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## PREVALENCE OF *Listeria* IN FRUITS AND VEGETABLES

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### ABSTRACT

*Listeria monocytogenes* contamination in fruits and vegetables has become an increasing cause for concern in recent years. Fruits and vegetables are considered to be a source of high nutrient value as a result of which their consumption as raw natural food in the form of salads and RTE food has been increasing. However, *Listeria monocytogenes*, a food-borne pathogen associated with them, has been known to cause serious listeriosis infection, with symptoms of varying severity such as fever, headache, tiredness, gastroenteritis, septicaemia, meningitis and even lead to abortion or stillbirth in pregnant women. This review article aims to provide collective data on the prevalence of *Listeria* spp. in fruits and vegetables. It also discusses different sources of contamination of fruits and vegetables and pathogenesis of *L. monocytogenes* leading to listeriosis infection in humans. *Listeria monocytogenes* has ability to cross different host barriers like placental, intestinal and blood-brain barrier. Being a psychrotrophic microorganism, *Listeria* grows and survives at low temperatures. Therefore, presence of *L. monocytogenes* may pose risk of food borne illnesses from consumption of raw or minimally processed fruits and vegetables. This review also provides an inventory of the current data on *Listeria monocytogenes* causing listeriosis outbreaks, some of them leading to major recalls of the produce. It is virtually impossible to get rid of *Listeria monocytogenes* from food environment because of its ubiquitous presence. Hence, there is a need to actively manage the elimination and exclusion of this organism.

**Keywords:** *Listeria monocytogenes*, Listeriosis outbreaks, Pathogenesis of *Listeria monocytogenes*, Prevalence in fruits and vegetables

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**INTRODUCTION**

Listeriosis is a serious illness caused by *Listeria* and it can be very dangerous for those people at risk. *Listeria* bacteria are found widely in nature. Eating food contaminated by certain types of *Listeria* bacteria can cause listeriosis. Fruits and vegetables, cold meats, cold cooked chicken, chilled sea food, cheese, ice cream and other dairy products are some examples of food which are at higher risk of *Listeria* contamination. The bacteria may be present in raw foods or may even contaminate food after it has been cooked or processed. Storing contaminated foods, also in refrigerator, may allow the *Listeria* bacteria to grow.

Older people (over 65-70 years), pregnant women, their unborn and newborn children, immune compromised people due to serious illnesses like cancer, leukaemia, AIDS, diabetes, kidney or liver disease, including organ transplant patients are at higher risk of listeriosis.

Symptoms in people at risk may include headache, fever, tiredness, aches and pains. Less common symptoms are nausea, diarrhoea and abdominal cramps. These symptoms may progress to more serious forms of illness, such as septicaemia and meningitis. In pregnant women, symptoms may be mild, but listeriosis can result in miscarriage, premature birth or in

rare cases, stillbirth. Blood, Cerebral Spinal Fluids, spleen and liver, which are the sterile parts of the body, are usually infected by *Listeria monocytogenes*.

The number of cases of listeriosis in humans has increased in the last years due to consumption of fresh fruits and vegetables. *Listeria monocytogenes* is one of the serious food borne pathogens associated with fruits and vegetables, causing human listeriosis.

For a healthy diet, fruits and vegetables became an important component. Fresh produce provide a variety of nutrients (vitamins, carbohydrates and proteins) and numerous health related benefits to human body due to the phytochemicals with healthy properties, thus increasing the consumption of vegetables is a policy common to several countries (Miceli & Settanni.,2019). 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 the fresh produce (Miceli & Settanni.,2019). The presence of pathogenic bacteria on fresh-cut products that are consumed raw can cause food-borne outbreaks (Miceli & Settanni.,2019).

The genus *Listeria* includes 21 species (Orsi & Wiedmann.,2016), with *Listeria grayi*, *Listeria innocua*, *Listeria ivanovii*, *Listeria*

*monocytogenes*, *Listeria seeligri* and *Listeria weishimeri* being those mostly characterised (Rocourt & Cossart., 1997) and *Listeria costaricensis* being the species more recently described (Nunez-Montero *et al.*, 2018). (Miceli & Settanni., 2019)

*Listeria monocytogenes* occurs ubiquitously in soil, water and manure, which increases the possibility of vegetables and fruits to be contaminated. *Listeria monocytogenes* bacteria have been isolated from market or restaurant produce such as Cabbage, Corn, Carrots, Lettuce, Cucumbers, Parsley and salad vegetables (Zhu *et al.*, 2017).

Storage of fruits and vegetables at low temperatures in the refrigerator is thought to reduce its spoilage and extend its shelf life. But being a psychrotrophic microorganism, *Listeria* grows and survives at low temperatures. For mesophilic microorganisms, spoilage can be prevented, when stored at low temperatures, but the presence of psychrotrophic pathogen such as *Listeria* might pose risk of food borne illness from consumption of raw or minimally processed fruits and vegetables. Technical reports describe that *Listeria monocytogenes* can grow under a wide range of growth conditions during food processing and storage, for example, at temperatures as low as -0.4°C and over a wide range of pH values from 4.3 to 9.4

(Zhu *et al.*, 2017).

Growth of *Listeria monocytogenes* is dependent on surface properties like topography, availability of nutrients, and moisture content. Surfaces of intact fruits and produce are different. Growth of food borne pathogens on intact produce surfaces is less likely to occur compared with cut produce surfaces, owing to the protective outer barriers on most intact produce (Marik *et al.*, 2019) (Han *et al.*, 2000) (Scallan *et al.*, 2011) (Takeuchi *et al.*, 2000) (Tian *et al.*, 2013). Nutrient and availability of moisture may be restricted by outer protective layers of the produce. Once the outer protective epidermal layer has been disrupted, food borne pathogen like *Listeria monocytogenes* can enter inside and increase its population. Produce that has been injured through peeling, cutting, slicing or shredding can provide microorganisms access to nutrients and water (Marik *et al.*, 2019). The surface topography of fruits and vegetables can be quite complex (Marik *et al.*, 2019). For example, studies have hypothesised that the extensive netting on the rind or surface of cantaloupe increased adherence, biofilm formation and growth of *Listeria monocytogenes* (Marik *et al.*, 2019).

*Listeria monocytogenes* is a matter of concern for growers and packers because it is encountered in the same environment where

fresh fruits and vegetables are grown. Fresh fruits and vegetables can be easily contaminated with *Listeria* bacteria from the soil, water and decaying vegetations. It can easily establish itself in common conditions in packaging facilities. These *Listeria* bacteria can be introduced into packaging facilities through raw fruits and vegetables from the fields and become established if proper sanitation practices are not carried out.

It has been observed that most food borne outbreaks in the world recently are due to contamination of fruits and vegetables with *Listeria monocytogenes*. In order to break this trend, it has become a food safety challenge now to prevent the contamination of fresh fruits, vegetables and other produce which are associated with outbreaks of Listeriosis.

#### **Contamination of Fruits & Vegetables By *L. monocytogenes***

Studies have found that, leafy vegetables were easily contaminated with soil and microorganisms, because of their larger surface area, and leafy vegetables were found to harbour more microorganisms than non-leafy vegetables.

*Listeria monocytogenes* is widely present in soil environment which may influence its presence and levels in fresh produce due to agricultural practices applied in pre-harvest, post-harvest, as well as handling procedures at

the harvest and the environmental biotic & abiotic factors characterizing the cultivation field.

The number of human listeriosis cases is on increase in the last years and the consumption of fresh fruits and vegetables is more frequently associated with these events. Contamination of fruits and vegetables by *Listeria monocytogenes* can occur anywhere during transportation or right in the farms, in homes, as well as in restaurants. This could happen due to mishandling and poor hygiene practices during processing and preparation of the foods.

Heavy rains may increase the contamination of crops especially a crop growing low to the ground, such as Cantaloupes, where there is contamination of soil. The splash of rains or even wash waters or irrigation water for that matter could splash bacterium onto the edible surface, thus contaminating the product. Locatelli *et al*, 2013 said that a bacterium such as *L. monocytogenes* is edaphic, and may last upto 84 days in some soils giving the higher possibilities of contamination in low growing produce.

The use of raw or improperly composted manure can contaminate the soil with *L. monocytogenes*. Major products used as composted manure for crops consists of cattle and chicken feces and animal carcasses, which

can contain food borne pathogens and ultimately contaminate soil, crops and water resources coming in contact with it (Nightingale *et al.*, 2004). The contaminated crop can cause cross contamination or even increase growth of the organism during harvesting period, which may further result in contamination of other produce and equipments.

Listeriosis outbreak in Jensen farms is a perfect example of how cross contamination played a key role in *Listeria monocytogenes* infection. In 2011, at Jensen Brother farm in Colorado, *L. monocytogenes* was found on conveyer belts, including shipping and packing equipments. This resulted in the cross contamination of Cantaloupes on the farm. When these Cantaloupes were distributed to a number of states, resulted in listeriosis infection, identified in 28 states (Rothschild., 2011) to affect 146 people and claiming lives of more than 33 people (Bill Marler., 2014). The machinery and equipments had dirt on them since it was impossible to clean them fully. In addition, the potato washing machine was used for washing Cantaloupes which resulted in cross contamination of Cantaloupes. Further, the trucks, including those used to haul rejected Cantaloupes sent to cattle feedlots, were parked next to the packing plant. This made it easy for the trucks to be

contaminated with *Listeria* from the cattle farms (Zhu *et al.*, 2017).

For sprouts, seeds are considered as a main source of contamination. It is not possible to wash off or inactivate pathogens from sprouts completely. The biofilms formed on structures like cotyledons, hypocotyls and roots, protect *L. monocytogenes*, making it resistant to antimicrobial compounds. (Miceli & Settanni., 2019)

*L. monocytogenes* can also be internalised in fruits and vegetables through cuts or puncture wounds, and result in increase in their growth. Studies have reported the internalization of pathogens in plants from soil by root uptake (Franz *et al.*, 2007). The common route of internalization of human pathogens has been proposed to be penetration at cracks in seed coats, invasion at lateral root junctions in seedling, and aerial tissues (Doyle & Erikson., 2008). (Calix & Juan., 2019). Food can also get contaminated in the retail environment due to poor hygienic practices. Common sources of contamination of fruits and vegetables by *L. monocytogenes* is shown in **Figure 1**.

The main control for reducing these risks of contamination are treatment with sanitizers, mostly involving chlorine (Calix & Juan., 2019). The wash treatments consists of three stages; the first being focused on eliminating debris and organic material, the second stage

uses a sanitizer in order to reduce cross contamination and reduce microbial load; and third stage uses non-chlorinated water to rinse the product (Allende *et al.*,2015) (Caleb *et al.*,2014) (Calix & Juan.,2019). Pietrysiak *et al.*, 2019 have described various methods to avoid microbial contamination during packing process and decontamination intervention techniques of apples.

According to EcoLab (2015) pathogens such as *Listeria* can be reduced upto 99.9% by antimicrobial wash.

Prevention of biofilm formation is an important control measure to reduce the prevalence and survival of *L. monocytogenes* in growing environments and on fresh produce (Zhu *et al.*, 2017). Overall maintaining good hygiene and sanitation practices can help prevent possible listeriosis infection. Possible control measures that can be taken to prevent food-borne listeriosis infection are shown in **Figure 2**.

Nowadays, one of the technique used for improving crop productivity and quality is Mulching, wherein a protective covering is spread or left on the ground to enrich the soil, prevent evaporation, maintain soil temperature, which changes the biological characteristics of the soil and help in cycling nutrients. Mulching can give cleaner products as it separates the soil from plants, but so far there is no direct

evidence of the effect of plastic mulching on *L. monocytogenes* (Miceli & Settanni.,2019).

### **Growth Ecology & Survival Of *Listeria monocytogenes***

*L. monocytogenes* is a Gram positive bacterium that occurs widely in agricultural, aquacultural and food processing environment. A higher prevalence has been found in soils closer to water, soils with higher moisture, soils recently cultivated, irrigated or rained upon, and soils close to pastures. Survival will also vary with soil type and conditions; moist and organic soils permit longer survival than dry, low- organic soils. *L. monocytogenes* is also a transitory resident of the intestinal tract in humans, with 2-10% of the general population being carriers of the microorganisms without any apparent health consequences.

The major transmission vehicle for listeriosis is considered to be food. Ready-to-Eat (RTE) foods are of particular significance in listeriosis infections. RTE foods are the ones that are normally eaten raw or that will not be cooked or reheated before serving. This will include fruits and vegetable salads, sandwiches, cooked meats, smoked fish, desserts, and foods that you have cooked in advance to serve cold and eaten without further listericidal steps. Sporadic cases and outbreaks of listeriosis have generally been

associated with those RTE foods that are held for extended periods at refrigeration or chill temperatures which allow growth to high numbers at the time of consumption (Buchanan *et al.*,2017).

Many strains of *L. monocytogenes* are relatively resistant to a number of environmental conditions, such as high salt or acidity in food as well as low humidity or low oxygen in food environments (Buchanan *et al.*,2017). Although *Listeria* growth is significantly reduced at low temperatures, the organism has been found to grow and survive at refrigeration temperatures between -0.5 and 9.3°C under laboratory conditions (Walker *et al.*,1990). The ecological and physiological traits of *L. monocytogenes* allow it to colonize food plant environments, survives hurdles in processing/ storage and proliferate in food products that support growth at low temperatures (Buchanan *et al.*,2017). The peptidoglycan of bacteria grown at refrigeration temperatures displays typical features of cold adaptation (Lebreton *et al.*,2016). In addition to an increased abundance of specific surface proteins, the cell wall architecture and chemical modifications of its peptidoglycan backbone are drastically modified, part of which is linked to the activity of cold-shock proteins (Lebreton *et al.*,2016). Several authors have concluded that it is

virtually impossible to permanently eradicate *L. monocytogenes* from food environments because of its ubiquitous presence in the environment and many potential avenues for entry into the facility. Therefore, elimination and exclusion of the organism must be actively managed, for e.g. by adequate hygiene design of a premise and equipment, effective cleaning and sanitation, personnel and movement of people and materials into areas where food products are exposed. This includes the disassembly of equipment for deep cleaning, among other important control measures for operations producing RTE foods, like to be associated with sporadic illnesses or outbreaks of listeriosis (CAS 2009)(Buchanan *et al.*,2017).

#### **Pathogenesis of *Listeria monocytogenes***

*Listeria monocytogenes* is a well known food-borne pathogen causing listeriosis and known to infect both animals and humans. It has ability to cross different host barriers like placental barriers, intestinal barriers and blood-brain barrier.

Listeriosis infection typically starts with ingestion of food contaminated with *Listeria monocytogenes*. Once it enters intestine, it can invade different epithelial regions of the intestine. *Listeria* can target either tip of the villi where apoptic cells are extruded or it can cross the intestinal epithelium through Goblet

cells. Crossing of the intestinal barriers starts by the interaction of the bacterial protein Internalin A with E-cadherin, a human specific receptor (Lebreton *et al.*,2016). After internalisation of *Listeria* bacterium, it is further transcytosed to Lamina propria and into the bloodstream. It then spreads to spleen and liver.

During pregnancy, at the syncytiotrophoblast, the bacterium binds to two surface receptors E-cadherin and C-met, via two surface proteins Internalin A and Internalin B, respectively, allowing crossing of placental barrier and infection of fetus which is otherwise protected. *Listeria* has the ability to alter host cell physiology before it enters inside, at the level of infecting organs. The bacterium starts this process by secreting the toxin Listeriolysin O (LLO) (Lebreton *et al.*,2016). This toxin creates pores in the host membrane, through which ions pass in or out. This disruption in the ion balance promotes bacterial entry inside the cell.

Mitochondria forms a highly active network in the cell, which constantly undergo fission and fusion. But when influenced by  $Ca^{2+}$  ions, fission becomes dominant. *Listeria monocytogenes* is benefited by this transient fragmentation of mitochondria.

Disruption of the host ion balance by LLO, also indirectly influences processes inside the

nucleus (Institut Pasteur, Paris, France). *Listeria* interferes with histone post-transcriptional modifications and thus alters the expression of host genes which are involved in immune responses.

In addition to histone dephosphorylation, LLO can alter other post-translational modifications such as SUMOylation (a post-translational modification process). UBC9, a critical enzyme of this pathway is degraded in response to LLO leading to impairment of host SUMOylation and alteration of several host protein activities including transcription factors. By all these processes, the bacterium can modulate cell functions of the host before its entry. (Pascale Cossart Laboratory, Paris, France).

When the bacterium binds to receptors E-cadherin and C-met on the cell membrane, it triggers cytoskeleton rearrangements which leads to internalisation of *Listeria* into the cytoplasm. The bacterium secretes LLO and Phospholipases once it is inside, to disrupt the cell membrane of the internalisation vacuole. It also becomes resistant to cell's inner defences by modifying its surface. It then grows and multiplies inside its host and trade-off distinct cellular functions by secreting virulence factors such as InlC and LntA.

Inside cells, one of the poles of the *Listeria* bacterium is covered with Act A, a bacterial

factor that engages small actin filaments rapidly grow to form comet tails (Lebreton *et al.*,2016), which helps *Listeria* to move further. This movement propels the bacterium to the neighbouring cells through cell membrane. The double membrane vacuole formed again is ruptured by the release of LLO toxin and bacterial Phospholipases, thereby spreading the infection further.

In response to bacterial infection, immune cells of the host, like neutrophils can destroy infected cells, hence controlling the spread of infection. *L. monocytogenes* can be totally eliminated with the help of adaptive immunity, but in immune-compromised individuals, it may lead to severe listeriosis symptoms.

### **Listeriosis Outbreaks & Major Recalls Associated With It**

Several outbreaks of listeriosis infection associated with fruits and vegetables have been reported in various parts of the world and it has become an increasing cause for concern. It has been also observed that most of these outbreaks are due to contamination of the produce with *Listeria monocytogenes*. For example, *L. monocytogenes* was responsible for death of 10 people in a food poisoning listeriosis outbreak in chopped Celery in Texas in 2010; In 2011, 30 people were infected by *Listeria*-contaminated in Melons in Colorado; and in 2014, a *Listeria* outbreak linked to

Caramel apple contamination was reported in California (Zhu *et al.*,2017).

In 1997, a serious *Listeria* outbreak occurred in two primary schools and a University in Italy, which was associated with canned corn contaminated by *L. monocytogenes*. Fever and gastroenteritis were the main symptoms in this outbreak. A total of 2930 people developed febrile gastroenteritis in these three institutes, including primary school students aged 6-10 years, adult staff in the primary school, and students at the University. Investigations showed that after eating food supplied by the same caterer, symptoms were shown. Outside these three institutes in the same area, no other cases were reported during 1997. (Zhu *et al.*,2017)(Aureli *et al.*,2000)

European Union has seen an increase in notifications of both listeriosis outbreaks and sporadic cases. EFSA reported that in 2013, 1763 confirmed human cases of listeriosis were reported in 27 member states (Buchanan *et al.*, 2017). Implicated food vehicles were Crustaceans, shell fish and molluscs and product thereof, cheese, meat and meat products, pig meat and product thereof, and vegetables and juices and product thereof (mixed salad) (Buchanan *et al.*, 2017).

Since 2010, US has experienced a number of listeriosis outbreaks attributed to foods considered to be "moderate risk" or "low risk"

by the existing risk assessments, including fruit and vegetables (e.g., Celery, lettuce, cantaloupe, sprouts, stone fruits, and caramel apples), as well as ice creams. (Buchanan *et al.*, 2017).

In 2010, the outbreak that was reported in Texas, was associated with diced, machine cut celery served in five different hospitals (Gaul *et al.*, 2013). There were over 55 patients, with a mean age of 80, having some or the other health problem. These patients were receiving corticosteroids or acid-reducing treatments, that could have increased their susceptibility to invasive listeriosis (Buchanan *et al.*, 2017).

An outbreak of listeriosis was reported in the United States between 2013 and 2015 involving 9 cases, all of whom were hospitalized, with fatalities. Epidemiological and microbiological evidence indicated that the source was frozen vegetables from a single producer in Washington state. Whole genome sequencing (WGS) showed that *Listeria monocytogenes* from frozen corn was similar to isolates from 8 human cases, and an isolate from peas from the same manufacturer yielded a different *L. monocytogenes* strain which was similar to that from an additional human case. A further outbreak of listeriosis occurred between 2015 and 2018 and involved 53 cases across 6 European countries. The source of the outbreak was traced to sweet corn grown and

frozen in Hungary. The use of WGS identified the outbreak strain (*L. monocytogenes*, serovar 4b, multilocus sequence type (ST)6) which was detected in multiple frozen foods and surface swabs from the single plant in Hungary where vegetables were processed. The food chain associated with this producer was highly complex and there was an international recall of products from this plant. (Willis *et al.*, 2020)

There was another outbreak in July 2015 to January 2016 due to *Listeria monocytogenes* contamination in packaged salads. This outbreak was reported to cause 19 illnesses in nine different states leading to hospitalizations of all the cases, with one reported death (Shah., 2019). The outbreak strain of *Listeria monocytogenes* was traced back to a particular processing facility in Springfield, Ohio. Several brands and varieties of packaged salads were recalled during this outbreak. (Shah., 2019)

A multi-state listeriosis outbreak in US due to eating of contaminated Cantaloupe, in 2011 infected 147 persons. The median patient age was 78 years; most ill people were over 60, and 99% of the patients were hospitalized, seven of which was related to pregnancy or newborns. (Buchanan *et al.*, 2017)

Similarly, 5 people became ill due to eating of Mung bean sprouts contaminated with *Listeria*

*monocytogenes* in 2014, all were hospitalized and there were 2 deaths (Buchanan *et al.*, 2017). FDA detected *L. monocytogenes* in sprouts and irrigation water samples collected during a routine inspection, and follow-up environmental samples also showed the presence of *L. monocytogenes* (Buchanan *et al.*, 2017).

In July 2014, a packing company in California recalled various stone fruits (Whole peaches, nectarines, plums, and pluots) due to concerns about *L. monocytogenes* contamination (Buchanan *et al.*, 2017).

Between 2014-2015, 35 people were affected with Listeria infection, after eating Caramel apples and 34 of them were hospitalised (Buchanan *et al.*, 2017). This outbreak of listeriosis was again reported in US. *L. monocytogenes* was isolated in the caramel apples as well as from apple packing facility by FDA. Subsequent research showed insertion of the stick into the apple may have created a local microenvironment at the apple-caramel interface that supports rapid growth, whereas the apple nor caramel alone do not support growth (Glass *et al.*, 2015) (Buchanan *et al.*, 2017).

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. (EFSA 2017) (Miceli & Settanni., 2019). The EU countries with the highest number of listeriosis notified in 2016 were Germany, France, Spain, UK, and Italy (Miceli & Settanni., 2019). However, this diseases has a low incidence in Europe, since only 1437 listeriosis infections were directly acquired in EU countries out of the total 2536 cases registered in the EU in 2016 (Miceli & Settanni., 2019).

Besides the 2011 contamination of Cantaloupes by the Jensen Brothers in Colorado, there was also 2016 contamination of *L. monocytogenes* in frozen vegetable blends due the possible contaminated Onions. Those products were produced by the Pictsweet Co., where Onions were from Oregon Potatoes Co. (Alicia Thomas, Master's degree thesis).

In 2015, Granny Smith and Gala Apples were recalled from Bidart Brothers of Bakersfield, Ca, which tested positive for Listeria in the packaging plant. (CDC, 2015). According to the CDC February 2015 Listeria outbreak report, there were a total of 35 cases, 7 deaths and 34 hospitalisations from 12 different states, and one in Canada.

A further case of Listeria meningitis occurred in England in February 2019 and this strain was also recovered from frozen sweet corn and cheese samples. The sweet corn was from one

of the recalled batches traced to Hungarian plant (Willis *et al.*, 2020). National surveillance of listeriosis in England and Wales reported that, in 2019 there were a total of 142 cases of listeriosis in England and Wales, out of which, 139 were reported in England and 3 cases were reported in Wales. There were 23 deaths reported during this period, out of these, 15 were known to have a clinical manifestation of invasive *L. monocytogenes* infection recorded as a cause of death. Out of the 139 cases reported in the England, 16 were reported in East Midlands, 7 cases were in East of England, 35 cases in London, 6 cases in North East, 19 cases in North West, 10 cases in South West, 18 cases in South East, 12 cases in Yorkshire and the Humber, and 16 cases were reported in West Midlands.

A multi-country outbreak of *Listeria monocytogenes* ST6 linked to blanched frozen vegetables (bfv) also took place in the EU(2015-2018) (EFSA BIOHAZ Panel *et al.*,2020). Hygiene of the raw materials, processing water, poor hygienic conditions of the food processing environment and temperature conditions used for storage and processing seem to be the main factors affecting the contamination and growth of *L. monocytogenes* in bfv.

There was a recent recall in June-July 2019 due

to possible *Listeria monocytogenes* contamination in packaged produce, such as butternut squash, cauliflower, zucchini, and a butternut squash based veggie bowl (Shah., 2019). In November 2019, several other recalls have been initiated in various produce types due to possible *Listeria monocytogenes* contamination in produce like veggie trays, broccoli, tomatoes, snap peas, veggie power blend, and stir fry blends to name a few, by numerous distributors and retailers. (Shah., 2019)

It is important to note that hospitalization and death rates are higher for patients with Listeriosis infection. This is because of the greater mortality rate due to *Listeria monocytogenes*, which is as high as 30% (Scallan *et al.*, 2011). A detailed summary of listeriosis outbreaks from the year 1979 to present due to eating of fruits and vegetables contaminated with *L. monocytogenes* is given in **Table 1**.

**TABLE 1: Food-borne outbreaks caused by *L. monocytogenes* in fruits & vegetables from 1979 to present**

Year	Product	Country	No. of cases	No. of deaths	Reference
1979	Raw vegetables (Tomato, celery, lettuce)	United States	20	5	Ho <i>et al.</i> , 1986
1981	Coleslaw	Canada	41	17	Schlech <i>et al.</i> , 1983
1997	Corn	Italy	1566	0	Aureli <i>et al.</i> , 2000
2008	Alfalfa sprouts	United States	20	0	Marik <i>et al.</i> , 2020
2010	Rockmelon	Australia	9	2	Popovic <i>et al.</i> , 2014
2010	Chopped celery	United States	10	5	Gaul <i>et al.</i> , 2012
2011	Cantaloupe, Melon	United States	147	33	Marik <i>et al.</i> , 2020
2011	Romaine lettuce	United States	99	15	Miceli & Settanni, 2019
2013	Vegetables	Germany	3	1	Ricci <i>et al.</i> , 2018
2014	Stone fruit	United States	4	1	Jackson <i>et al.</i> , 2015
2014	RTE salad	Switzerland	32	4	Stephan <i>et al.</i> , 2015
2014	Mung bean sprouts	United States	5	2	Marik <i>et al.</i> , 2020
2015	Caramel apples	Canada & United States	35	7	Marik <i>et al.</i> , 2020
2016	Frozen vegetable products	United States	9	3	Marik <i>et al.</i> , 2020
2016	Packaged salads	Canada & United States	33	4	Marik <i>et al.</i> , 2020
2018	Rockmelon	Australia	19	8	Marik <i>et al.</i> , 2020
2019	Frozen sweet corn	England	139	23	National surveillance report of listeriosis in England

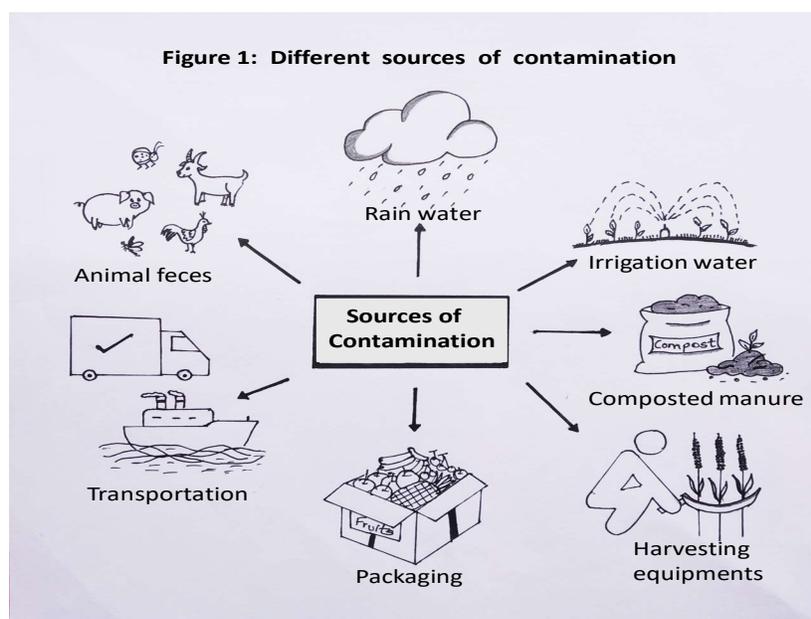
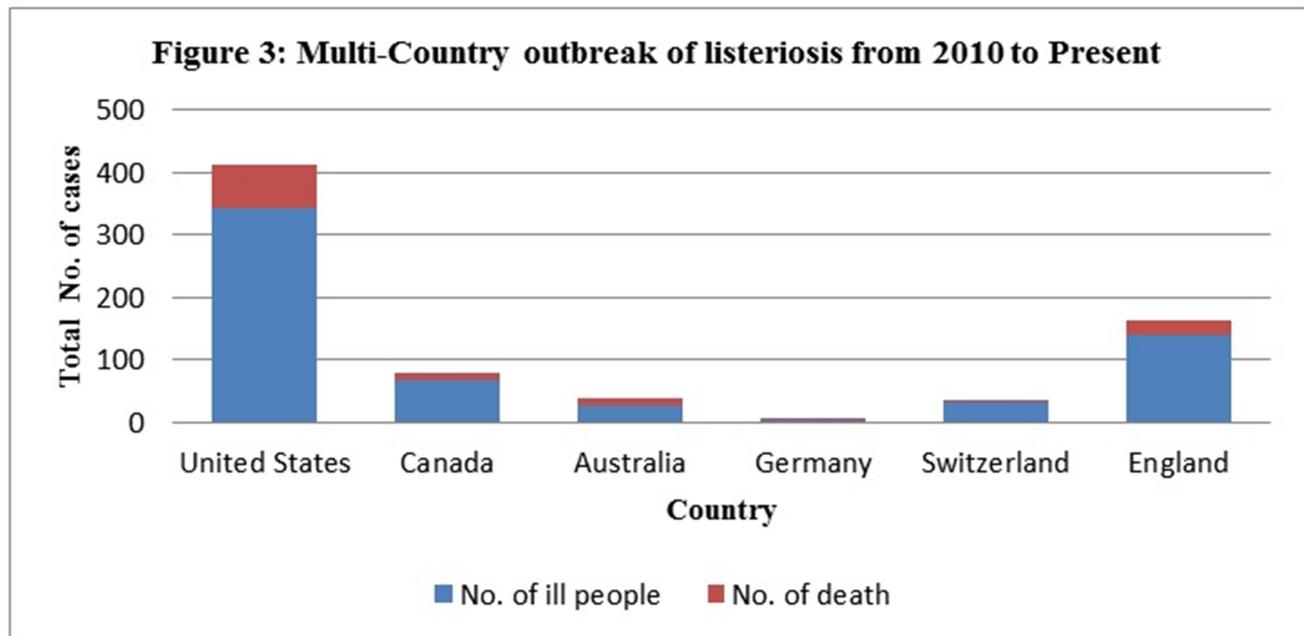
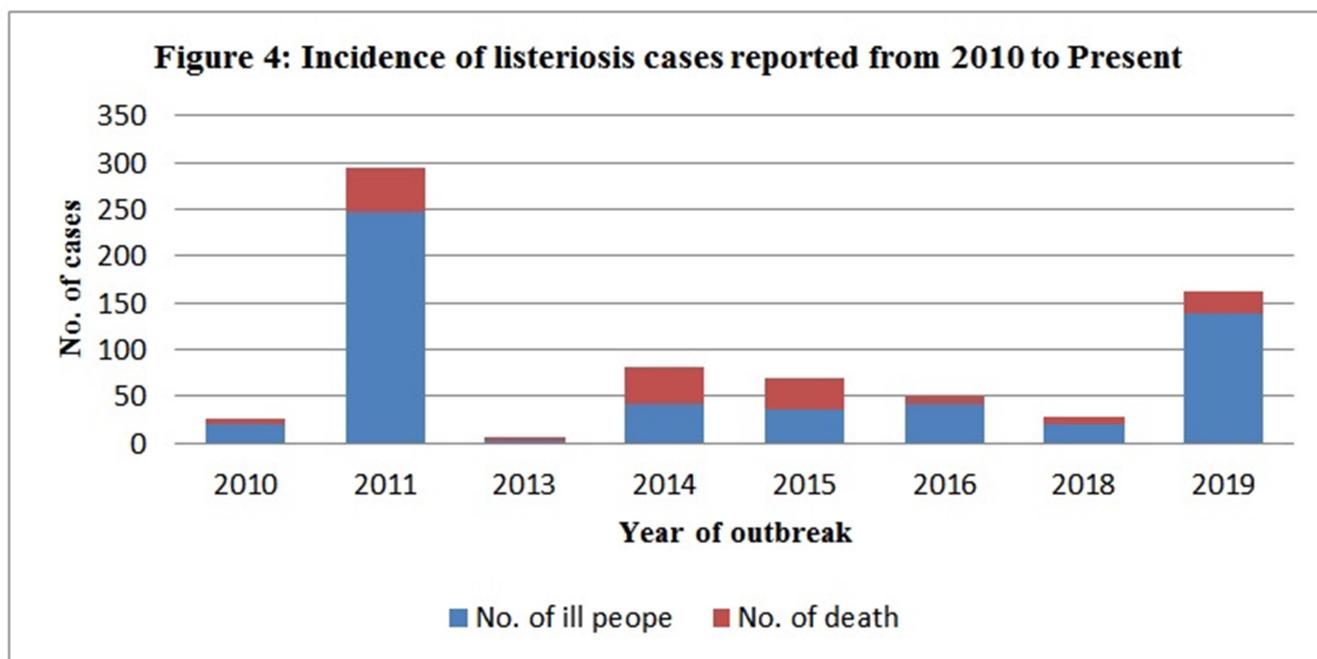


Figure 2: Control measures to prevent food-borne Listeriosis





## DISCUSSION

In recent years, *Listeria monocytogenes* has caught attention for causing several outbreaks related to different fruits and vegetables across the world. Since *L. monocytogenes* occurs ubiquitously in soil, water and composted manure, this increases its possibility of contaminating fruits and vegetables. Many studies have shown the presence of *L. monocytogenes* on fruits and vegetables sourced from its cultivation farms. Zhu and Hussain in 2014, conducted a study for investigating prevalence of *Listeria* spp. in four different vegetables commonly used as salads, sampled from two fruit and vegetable markets in Canterbury region of New Zealand. All the samples were tested positive for *Listeria* in this study. They successfully concluded that,

*Listeria* spp. have been frequently detected in market produce such as Cabbage, corn, lettuce, sprouts, cucumbers, potatoes, parsley and watercress. In a study conducted by Tang *et al*, 2017 in Terengganu, found *Listeria monocytogenes* in 3.8% and 7.3% of vegetables sourced from commercial farms and local farms respectively from a total of 327 vegetable samples.

Many other research reports also indicated the presence of *Listeria* spp., *Listeria monocytogenes* in particular, on the surface of fruits and vegetables. Althaus *et al* found that the samples of RTE lettuce were infected by *L. monocytogenes* (Zhu & Husaain, 2014). In another report, Uzeh and Adepoju showed *L. monocytogenes* was present on salad vegetables (sliced Cabbage, lettuce and carrot)

(Zhu & Hussain, 2015) (Uzeh & Adepoju, 2013). Storage temperature was linked to the growth of *L. monocytogenes*. Chen *et al.*, 2019 also concluded in his study that 5.49% of fresh vegetables collected from 43 representative cities/ regions markets in China were positive for *L. monocytogenes*.

Relative humidity is likely an important factor in assessing the likelihood of *Listeria monocytogenes* growth and/or survival on intact produce and may be a potential control strategy for limiting *L. monocytogenes* growth and/or survival on intact produce during certain activities for e.g. transportation and storage. (Marik *et al.*, 2019). Usually, for growth of *L. monocytogenes*, the outer surface environment of fruits and vegetables was not favourable, but it was observed that mishandling and storage conditions of these produce, enhanced the survival of *L. monocytogenes* along the supply chain to the consumers.

Data from Zhu and Hussain, 2014 study and several other study reports strongly suggested that *Listeria* spp. could pose a serious threat to the safety of fresh fruits and vegetables produce, and products that contain them as a main ingredient. Hence, it is necessary to take appropriate measures to minimize the contamination. Several methods have been developed to control the levels of *L. monocytogenes* on the surface of fresh

vegetables and fruits. for e.g., using balsamic vinegar from Moden approved more effective than washing, in reducing pathogens including *L. monocytogenes* (Zhu & Hussain, 2014). It is also suggested that dry place with less humidity is more fitful storage condition for fresh produce (Zhu & Hussain, 2014).

Studies have shown that, highest number of listeriosis outbreaks have occurred in both United states and Europe. Figure 3 shows the total number of listeriosis cases from the year 2010 to present, due to eating of contaminated fruits and vegetables, in six different countries i.e. United States, Canada, Australia, Germany, Switzerland and England. It shows that from the year 2010 to present, United States have had the highest number of listeriosis cases, followed by England (Figure 3). In England, the outbreak was recent, i.e. in 2019, which was due to eating of frozen sweet corn.

Similarly, figure 4 shows the incidence of listeriosis cases reported from the year 2010 to present, due to eating of contaminated fruits and vegetables by *Listeria monocytogenes* across the world. It shows that, in 2011 the world had highest number of listeriosis cases due to eating of contaminated fruits and vegetables. Listeriosis outbreaks revealed that improper sanitation and cleaning practices of fruits and vegetables, their packing and retail environment may lead to contamination of the

produce with *L. monocytogenes*.

Willis *et al.*, 2020 conducted a study to provide data on the microbiological quality and safety of frozen fruits and vegetables in catering premises and on retail sales in England. A total of 1050 samples of fruits and vegetables were collected from various premises by sampling officers from 91 Environmental Health Departments in England between December 2018 and April 2019. Out of these, *Listeria monocytogenes* was detected in 78(7%) of the total samples and comparatively, more was detected in vegetables than in fruits.

## CONCLUSION

Fresh fruits and vegetables are considered to be in the top list of healthy foods with lots of nutritional value, that is why all the people, including sick and vulnerable populations are inspired to eat more of these produce. Having said that, it would be frightening situations to have where *L. monocytogenes* was routinely isolated from such food sources. Hence in order to implement proper mitigation strategies to reduce their food safety risks, it is important to understand the prevalence of *L. monocytogenes* contamination in fruits and vegetables. In USA, a survey conducted by Luchansky *et al.*, for *Listeria monocytogenes* prevalence on raw cut vegetables (1,689), low acid cut fruits (2,408), and sprouts (2,652) showed that 18, 9 and 3 samples, respectively, for each product

type were positive for *Listeria monocytogenes* (Shah, 2019). Once present on the produce, *Listeria monocytogenes* can survive for long time periods as this pathogen is adapted to such conditions.

A product recall can ruin manufacturer's reputation and can lead to financial crisis due to loss of large consignments of the produce. Small scale producers may not be able to recover from such recalls. Therefore, it is necessary to monitor and analyze environmental contaminant and health related organism like *L. monocytogenes*.

It is also very important to make consumers aware of such situations, so that cross contaminations at homes can be prevented. Consumers should discard recalled and food borne disease outbreak associated products with longer shelf life, mainly frozen vegetables/fruits, whenever such information is available.

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