for water for agriculture purposes has increased as a consequence of changes in the precipitation patterns and increase in the world's population (Forsslund et al. 2010). The increasingly limited water supplies call for alternative solutions, and the European Water Framework Directive (2000/60/EC) encourages and promotes the use of treated urban wastewater in agriculture. This agronomic practice is becoming common; it occurs in many Countries (Scott et al. 2004) because it is of high value for the horticultural crops. However, when irrigation is performed with wastewater, the presence of microbial pathogens (Hamilton et al. 2005; Toze 2006) has to be considered.

A recent review article published by Jongman and Korsten (2018) discussed the global water challenges associated with irrigation water and deeply analysed the microbial quality of source water for irrigation, including groundwater, municipal water, rainwater, surface water, and wastewater and reported the crop contaminations for leafy greens. In particular, the authors highlighted the aspects related to the microbiological quality of roof-harvested rainwater that was not the object of any previous reviews, reporting the presence of *Salmonella*

spp., *Campylobacter* spp., and *L. monocytogenes*, but concluded that roof-harvested rainwater is an alternative source of water for irrigation of leafy green vegetables. However, all unprotected sources represent a risk for *L. monocytogenes* transfer. Ssemanda et al. (2018) found a prevalence of 1.0% *L. monocytogenes* in farm vegetables in Rwanda where all the water used for irrigation was from marshlands, rivers, lakes, and runoff lagoons.

The microbiological safety of fresh produce is greatly influenced by the irrigation system applied (Brackett 1999; Aruscavage et al. 2006; Warriner et al. 2009). To this end, the irrigation types that mostly represent a risk for leafy vegetables are spray and flood, since the contaminated water makes direct contact with the edible plant portions and also determines the transfer of soil particles (U.S. Food and Drug Administration 1998). Settanni et al. (2012) demonstrated that watering pots containing soil artificially contaminated with *L. monocytogenes* from below (sub-irrigation) did not determine the contamination of the aerial parts of leafy vegetables. For this reason, the transmission of pathogens by drip irrigation with contaminated water is very low in comparison to the irrigation performed by overhead sprinklers. Water is not only responsible for vegetable contamination in pre-harvest, since during the application of post-harvest operations it might also cause problems related to the development of foodborne pathogens (Berger et al. 2010; Macarisin et al. 2014).

Fertilisation

To achieve high yields with appropriate fruit and leaf size and good taste, the plant should be provided with good cultivation practices, namely proper fertilisation (Dzida and Pitura 2008). Although the soil is a natural environment for a wide variety of human pathogens, their presence is broadened considerably by the addition of animal wastes (Whipps et al. 2008). Fields that contain animal manure are more likely to be contaminated with enteric pathogens because of their ability to survive in soils for months or years (Doyle and Erickson 2008).

Inadequate composting is responsible for the transmission of undesired microorganisms from manures to the soil (Beuchat and Ryu 1997; De Roever 1998; Natvig et al. 2002; Santamaria and Toranzos 2003). Hutchison et al. (2004) reported that spreading livestock manure on the top of agricultural soil and letting it age without mixing them into the soil significantly reduced counts of pathogenic bacteria such as *E. coli* O157:H7, *S. enterica*, *L. monocytogenes*, and *Campylobacter jejuni*. *L. monocytogenes* can persist in a viable state for months when sewage sludge is applied to soil (Watkins and Sleath 1981). However, the survival of pathogens in the soil is time-dependent and, as such, the longer the interval between application of the contaminated vehicle and the harvest of the plant, the greater the likelihood that the product would not be contaminated. Consequently, the level

of contamination and the time in the growing cycle at which the contamination event takes place would be important indicators for whether contamination of the plant at harvest is likely (Doyle and Erickson 2008).

As reported above, leafy vegetables are greatly affected by the contact with soil particles for what concerns their microbiological aspects. For this reason, these vegetables have been deeply investigated for the influence of fertilisers on their hygiene quality. From this perspective, lettuce is the species investigated more extensively. The study of Oliveira et al. (2011) aimed to investigate the transfer of *L. innocua* from soil to lettuce. For this purpose, the researchers applied artificially contaminated compost or performed the irrigation with contaminated water. After sprinkling, the population of *L. innocua* on lettuce leaves was at high levels, but it was undetectable under field conditions. The transfer of *L. innocua* was not registered when soil was contaminated through compost. In general, the levels of *L. innocua* in the soil and on lettuce were highly similar independently of the manner of contamination (via application of contaminated compost or contaminated water). Girardin et al. (2005) investigated the behaviour of *L. innocua* in parsley cultivated in fields amended with bovine manure or municipal waste sewage sludge. Both sources of organic matter were composted before application, and the irrigation was performed by sprinkling using groundwater. After 90 days, the levels of *L. innocua* detected in the soil were 7 orders of magnitude lower than those registered at the beginning of the experimentation. During the first 30 days, this indicator was found at very low numbers on the leaves of parsley. The effect of the temperature was clearly evidenced since a high survival of *L. innocua* was observed during winter. In that study, *L. innocua* was mostly transferred from soil to leaves through the splash effect due to rain or irrigation, while the internalisation from the soil did not occur. For this reason, a period of 90 days represents a right delay between the fertilisation with contaminated material and harvest to contain the risk of transfer of the pathogenic *L. monocytogenes*.

Consumers are requesting organic foods more and more. The increasing demand for these foods, especially fresh produces, generated food safety issues related to the cultivation practices. Stuart et al. (2006) also indicated the dangers that farming operations pose to environmental sustainability and considered the ecological aspects of the guidelines for food safety. In this direction, recycled organic wastes are being used as alternative sources for enhancing soil since they increase the organic content of soil and stimulate its biological activity. The application of the organic wastes in place of synthetic fertilisers also improves the physical structure of the soil and allows an increase of the plant yield and resistance to diseases (Bulluck et al. 2002; Bulluck and Ristaino 2002). However, the work of Maurice Bilung et al. (2018) showed the occurrence of *Listeria* spp., both in organic and conventional vegetable farms, but, so far,

only limited information on the comparison of conventionally, organically and hydroponically vegetables contaminated by *L. monocytogenes* are available in the literature (Maffei et al. 2016).

The agricultural practice referred to as “fertigation” is the injection of water-soluble fertilisers into an irrigation system to distribute nutrients fusing irrigation water. This technique is practised extensively in commercial agriculture and horticulture and could be a source of pathogen distribution. López-Gálvez et al. (2014) found that the concentrated stock solutions used for fertigation of a hydroponic system contained high levels of *Listeria* spp. (around 4.5 log cfu/100 mL), especially in the case of the potassium nitrate solution. Selma et al. (2007) reported that fertiliser solutions could be an additional source of *Listeria* spp., which increases the levels of *Listeria* spp. initially present in surface water or reclaimed water.

Harvest

During harvest, the handling of the vegetables can greatly contribute to the microbiological contamination (Beuchat 2002). Furthermore, Park et al. (2013) stated that the personal hygiene of field workers’ represents an additional factor that influences the pre-harvest microbial contamination of fresh produce. The increasing global demand for vegetables has determined the diffusion of mechanised horticulture (Siddiqui et al. 2011). Hence, large-scale planting and mechanical handling during harvest are necessary (Hussein et al. 2018).

Root crops (carrots and potatoes) but also some leafy vegetables are mainly produced on a large-scale in European countries with harvesting machine and automatic grading, giving little possibility for direct contact between workers’ hands and harvested products (Monaghan 2014). However, small-scale growers perform manual harvesting and grading of vegetables, with the consequent prolonged and intense contact between vegetables and workers’ hands (Monaghan and Hutchison 2016). The hands of workers in the food sector contaminated by human pathogens can be implicated in foodborne outbreaks (Todd et al. 2010). Contaminated workers’ hands were implicated in the transfer of *S. enterica* serotype Enteritidis on lettuce (Waitt et al. 2014) and *E. coli* O157:H7 on strawberries (Shaw et al. 2015). Hand contamination was also the cause of transferred contamination of generic *E. coli* to carrots (Monaghan and Hutchison 2016). A similar contamination route could be supposed for *L. monocytogenes*.

The majority of investigations have been focused on food handlers in the post-harvest chain until restaurants or other high-throughput food preparation areas, but the implementation of correct hand hygiene is of relevance also for farm workers. The application of hand sanitisers and the use of portable latrines, as well as adequate training of the workers in their use brought about a reduction of spinach contamination by generic *E. coli* (Park et al. 2013).

Biological control of *L. monocytogenes*

Bio-based agents might represent a useful and sustainable tool in the prevention of attachment of *L. monocytogenes* in vegetables. From this perspective, a successful strategy could be designed on the application of bacteriocins, ribosomally synthesised, extracellularly released low-molecular-mass peptides or proteins, which have a bactericidal or bacteriostatic effect on other (usually closed related) species (Jack et al. 1995). Among soil bacteria able to compete in situ with *L. monocytogenes* by bacteriocin production, *Bacillus* genus members are particularly indicated. The species *B. cereus*, *Bacillus coagulans*, *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus thermoleovorans*, and *Bacillus thuringiensis* are producers of cerein (Bizani et al. 2005), coagulins (Hyronimus et al. 1998), megacins (von Tersch and Carlton 1983), subtilin (Jansen and Hirschmann 1944) and subtilosin (Zheng and Slavik 1999), and thuricin (Cherif et al. 2001), respectively. Among these bacteria, *B. thuringiensis* is the most promising species to reach the scope of controlling *L. monocytogenes* in the soil, since it is widely and commonly used in agriculture, mainly for the biological control of pathogenic insects due to the production of δ -endotoxins (Beegle and Yamamoto 1992).

Soil bacteriocin-producing *Bacillus* isolates with anti-*Listeria* activity have been proposed as alternative antibacterial agents for use in the food industry (Aunpad et al. 2011), but so far, no soil applications have been evaluated. Generally, bacteriocins are used as food biopreservatives (Settanni and Corsetti 2008), since they confer a rudimentary form of innate immunity on foodstuffs (Cotter et al. 2005) and they are, generally, produced by lactic acid bacteria (LAB) (Field et al. 2018). However, some class II bacteriocins active against *L. monocytogenes* (Larsen et al. 1993) with potential food applications have been characterised from soil LAB isolates, such as durancin produced by *Enterococcus durans* (Yanagida et al. 2005) and bacteriocin LB44 by *Pediococcus pentosaceus* (Kaur and Tiwari 2018). In general, bacteriocin-producing LAB might be associated with the soil (Yanagida et al. 2006). Hence, bacteriocin production plays an important role in bacterial competition strategies in the soil, and this phenomenon should be better investigated and exploited to adopt biological measures to control *L. monocytogenes* in open fields during vegetable cultivation.

Upcoming technologies: an opportunity to prevent *L. monocytogenes* attack?

To keep agriculture on the cutting edge, many farmers now implement a variety of technologies, including precision agriculture, remote sensing, computers, the Internet, specialised softwares, global positioning, drip irrigation, and

biotechnology (International Food Information Council Foundation 2018). Since information and communication technologies have become a global tool to plan activities in modern agriculture at different levels (Zahedi and Zahedi 2012), traceability technologies, which are already available for farms (Bai et al. 2017), should be developed and targeted to monitor bacteria and follow their route of contamination to implement traceability systems. In view of their application in the prevention of *L. monocytogenes* attack on agro-based products, upcoming technologies show great potential to set the cultivation systems to hamper the growth of this ubiquitous foodborne pathogen.

Concluding remarks and future perspectives

While still relatively rare, human listeriosis is one of the most serious food-borne diseases under EU surveillance, causing hospitalisation, high morbidity, and high mortality, particularly among the elderly and immunocompromised people as well as pregnant women and infants. In recent years, an increased incidence of foodborne illnesses has been caused by agents vectored by fresh vegetables. Thus, *L. monocytogenes* continues to be one of the more lethal foodborne pathogens associated with vegetables.

Table 3 Influence of agronomic practices and pre-harvest conditions on the attachment and development of *Listeria monocytogenes* in vegetables

Agronomic practices and pre-harvest conditions	Impact on <i>L. monocytogenes</i> contamination
Agronomic practices	
Seed germination and seedlings	<p>Planting procedures can be responsible for the transfer of <i>L. monocytogenes</i>.</p> <p>The contamination of seedling may occur during their production in nurseries, from which contaminated plants can be transferred to large cultivation areas.</p> <p>Seeds are also considered the main source of contamination for sprouts.</p>
Cultivation system	<p>Protected cultivation is assumed to be safer than open field due to the limitation of some risk factors linked with sources of pre-harvest contamination.</p> <p>The nutrient solution used in hydroponic cultivation systems may be the vector of microorganism and spread contaminations among plants but the contamination could be related to vegetable species.</p> <p>Tillage or cover crops can modify soil microbial biomass and composition but no apparent effect of tillage or cover crop on the presence of <i>Listeria</i> was registered. <i>L. innocua</i> is able to overwinter and persist for more than 10 months in the soil, thus human pathogens cannot be eliminated by leaving a field fallow over the winter.</p>
Irrigation water and irrigation system	<p>All unprotected water sources represent a risk for <i>L. monocytogenes</i> transfer.</p> <p>The likelihood of <i>Listeria</i> spp. and <i>L. monocytogenes</i> isolation from field soils is highest during the 24 h immediately following irrigation. Possibility to limit the contamination of fresh vegetables with <i>L. monocytogenes</i> simply avoiding irrigation before harvest.</p> <p>Watering pots containing soil contaminated with <i>L. monocytogenes</i> from below (sub-irrigation) did not determine the contamination of the aerial parts of leafy vegetables. Thus, the transmission of pathogens by drip irrigation with contaminated water is very low in comparison to the irrigation performed by overhead sprinklers. <i>L. innocua</i> can be transferred from soil to leaves through the splash effect due to irrigation.</p>
Mulching	<p>There are no direct evidences on the effect of plastic mulching on <i>L. monocytogenes</i>.</p>
Fertilisation	<p>Inadequate composting is responsible for the transmission of undesired microorganisms from manures to soil. <i>Listeria monocytogenes</i> is able to persist in viable state for months when sewage sludge are applied to soil.</p> <p>The level of contamination and the time in the growing cycle at which the contamination event take place would be important indicators for contamination of the plant at harvest.</p> <p>A period of 90 days represents a right delay between the fertilisation with contaminated material and harvest to contain the risk of transfer of <i>L. monocytogenes</i>.</p>
Harvest	<p>The concentrated stock solutions used for fertigation may contain high levels of <i>Listeria</i> spp.</p> <p>During harvest, the handling of the vegetables can greatly contribute to the microbiological contamination. The hands of workers in the food sector contaminated by human pathogens can be implicated in foodborne outbreaks. A similar contamination route could be supposed for <i>L. monocytogenes</i>.</p>
Environmental factors	
Rain	<p>Rain events are associated with an increased prevalence of foodborne pathogens in production environments. The splash effect of rain can transfer <i>Listeria</i> spp. from soil to leaves.</p>
Wind	<p>Run-off and soil particles or dust driven by the wind might be responsible for the diffusion of this pathogen.</p>
Wild and domestic animals	<p>Their faeces might contain several pathogens of intestinal origin.</p>
Birds	<p>They can transmit bacteria over long distances, from contaminated places to the agricultural environment.</p>

During the pre-harvest phases, pathogen populations, including *L. monocytogenes*, can establish themselves on growing crops, especially on root and leafy vegetables (Table 3). The presence of *L. monocytogenes* on seeds might facilitate its entrance into plants during germination. Thus, seed decontamination represents an efficient operation to reduce microbial plant internalisation and diffusion. Soil contamination plays a defining role on the hygienic characteristics of the product since *L. monocytogenes* persists in soil for long periods under a wide range of thermal and humidity conditions. To this end, the hydroponic systems have been found to reduce the microbial contaminations of vegetables, but the contamination of the nutrient solutions has to be taken under microbiological control because their massive contaminations are responsible for *L. monocytogenes* transfer. Analysing the cultivation systems and the abiotic and biotic factors, it seems there is a correlation among the agricultural practices and the environmental conditions with the contamination of the vegetable species. This strongly suggests that the choice of vegetable species should be determined after considering how all these parameters influence the attachment and development of *L. monocytogenes*. Furthermore, farmers' markets are gaining popularity due to the increased consumer demand for locally grown fresh produce. The application of organic wastes and manure accentuated the safety concerns of vegetables. Actually, the risk of human pathogen internalisation in vegetables is very low at harvest, but its occurrence during the first stages of plant development might determine a prolonged persistence in the rhizosphere. Safety issues may also arise when, at harvest, the hygienic habits of workers are scarce.

The application of pre-harvest food safety procedures can contain and reduce the burden of foodborne pathogens in humans at the post-harvest stages. Indeed, an effective pre-harvest intervention to control foodborne pathogens is currently lacking; due to the importance of bacteriocin production by soil bacteria in bacterial competition strategies, having in place control measures based on the inhibition of *L. monocytogenes* through in situ release of bacteriocins during vegetable cultivation seems to be a promising route to be investigated and exploited in open fields. Furthermore, the application of transgenic technology in agriculture based on the production of anti-*Listeria* molecules by plants could be of great usefulness to prevent the attachment of *L. monocytogenes*. However, the early detection and monitoring of pathogens during the pre-harvest steps is still of relevance for the safety aspects at harvest.

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