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Review

Outbreaks of listeriosis associated with deli meats and cheese: an overview

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Abstract: Microbial pollution of foods by undesirable microorganisms is a global food safety issue. One of such undesirable microorganism is the psychrotrophic, pathogenic specie of Listeria—*Listeria monocytogenes* that survives at low temperature. The source of contamination of this microbe into foods can be many including the food processing facilities due to improper sanitation procedures. The review of the literature on this important topic shows there are increasing concerns as regards contamination from Listeria in foods leading to many cases of listeriosis disease and food recalls. Ready-to-eat products, such as delicatessen (deli) meats and soft cheeses have repeatedly been identified by foodborne disease control programs as sources of outbreaks and products that put humans at risk for listeriosis. Although, most listeriosis cases tend to be sporadic in occurrence, outbreaks do occur frequently. Due to the global phenomenon of outbreaks associated with Listeria in deli meats and cheese, it requires an urgent attention from national and international authorities through rigorous procedures for its identification, surveillance procedures that can bring more awareness to the general public. There is also a need for more reports on the cases of Listeria particularly in developing countries, the standardization of identification procedures, and an improvement on national control programs by adequate surveillance.

Keywords: contamination; cheese; deli meats; *Listeria monocytogenes*; listeriosis; prevalence

1. Introduction

Microbes can either be desirable or undesirable in foods and may have positive or negative impacts on impact human's health. Undesirable microbes that are pathogenic enter the food chain

either through contamination from soil, water, air or contact with unhygienic equipments including humans. The most common types of bacteria that have caused outbreaks resulting in food poisoning are *Campylobacter spp, Salmonella spp, Escherichia coli, Staphylococcus aureus, Toxoplasma, Clostridium perfrigens* and *Listeria monocytogenes*. *L. monocytogenes* is a facultative, Gram-positive, intracellular and ubiquitous bacterium which is widespread in the soil environment, decaying vegetation, water and may be part of the fecal flora of many mammals, including healthy human adults [1]. Out of the currently known seventeen species of Listeria in the natural environment, the two pathogenic species are *L. monocytogenes* and *L. ivanovii*, the remaining non-pathogenic species are *L. welshimeri, L. innocua, L. seeligeri, L. grayi, L. marthii, L.aquatic, L. booriae, L. cornellensis, L. fleischmannii, L. floridensis, L. grandensis, L. newyorkensis, L. riparia, L. rocourtiae and L. weihenstephanensis* [2,3].

Listeriosis results when a person is infected and develops symptoms of Listeria infection, the illness is associated with high mortality [4]. Before the 1980s, human listeriosis was an obscure disease although there were large outbreaks of considerable morbidity and mortality [5]. Listeriosis was first recognized as a foodborne pathogen in the early 1980s and since then the disease has risen among human and animals in Europe and North America. It is the second most common cause of death from food borne disease in the US &France and the fourth in England & Wales [6]. Most of the people at high risk of listeriosis are pregnant women, newborn babies, elderly people (over 60 years). The bacterium is known to cross the intestinal barrier, disseminate via the bloodstream and can reach the liver, spleen, central nervous system and foetus [7]. Since the early 1980s when the food pathogen status of Listeria was established, there have been rigorous efforts by the food industry and regulatory agencies to control the growth of this ubiquitous organism in food production, processing, distribution and consumption chain [8].

L.monocytogenes, unlike most other foodborne pathogens, can grow at refrigeration temperatures and in foods with fairly low moisture content and high salt concentration. This ability to persist and multiply in the food environment makes *L.monocytogenes* especially difficult to control. They have been reported in smoked seafoods, hot dogs, soft cheeses from unpasteurised milk, and deli-meats. With prompt investigations into Listeria contaminations, adequate reports of listeriosis cases and their interventions will help policy makers in ensuring the safety of consumers from Listeria outbreaks worldwide. For instance, an investigation into a large multi-state outbreak of listeriosis reported in the US traced to turkey deli-meats in 2002 helped policy makers to make changes that helped to prevent future *L. monocytogenes* contamination in ready-to-eat meat (RTE) and poultry products [9]. By ensuring that the food contact surfaces where RTE foods are processed or served in retail or home environment is clean and sanitized will further help to prevent Listeria cross-contamination.

This article reviews the outbreaks *L. monocytogenes* in foods with special emphasis on deli meats and cheese worldwide. The first section is a brief introduction on its characteristics as a foodborne pathogen. Section 2 highlights some of the outbreak of Listeria in several foods in many countries worldwide in the last twenty years. In addition, this section reports on the prevalence of these outbreaks in deli-meats and cheese. Section 3 describes their identification, sampling methods and characterization in foods. Section 4 describes efforts towards the prevention of Listeria and other containment strategies.

2. Global Outlook of Listeria Outbreaks (1995 to 2016)

Listeria are frequently present in raw foods of both plant and animal origin around the world and they are also present in cooked foods due to post-processing contamination. They have been isolated in foods such as raw and pasteurized fluid milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw meat and cooked sausages, raw and cooked poultry, raw meats, and raw and smoked seafood [10]. RTE foods such as cold cuts or deli meats, cheeses and other dairy products are ideal sources for contamination since they are not cooked before consumption. In food handling operations, *L. monocytogenes* are able to grow at refrigeration temperature between 4 ℃ to 10 ℃, freezing temperatures have little detrimental effect on this ubiquitous microbe. In addition, they can still survive when the desired temperature is not reached during pasteurization especially in large packages. *L. monocytogenes* have also been isolated from food processing environments, particularly those that are cool and wet.

L. monocytogenes was estimated to infect 23, 150 people globally and killed 5,463 (23.6%) of the infected people in 2010 [11]. However, most of the cases involving Listeria are not reported in developing countries due to ineffective control or surveillance strategies. This makes it problematic in having a comprehensive global outlook on the outbreaks of Listeria and listeriosis in humans. Snapshots of some cases involving Listeria contamination worldwide from 1995 to 2016 are shown in Table 1. These cases were either from the raw food samples, the processing plants of such foods or the processed foods in different countries. The method of detection varied from country to country. In the year 2016, a legal tussle is being waged against a major US food company in the contamination of salad products by *L. monocytogenes*.

These reported cases were from clinical listeriosis tests on patients or through a national survey.

L. monocytogenes are known to have13 distinct O-antigenic patterns, which comprises the serovars; 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e, 4ab, and 7 [39]. However, the pathogenicity of *L. monocytogenes* are divided into three lineages [40]; Lineage I (Highly pathogenic, with epidemic clones and responsible for most outbreaks e.g. 1/2b, 3b, 4b, 4d, 4e), Lineage II (medium pathogenic, sporadic cases e.g. 1/2a, 1/2c, 3c, 3a) and Lineage III (low and rarely cause human diseases e.g. 4a, 4c). These strains of *L. monocytogenes* show differences in their virulence and epidemic potential, it was shown that a limited subset of clones that mostly belong to serotypes 1/2a, 1/2b and 4bwere responsible for a large part of clinical cases worldwide, they also account for 98% of listeriosis outbreaks [39,41].

Although the disease burden of listeriosis on population level is low, on individual level the impact is high, largely due to severe illness and a high case fatality [27]. Despite this low incidence of listeriosis that represents less than 0.1% of all food-borne illnesses they cause infections with very high mortalities 20 to 30% deaths [42,43].

The approximate infective dose of *L. monocytogenes* was estimated to be 10–100 million colony forming units (CFU) in healthy hosts, and only 0.1–10 million CFU in people at high risk of infection. On the mild end of the spectrum, listeriosis usually consists of the sudden onset of fever, chills, severe headache, vomiting, and other influenza-type symptoms [44]. Along these same lines, the US Centre for Disease Control notes that infected individuals may develop fever, muscle aches, and sometimes gastrointestinal symptoms such as nausea or diarrhea [45].

Table 1. Select reports of Listeria outbreaks globally from 1995 to 2016.

Country	Year	Food source	No. of cases	References
France	1995	Cheese	36	[12]Goulet et al., 1995
Israel	1995–1999	N.A. [a]	N.A.	[13]Siegman-Igra et al., 2002
Italy	1997	Corn salad	2	[14]Aureli et al., 1997
Sweden	1997	Rainbow trout	9	[15] Ericsson et al., 1997
Finland	1998–1999	Butter	25	[16]Lyytikäinen et al., 2000
USA	1998–1999	Frankfurter	108	[17]Kathariou et al., 2006
France	2000	Salmon fish	N.A.	[18] Dauphin et al., 2001
Japan	2001	Cheese	86	[19]Makino et al., 2005
France	2002	Raw sausage	11	[20]de Valk et al., 2005
Canada	2002	Soft cheese	130	[21]McIntyre et al., 2002
USA	2004	Deli meats	108	[22]Mead et al., 2006
Switzerland	2005	Cheese	10	[23]Bille et al., 2005
Germany	2006/7	Jellied pork	189	[24]Pichler et al., 2009
Austria	2008	Milk and cheese	12	[25]Koch et al., 2010
Canada	2008	Deli meats	57	[26]Currie et al., 2015
Netherlands	2008–2013	N.A.	406	[27]Friesema et al., 2015
Austria	2009–2010	Acid curd cheese	25	[28]Pichler et al., 2011
Australia	2010	Chicken wraps	36	[29]Popovic et al., 2010
USA	2010	Celery	10	[30]Gaul et al., 2013
Belgium	2011	Cheese	12	[31]Yde et al., 2012
USA	2011	Cantaloupe	147	[32]McCollum et al, 2013
South Africa	2012	RTE foods	51	[33]Nyenje et al., 2012
South Korea	2014	Ham and sausage	25	[34]Park, M. et al., 2014
Macedonia	2014	Home made sausage	7	[35]MCID, 2014
UK	2014	N.A.	169	[36]Whitworth, 2015
USA	2014–2015	Caramel apples	35	[37]CDC, 2015
USA	2016	Lettuce salad	19	[38]CDC, 2016

[a]not available

Most cases of listeriosis are sporadic and have been reported in high-income countries, where incidence is quite low but fatality rate is high. Most healthy adults and children who consume Listeria contaminated food experience only mild to moderate symptoms. The infection is usually self-limited, since in healthy hosts, exposure to Listeria stimulates the production of tumor necrosis

factor and other cytokines, which activate monocytes and macrophages to eradicate the organism. Few people with normal immune function go on to have more severe, life-threatening forms of listeriosis, which is often characterized by septic shock, meningitis and encephalitis [1].With ageing population and many immune-compromised individuals in high income countries, listeriosis has become a major issue. Since the US Center for Disease Control and Prevention(CDC) began tracking outbreaks in the 1970s, *L monocytogenes*is the third most costly foodborne pathogen in the USA per case in 2010, after *Clostridium botulinum* and *Vibrio vulnificus* [46]. The global burden of human listeriosis was estimated for 2010 to result in 172, 823 disability-adjusted life years (DALYs) [11]. DALYs are useful in comparing diseases and health conditions thereby it will help policy makers to allocate resources in the prevention of listeriosis [47]. It was estimated that the annual benefit from safety measures of *L. monocytogenes* in the USA far exceeds its cost. The estimated benefit and cost was US$ 2.3 billion to 22 billion, and US$ 0.01 billion to 2.4 billion respectively [48]. Foodborne outbreaks due to severe pathogens, such as *L. monocytogenes* and those that result in product recalls, are typically the most costly from the individual and societal perspectives. In Canada, the costs that were associated with the cases of Listeria outbreaks (including medical costs, nonmedical costs, and productivity losses) and those incurred by the implicated plant and federal agencies responding to the outbreak were estimated to be nearly $ 242 million Canadian dollars [49].

Some RTE products may not undergo thermal or other processing sufficient to inactivate *L. monocytogenes*. In those products receiving a listericidal treatment, the presence of the pathogen is generally associated with recontamination from environmental sources prior to final packaging. Other RTE foods may be contaminated at the point of sale, for example, due to slicing of processed meats. Within the home, opened packages may be contaminated from *L. monocytogenes* present within the refrigerator or in other refrigerated foods, from the kitchen environment or from family members. In a risk assessment by the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture Food Safety and Inspection Service (FSIS), deli meats were identified as having the highest predicted relative risk for causing listeriosis cases and deaths among 23 categories of RTE foods that are potentially contaminated with *L. monocytogenes*, on both a per serving and per annum basis. The risk assessment estimated that about 90% of human listeriosis cases in the United States are caused by consumption of contaminated RTE deli meats [50].

2.1 Listeria in Deli meats

Deli shortened from delicatessen is a delicacy or specialty food that is popular in many developed countries around the world.

The three major categories of deli meats are 1) freshly cooked meat with salt and spices as in turkey and roast beef 2) moderately processed, ground meat that has been emulsified into a soft cake-like batter where flavors, additives, binders are added, it is then cased in a cylindrical package and cooked for several hours as in turkey, bologna and chicken deli 3) very processed, as the second category but aged for several days and fermented as in hot dogs, liver sausage [51].

The prevalence of *L. monocytogenes* outbreaks in deli meats and other meat products in 16 countries are shown in Table 2.

Table 2. Prevalence of *L. monocytogenes* outbreaks in deli meats and meat products.

Country	Product	Prevalence (%)	Reference
Canada	frankfurters & wieners	5	[52]Bohaychuk et al., 2006
China	ready-to-eat meat	1.4	[53]Yang et al., 2016
Spain	pate	5.4	[54]Dominguez et al., 2001
Switzerland	beef	6.3	[55]Fantelli & Stephan, 2001
Belgium	cooked meat products	1.1	[56]Uyttendaele, 2009
Sweden	meat products	1.2	[57]Lambetz, et al., 2012
Northern Spain	store packaged deli-meat	8.5	[58]Garrido et al., 2009
Estonia	raw meat products	18.7	[59]Kramarenko et al., 2013
UK	RTE salad with meat	6.0	[60]Little et al., 2009
Spain	RTE cooked ham	12.5	[61]Cabedo et al., 2008
Spain	RTE dried sausage	16.9	[61]Cabedo et al., 2008
Italy	vacuum packed salami	20.5	[62]Di Pinto et al., 2010
China	RTE meat products	7.4	[63]Yu & Jiang, 2014
Greece	RTE meat products	1.9	[64]Materagas et al., 2010
USA	deli meats	0.4	[65]Pradhan, et al., 2011
Trinidad & Tobago	RTE deli meats	8.1	[66]Syne et al., 2011
Korea	RTE kimbab	6.7	[67]Cho et al., 2008
Mexico	RTE turkey frankfurters	11.7	[68]Casteneda et al., 2013

The incidents of nationwide or multi-state Listeria outbreaks linked to deli-meats are commonly reported in Canada and USA. In 2004, there was a nationwide outbreak in 24 states of the US with 108 reported cases that led to 14 deaths, 4 miscarriages in pregnant women [22]. In nine US states, there were 54 cases with 8 deaths reported in 2002 [9]. In 2000, 30 cases were also reported in eleven US states, with 4 deaths and 3 miscarriages [69]. In 2008, 57 cases of listeriosis and 24 deaths in Canada were linked to contaminated delicatessen meat from one meat processing plant [26].

In 2003, there was a recall of ready-to-eat (RTE) meat products by a large processing plant in Trinidad as a result of contamination by *L. monocytogenes*. Of the 32 processed RTE meat products tested from this plant, 11 (34.4%) were positive for Listeria spp., E. coli, Salmonella spp. and Campylobacter spp. either in combination or singly [70].

The USDA reported that approximately 83% of Listeria cases and deaths in the US are associated with deli meats sliced and packaged at retail outlets [71]. Mechanical slicers were reported to be an important source of Listeria cross-contamination in deli meats at retail outlets. It was recommended that these outlets should hold their products at a temperature of 5 ℃ or below, maintain person hygiene and wear personal gloves while serving customers, use growth inhibitors and maintain a log on the methods and frequency of cleaning surfaces [50]. The Food and Drug

Administration (FDA) Food Code specified a minimum frequency of every four hours for slicers to be fully disassembled, cleaned, and sanitized by retailers to reduce Listeria illnesses and outbreaks [72]. Further details on prevention and intervention strategies are discussed in section 4.

2.2 Listeria in Cheese

Foods can be contaminated after processing by the introduction of unpasteurized materials. This can occur during the preparation of some cheeses especially when unpasteurized raw milk is used. Listeria can also be spread by contact with contaminated hands, equipments and counter tops. The centralized production of prepared RTE food products increases the risk of higher levels of contamination, since it requires that foods be stored for long periods at refrigerated temperature which favors the growth of Listeria. During the preparation, transportation and storage of foods, the organism can multiply to reach a threshold needed to cause infection. The ability to identify the critical control points in the manufacture of cheeses will help to prevent Listerial contamination and how it behaves under varying post-processing packaging conditions [73].

Table 3. Prevalence of Listeria monocytogenes outbreaks in cheese

Country	Product	Prevalence (%)	Reference
Ethiopia	cottage cheese	1.0	[75]Gebretsadik et al., 2011
Portugal	soft cheese	46	[76]Pintado et al., 2005
Portugal	hard & semi-hard cheese	24	[77]Guerra et al., 2001
Spain	soft cheese	1.0	[78]Vitas et al., 2004
Turkey	soft cheese	5.0	[79]Akpolat et al., 2004
Turkey	Tulum cheese	4.8	[80]Colak et al., 2007
Brazil	soft & semi-soft cheese	6.7	[81]Abrahao et al., 2008
Iran	white cheese	9.2	[82]Arslan & Özdemir, 2008
Mexico	Quesco Fresco cheese	3.4	[83]Moreno et al., 2007
Portugal	soft cheese (from raw ewe's milk)	46	[84]Pintado et al., 2005
Turkey	white cheese	2.4	[85]Aygun & Pehlivanlar, 2006
Ethiopia	cottage cheese	1.7	[86]Derra et al., 2013
USA	fresh soft cheese	0.17	[87]Gombas et al., 2003
Sweden	soft & semi-soft cheese	0.4	[58]Lambetz et al., 2012
Spain	RTE fresh salty cheese	1.3	[62]Cabedo et al., 2008
Italy	cream cheese	1.9	[63]Di Pinto et al., 2010

It is important to adhere to strict hygienic operations, rigorous safety procedures as the equipments or additives may be a source of Listeria contamination. Artisan cheesemakers believe that raw milk makes tastier cheese, but when the raw milk is not pasteurized, the risk of Listeria contamination is greater [74].The prevalence of Listeria outbreaks in cheese from different countries are shown in Table 3.

Sporadic cases of neonatal listeriosis have been reported in Japan. Since *L. monocytogenes* has been often isolated from foods in Japan, food-borne outbreaks potentially could have occurred. In 2001, *L. monocytogenes* serotype 1/2b was isolated from a washed-type cheese during routine *Listeria* monitoring of 123 domestic cheeses [18]. Similarly in Switzerland 10 cases of Listeria outbreaks in a locally made and distributed soft cheese (known as 'tomme') were reported in 2005. Out of the 10 cases, 8 were in older immune compromised patients who became ill with bacteraemia (three deaths), and two cases were in pregnant women who had septic abortion [24]. All the cases were due to a serotype 1/2a isolate with one of two pulsovars found by PFGE.

3. Identification, Sampling and Characterization of *L. monocytogenes*

The employed detection methods will play vital roles in a better understanding of the control programs and the interventions to be applied. In the identification of Listeria spp. based on Gram-staining, catalase, oxidase and motility tests, were used in the past as they can be distinguished by haemolysis test, CAMP test and sugar fermentation [88]. *L. monocytogenes*is is known to show a narrow β-haemolysison blood media and ferments D-rhamnose but not D-xylose. The CAMP test is positive with β-haemolytic *Staphylococcus aureus*, showing enhanced haemolysis at the intersection of the cultures of *L. monocytogenes* and *S. aureus*. *L. monocytogenes* can be distinguished from other Listeria spp. with commercial test kits such as API Listeria [89]. There are current modified rapid methods of enrichment, these include biosensory and bacteriophage based detection methods. These molecular tests are rapid and reproducible enabling multi-parameter testing with automation possibilities [90].

Several methods are available for typing *L. monocytogenes* strains in the surveillance of foodborne disease outbreaks including serotyping, genoserotyping (PCR serogrouping), polymerase chain reaction-restriction fragment polymorphism (PCR-RFLP), and pulsed-field-gel-electrophoresis(PFGE). PFGE has a high discriminatory power in outbreak surveillance, but it provides limited information about phylogenetic relationships and overall clonal structure [91].

Even though the cosmopolitan distribution of *L. monocytogenes* is worldwide, the geographic distribution of its clones remains largely unknown. Sequence-based typing methods such as multi locus sequence typing (MLST) and multi-virulence-locus sequence typing (MVLST) have been used to classify isolates into high level groups (epidemic clones and clonal complexes), allowing researchers to group geographically and temporally unrelated isolates into a common ancestor strain [92]. The European Food Safety Association Panel on Biological Hazards recommended the application of molecular typing methods [93].

Their recommendations include PFGE, which was established as the gold standard approach for epidemiological studies in foodborne outbreaks. PFGE has the advantage of being widely used for small-scale epidemiology, listeriosis surveillance and outbreak investigations but it is considered to be time-consuming and requires stringent standardization for inter-laboratory data comparison. PFGE also provides limited information on the phylogenetic relationships among strains, which

limits the understanding of the evolution of important phenotypic traits such as virulence [94]. EFSA also recommended MLST as a useful method for microbial ecology which is based on core genome sequences that can be used to investigate broad population structure of several bacterial species. In the case of *L. monocytogenes*, several studies have used MLST including a recent analysis of more than 1000 isolates, across wide temporal, geographical and source diversity [95]. However, MLST has a drawback since is neither rapid nor cheap and has limited discriminatory power within *L. monocytogenes*.

Other molecular typing methods that have been suggested include multi-locus variable-number tandem repeat analysis (MLVA), multi-virulence-locus sequence typing (MVLST), single nucleotide polymorphism (SNP) and clustered regularly interspaced short palindromic repeats (CRISPR) [96].

Although the genetic markers that are associated with enhanced virulence of *L. monocytogenes* are yet to be identified, however previous studies have identified multiple distinct mutations that leads to a premature stop codon (PMSC) in the key *L. monocytogenes* virulence gene, internalin A (in1A) [97,98]. This virulence factor (interlin InlA; encoded by *inlA*) facilitates the uptake of *L. monocytogenes* by non-professional phagocytic cells that express the human isoform of E-cadherin, and this was noted to play a critical role in crossing the intestinal barrier at the initial stages of an infection [99].

In recent years, high-throughput next-generation sequencing (HT-NGS) has become increasingly popular, replacing classical microbiology and first-generation bio-molecular technologies in several fields, as HT-NGS are known to deliver sequence data thousands folds more cheaply than Sanger sequencing [100]. By combining the laboratory and epidemiological data from PFGE and whole genome sequencing (WGS) have markedly improved the detection of listeriosis and have increased the number of solved outbreaks. WGS is known to compliment the time consuming PFGE and leads to a faster detection of Listeria as shown in the 'PULSE NET and LISTERIA' initiative [101].

4. Prevention and Containment Strategies

It is important that hygienic practices are regularly maintained by those in contact with foods, the food contact surfaces need to be clean in order to prevent cross-contamination from the processing facilities. The growth of Listeria in cold and damp area of food factories can be prevented by controlling the flow of water in these processing facilities. An interagency report on the risk assessment of *L. monocytogenes* in deli meats and cheeses identified measures that will reduce their outgrowth to include: cleaning and sanitizing the slicer which is a key route of contamination, use of growth inhibitors to mitigate their growth, strict temperature control during refrigeration and wearing of gloves [102].

Listeria are known to survive for extended periods in acidic conditions and can form biofilms to grow in the absence of oxygen. In recent years, biofilms have been shown to be important for harborage of various food-borne pathogens thereby increasing the risk for food safety violations [103]. Contamination of food products are fostered by biofilms on equipments and their formation is favored by the accumulation of food residues, spoilage organisms and food-borne pathogens. Many food manufacturing processes may provide niches of low sanitation level, where biofilms can prosper, including stainless steel (e.g. ultrafiltration membrane, valves, air separator), rubber surfaces (e.g. belt, packing machine, air separator, liner, short milking tube) and floors [104].

L. monocytogenes may increase or decrease in biofilms, depending on the competing microbes that are present. This organism was shown to increase in mixed biofilms with *Pseudomonas fragi* [105], *Pseudomonas fluorescens* [106] and *Flavobacterium* spp. [107]. In addition, large amounts of exopolysaccharides (EPS) may improve the conditions for adherence by entrapment [105]. The number of *L. monocytogenes* cells has been observed to decrease in a mixed biofilm with *Staphylococcus sciuri* [108] or with *Staphylococcus xylosus* and *Pseudomonas fragi* [109]. In a recent study on *L. monocytogenes* from retail deli environments, it was shown that *L. monocytogenes* vary in their ability to adhere and form biofilms [110]. The results also indicated that persistent strains had enhanced adhesion on day 1 of a 5-day adhesion-biofilm formation assay and there was no significant difference in sanitizer tolerance between persistent and transient strains. The major findings from this study suggested that foods contaminated with persistent *L. monocytogenes* strains from the retail environment are likely to have wild-type virulence potential which may persist due to increased adhesion and biofilm formation capacity rather than sanitizer tolerance, thus posing a significant public health risk since *L.monocytogenes* are able to form biofilms and tolerate sanitizers for their survival. Sanitizers extend the lag time of Listeria growth and are less effective against the biofilms formed thereby increasing the risk of outbreaks.

Since research has proven that the control of *L.monocytogenes* is directly associated with the removal of biofilm and the persistence of *L. monocytogenes* in areas that are used for food storage, processing, production and transportation. Therefore, it is important to mitigate biofilms in food production environments [111]. The interplay of these mechanisms along with their virulence potential and persistence in deli environments is an important area that needs to be studied further. During routine hygiene monitoring in food processing environments, drain water samples are often taken without inclusion of drain biofilm matrix. As part of an efficient monitoring program, it was hypothesized that by including both drain water and drain biofilm samples in *Listeria* monitoring programs, this might yield more reliable insights into the presence of *Listeria* and the composition of microbial communities originating from floor drains [112].

There has been a lot of work carried out to prevent or reduce Listeria contamination in USA foods. The Listeria rule as described in [113] established three alternative ways to address post – lethality contamination of *L. monocytogenes* in RTE meat and poultry products. Under Alternative 1, the establishment applies a treatment to the product after its exposure to the processing environment (post-lethality treatment) and uses a growth inhibitor (antimicrobial agent or process) to prevent the growth of *L. monocytogenes* in the product up to its declared shelf life. Under Alternative 2, the establishment can use either a post-lethality treatment or an antimicrobial agent/process to control *L. monocytogenes.* Alternative 3 requires the establishment to have a sanitation program controlling *L. monocytogenes* contamination in the processing environment and on the product. The three USDA alternatives decreased the incidence of Listeria to less than 0.1% in intact packages. The prevention and containment intervention strategies can be classified as:

4.1 Physical treatments

They include thermal and non-thermal treatments such as irradiation UV light, pulsed light, and the use of high pressures. Thermal treatment above 70 ℃ is the most common traditional intervention strategy against *L. monocytogenes.* However, it is well known that the microorganism may develop an adaptive response during exposure to sublethal food processing interventions and the pathogenic

potential is preserved [114]. The use of UV light as an alternative treatment to traditional thermal treatment to disinfect surfaces of equipments in meat plants is gaining acceptance. Studies have demonstrated that UV light has the potential to reduce bacterial contamination in food surfaces and as post-lethality treatment to control *L. monocytogenes* in meat and poultry processing facilities [115].

The efficacy of Pulsed Light as an alternative to thermal treatment in reducing *L. monocytogenes* on the surface of 2 RTE dry cured meat products was investigated. The results showed a maximum log reduction of 1.5–1.8 cfu/cm^2 at 11.9 J/cm^2 pulsed light [116] irradiation employs the use of ionizing radiation from ^{60}Cobalt, ^{137}Caesium, or machine generated electron beams, it can either be used alone or in combination with other treatments. The applied dosage is important as too high dosage can have negative impacts on the sensory quality of the product [117].

It was possible to control *L. monocytogenes* on frankfurters and cooked pork chops with irradiation and modified atmosphere packaging (MAP) containing a high concentration of CO_2 [118]. The potential use of pectin-nisin films alone or in combination with ionizing radiation for preventing listeriosis due to post-processing contamination of ready-to-eat meat products was very promising [119].

In an investigation on the application of Intense Light Pulses (ILP), ILP was shown to reduce *L. monocytogenes* and *E. Coli* 0157:H7 on the surface of stainless steel knife in meat contacts. The results further suggested ILP can be effective in meat processing lines as an intervention strategy to prevent cross-contamination between the equipment and the final product [120]

High hydrostatic pressure (HHP) has been applied to decrease the count of *L. monocytogenes* in foods. Recently, it was reported that treatment at 600 MPa for 3 min resulted in an immediate 3.9–4.3 Log CFU/g reduction of *L. monocytogenes* count in RTE sliced ham [121]

HHP was shown to be effective in slowing the growth of microorganisms that will shorten the shelf life of Queso Fresco cheese. The effect of HHP on the survival of a 5-strain rifampicin-resistant cocktail of *L.monocytogenes* in Queso Fresco cheese was evaluated as a post-packaging intervention. The result from this evaluation showed that HHP at 600 MPa was most effective in reducing L. monocytogenes to below the detection level of 0.91 log10 cfu/g when held at either 20 or 40 ℃ for 5, 10, 15 or 20 min [122].

4.2 Chemical treatments

Under the USDA/FSIS rule, alternative 3 in the control of *L. monocytogenes* rigid sanitation control measures are to be incorporated into the Hazard Analysis Critical Control Point (HACCP) plan, Sanitation Standard Operating Procedures (SSOP), or prerequisite program. The potential for the contamination of Refrigerated or Frozen-Ready-To-Eat (RF-RTE) food and food-contact surfaces with *L. monocytogenes*, can be minimized through the establishment and use of a sanitation program that includes a written SSOP and a sanitation maintenance schedule for areas where RF-RTE foods or food-contact surfaces are processed or exposed [123].

Sanitizers that contain quaternary ammonium compounds (QACs), peroxyacetic acid, iodine, or chlorine have been used to control *L. monocytogenes* in various situations. The Food and Drug Administration (FDA) recommends the use of QACs for many applications, because QACs have been found to be effective against *L. monocytogenes* and leave a residual germicidal effect on

surfaces. FDA also noted that peroxyacetic acid sanitizers have been shown to be effective against biofilms containing *L. monocytogenes* [124].

In order to discourage the excessive use of sanitizers such as chlorine in limiting the growth of *L. monocytogenes* in deli meats and cheese, electrolyzed oxidizing water (EOW) is a suitable alternative as an effective sanitizing agent that finds application in post-processing interventions. However, it was observed that treatments with the acidic EOA or basic EOB were not enough to meet regulatory requirements for control of *L. monocytogenes* on RTE meats. As such, there is a need for additional interventions to control the pathogen on RTE meats [125]. Lauric arginate is labeled as GRAS and it may be used successfully as part of an integrated cleaning and sanitation program [126].

Sanitizer on food contact surface by definition is required to bring about a 5 log reduction after a 30s contact time. Lauric arginate tested at 100 and 200 ppm concentrations showed a maximum of 2.5 log CFU/coupon at 100 ppm concentration with exposure time of 15 min at low bacterial concentration levels of 3 log CFU/coupon; therefore, it does not meet the requirements of being either a cleaner or sanitizer when used alone [126]

4.3 Natural interventions

These include the use of probiotics, probiotic metabolites and organic acids, phytochemicals. Plant based secondary metabolites with generally recognized as safe (GRAS) bioactive substances can be used to control the growth of *L. monocytogenes*. The antimicrobial activity of plant extracts was attributed to the presence of different compounds especially the low molecular weight phenols, terpenes and ketones that also show antimicrobial activities in their pure form [127]. The essential oils (EOs) from different plants are known to have different minimum inhibitory concentration against *L. monocytogenes* in vitro. EOs with the strongest anti-bacterial properties against food pathogens contain a high percentage of phenolic compounds such as carvacrol, thymol and eugenol [128]

The antimicrobial effects of natural occurring peptides such as nisin were shown to have inhibitory effects on *L. monocytogenes.* However, some Gram-positive bacteria that are repeatedly exposed to increasing nisin concentrations can acquire nisin resistance. The emergence of nisin tolerance has been reported in several species of bacteria, including *L. monocytogenes* [129,130]. As a result of this resistance, the use of natural antimicrobials from a broad variety of natural sources is been explored. Soy peptide in combination with nisin was shown to restrict the motility and aggregation of *L. monocytogenes* [131]. Recently, it was shown that there is a synergistic interaction of nisin and *p*-Anisaldehyde (AS) which is extracted from anise seed oil, derived from *Pimpinella anisum* L. seeds can be used to control a diverse array of *L. monocytogenes* strains [132].

Organic acids such as acetic, lactic, malic, and citric are natural constituents of many foods and they have been used in food preservation since ancient time. Cured meats, such as sausage, ham and frankfurters are more susceptible to the listericidal effects of organic acids probably as a result of the presence of salt, nitrite and other preservatives [133]. By dipping frankfurters with a solution containing 2.0% of acetic acid, 1.0% of lactic acid, 0.1% propionic acid and 0.1% of benzoic acid followed by a steam prevented the growth of *L. monocytogenes* for 14 weeks at 7 ℃ [134]

The applications of plant-based strategies for *L. monocytogenes* control in foods are likely to grow, due to the consumer demand for natural products (green label) and the industry interest for additive decrease (clean label) [114].

4.4 Active packaging

A combination of these intervention methods, incorporated preservatives into edible films for active packaging are of interest to researchers. In addition, commercial anti-listeral phage preparations which can be employed in the presence or absence of chemical preservatives are also

The use of chitosan edible films in active packaging is useful in reducing *L. monocytgenes in foods*. However, as chitosan are not able to migrate from the film to the food matrix and it is important to add other preservatives such as bacteriocins, EOs and organic acids since chitosan film allow the gradual release of these preservatives into foods [133].

Trinetta and co-workers demonstrated the effectiveness of sakacin A-containing pullulan films to control *L.monocytogenes* growth and the applicability of active pullulan films as a means of delivering a bacteriocin directly to a food surface. Pullulan films require less antimicrobials, they demonstrate longer antimicrobial activity and allow for controlled migration of the molecule from film to the food matrix, when compared with the direct addition of sakacin A to RTE meat products [135].

5. Conclusion

The prevention of the outbreaks of listeriosis associated with deli meats and cheese during their processing, storage and distribution is vital. Listeriosis as a food-borne disease is a big economic burden to the food industry and the public health services. Modern methods of identification that are rapid, accurate and reliable will help in its control. In order to improve the chances of listeriosis to be included as a disease in the international priorities of the World Health Organization, the quantification of the burden of Listeria and listeriosis especially in developing countries need to improve.

Conflict of Interest

The author declares no conflict of interests in this paper.

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