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A Review on *Listeria monocytogenes* in Food of Animal Origin

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Abstract

Listeria monocytogenes is the most important specie in the genus Listeria causing human and animal health threat. Among the species only *L. monocytogenes* and L. ivanovii are considered pathogens *L. monocytogenes* is ubiquitous in the environment and can be found in soil, water, faeces,silage, effluents foods and sewage. Even though freezing can lead to a reduction in *L. monocytogenes* numbers, it can grow at temperatures as low as 0°Cin food during refrigerated storage. Human listeriosis is usually associated with older adults, pregnant women, newborns and adults individuals with inadequate immune system. Ingestion is the main transmission methods then it enters the intestine and binds to the receptors on the host cells to instigate adhesion and internalization. The incidence of human listeriosis is low; the mortality rate is from 20% to 30%and with the highest hospitalization rate. In general, *L. monocytogenes* can contaminate all types of animal origin food moreover contamination may also occur after cooking by cross-contamination environmentally or via workers, surfaces and equipment's. There have been recent outbreaks of food poisoning associated with L. monocytogenesin different country. One-broth Listeria method, PCR (RTi----PCR), antibodybased tests, ELISA, immune-capture methods, molecular methods targeting different genes and biosensor methods are different test methods used for analysis of L. monocytogenes. Highest resistance was detected against antibiotics so recommended that effective cleaning and sanitation programs and safe handling procedures are important for ensuring a safe, high quality food for consumer.

Keywords: Listeria, Food, Animal, Contamination, Characteristics.

Introduction

Listeria monocytogenes is the most important specie in the genus Listeria causing human health threat and spread worldwide with specific host range [1]. Even though *L. monocytogenes* is mostly responsible of human listeriosis but occasionally infection with other species such as L. seeligeri and L. ivanovii has been reported. Starting in the 1960s, as a result of the introduction and widespread use of refrigerators, processed foods and extended shelf life foods became more associated with listeriosis due to *L. monocytogenes* [2]. The disease primarily affects older adults, pregnant women, newborns and adults with weakened immune systems. Today, listeriosis is regarded as a food-borne disease of serious public health concern. The incidence of human listeriosis is low (0.1–11.3 cases per million inhabitants), but it is increasing in Europe [3]. The mortality rate is reported to be 20–30% [4]. In sum, the available literature shows that *L. monocytogenes* has been reported from a wide variety of food types and responsible for outbreaks and clinical manifestations in various countries of the world [4]. Most cases of listeriosis are

sporadic. Despite this, foodborne outbreaks due to *L. monocytogenes* have been associated with cheese, raw (Unpasteurized) milk, deli meats, salad, fish and smoked fish, ice cream and hotdogs [4] also includes poultry and ticks [4].

Listeria commonly present in the dairy environment, on the farm and in the processing plant. On the farm, animals have some pathogens itself, but Listeria is frequently present in manure and fermented silage. It is most abundantly present in the humid areas, stable water including drains, coolers, washing areas and floors [5]. The strong relation between occurrence of *L. monocytogenes* in raw milk and the infection of the disease frequency was observed. Hence, a decrease in the number of cases per year in all populations was observed when unpasteurized milk was tested [6]. With increasing the consumption of manufactured ready-to-eat foods in the whole world, it has become known as an important opportunistic human foodborne pathogen [7].

Despite an increasing rate of Listeriosis reported in several European countries in recent years and other outbreaks in the United States [8] Canada [9,10] and China [11] the occurrence and prevalence of the organism in food borne disease in Africa for examples, Nigeria is hardly reported [12]. But In Ethiopia, there is limited data regarding the prevalence of *L. monocytogenes* in animal origin foods [13].

Lsteria monocytogenes is an important food bore pathogen that cause septicemia, meningitis and gastroenteritis particularly in children, the elderly and immune suppressed individuals. It causes miscarriage in pregnant women. In addition to being a public health risk, *L. monocytogenes* is an economic burden on the ready-to-eat (RTE) food industry. Ready-to-eat foods are the most vulnerable to *L. monocytogenes* as they do not have a heating or other antibacterial step between production and consumption. The economic burden includes the cost of analysis of samples, the costs, both financial and reputational, of recall of a contaminated product and the possible litigation costs, if the food is shown to have caused disease [14]. The standard microbiological methods for identification of Listeria species are laborious and time consuming, requiring a minimum of five (5) days to recognize listeria species and about 10 days to identify *L. monocytogenes* by confirmation test [15].

Therefore, the objective of this paper is to review Listeria Monocytogens associated with food of animal origin and its diagnosis, analysis, control and prevention.

General characteristics of *Listeria monocytogenes.*

Description of the Organism

Listeria monocytogenes is a Gram-positive, non- acid fast, non-spore forming, rod-shaped, facultative anaerobic an intracellular bacterium. It belongs to the genus Listeria along with six (6) species:L. monocytogenes, L. innocua, L. seeligeri, L. welshimeric, L. ivanovii and L. grayi. Of which L. ivanovi and L.monocytogenes are pathogenic for mice and other animals [16]. Only two are considered pathogens: *L. monocytogenes* which infects humans and animals, and L.ivanovii which infects ruminants (Although there have been rare reports of L. ivanovii being isolated from infected humans) [17,18]. There are thirteen known serotypes of L. monocytogenes: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7. The serotypes most often associated with human illness are 1/2a, 1/2b and 4b [19]. There are no strains of *L. monocytogenes* with unique properties that lead to persistence and there are no mechanisms that can protect the organism when present in acidic juices, yogurt and salad dressing and modified CO2 atmosphere [20].

Morphology, Growth and Survival Characteristics

They are facultative anaerobe that grows best under reduced oxygen and increased carbon dioxide concentration. Growth occurs at 4 to 45°C with an optimum temperature of 30 to 37°C. Simple, laboratory media support growth preferably at an alkaline or neutral PH. Listeria tolerates 0.04% potassium tellurite 0.025% thallium acetate, 3.75% potassium thiocyanate, 10% NaCl and 40% bile in media. Most strains grow over a pH range of 5.5 to 9.6 [21]. *Listeria monocytogenes* has become known as an important opportunistic human food borne pathogen [7]. Freezing can also lead to a reduction in *L. monocytogenes* numbers [22]. As *L. monocytogenes* can grow at temperatures as low as 0°C, it has the potential to grow, albeit slowly, in food during refrigerated storage. *L. monocytogenes* will grow in a broad pH range of 4.0–9.6 [22]. It becomes more sensitive to acidic conditions at higher temperatures [2]. Like most bacterial species, *L. monocytogenes* grows optimally at a water activity (aw) of 0.97.

However, *L. monocytogenes* also has the ability to grow at an awof 0.90 [22]. Survival in the presence of salt is influenced by the storage temperature. Studies have indicated that in concentrated salt solutions, the survival rate of *L. monocytogenes* is higher when the temperature is lower [22]. It has greater heat tolerance than other non-spore forming bacteria; however, short-time high temperature pasteurization is effective for killing listeria [21]. *L. monocytogenes* can grow under both aerobic and anaerobic conditions; although it grows better in an anaerobic environment [22]. *Listeria monocytogenes* can consume limited numbers of carbon sources for the energy with glucose. Thus, this microbe is essentially able to utilize different sources of energy for the survival or growth in gastrointestinal phase e.g. degradation of proteins, polymers of carbohydrates, lipids and nucleic acids [23].

Epidemiology

Persistence of Listeria monocytogene

L. monocytogenes is ubiquitous in the environment and can be found in soil, water, faeces,silage, effluents foods and sewage [24]. It has the ability to form biofilms which can contribute to its ability to colonize food processing facilities. It is also resistant to many of the stresses imposed in food processing such as salt (Up to 10% salt), temperature (Refrigeration temperatures) and detergents (Many detergents). Therefore, it can survive in food processing environments and become persistent. Such persistence of *L. monocytogenes* has been shown, often for many years, at larger scale and smaller artisan facilities of different production sectors [25-27] Because L.monocytogenes is ubiquitous in the environment and frequently present in the processing environment, it can contaminate foods includes fish, mammal, crustacean, poultry,and ticks [1] meat, soft cheeses and ready to eat products are frequently [28] in cooked foods due to post-processing contamination [14] the food processing environment [29-31] in smoked fish products and processing facilities [26] and ready-to-eat food producing facilities [32]. Different rates of Listeria species prevalence were observed among the various geographical areas under investigation that there was a difference in contamination index of animal origin by a range of pathogens between two different geographical locations in which different risk factors for each study region [28].

In Ethiopia, Addis Ababa some studies show that there is the distribution of *L. monocytogenes* in foods of animal origin [13, 33]. *L. monocytogenes* can also be isolated from the surface and underground waters, improperly fermented silage, sewage sludge, slaughter wastes, animal and human faeces, foodstuffs, and food industry plants [34]. The occurrence of L.monocytogenes in surface waters seems to be related to direct upstream land use, specifically, crop land and proximity to a dairy farms [34]. Silage is the most common feed to harbor L. monocytogenes. Chemical quality of silage, i.e. its PH and aerobic deterioration, affects the presence of *L. monocytogenes* and the pathogen iscommonly found on poor quality silage [34].

Listeria is most abundantly present in the humid areas, stable water including drains, coolers, washing areas and floors [5]. Epidemiological patterns of human listeriosis include a background level of sporadic cases with occasional outbreaks [35, 36]. A minimal infective dose has not been determined in human infection studies and estimates vary from 102 colony-forming units (cfu) to 109cfu, depending on the immunological status of the host [37].

Host factors that influence Listeriosis in Humans

Saha *et al.* [38] suggested that the first report was in 1929 about human listeriosis, and in 1936 first perinatal case was reported. Human llness is caused by eating the contaminated food which causes the infection [39] which may lead to serious and potentially life-threatening listeriosis. The presence of listeriosis is usually associated with young, old, pregnant and immunocompromised individuals with inadequate immune system [40] typically presents as septicemia, meningitis, or meningoencephalitis, intrauterine infection; and sometimes death are reported [40]. Less frequently reported, but also at a greater risk, are patients with diabetes, asthma, cirrhosis (Liver disease) and ulcerative colitis (Inflammatory bowel disease) [19].

Virulence and infectivity

When *L. monocytogenes* is ingested, it may survive the stomach environment and enter the intestine where it penetrates the intestinal epithelial cells.Furthermore, acidic conditions in the gastrointestinal tract and in macrophages following phagocytosis can be encountered by the pathogen and acid tolerance thus enhances the virulence [41]. The organism is then taken up by macrophages and non-phagocytic cells.

The *L. monocytogenes* surface protein internal in is required for this uptake by non-phagocytic cells, as it binds to the receptors on the host cells to instigate adhesion and internalization. The bacterium is initially located in a vacuole after uptake by a macrophage or non-phagocytic cell. *L. monocytogenes* secrete listeriolysin O protein, which breaks down the vacuole wall and enables the bacteria to escape into the cytoplasm. Any bacteria remaining in the vacuole are destroyed by the host cell. Once located in the cytoplasm of the host cell, L. monocytogeneis able to replicate. *L. monocytogenes* is transported around the body by the blood, with most *L. monocytogenes* being inactivated when it reaches the spleen or liver. L. monocytogeneis able to utilize the actin molecules of the host to propel the bacteria into neighboring host cells. In the case of invasive listeriosis, this ability to spread between host cells enables *L. monocytogenes* to cross the blood-brain and placental barriers [42, 43].

Reservoir and Mode of Transmission

Contamination may also occur via tools or hands, footwear and gloves and aprons of the personnel involved in manufacturing [34]. All food contact surfaces can be a source of contamination, but concern especially rests on complex machinery that is hard to clean and equipment that has contact with large production like dairy processing plant [34]. Because of the ubiquitous nature of L. monocytogenes, it is unrealistic to eliminate the organism from the food chain and possible contamination sites will exist throughout the chain [34]. Calves which develop septicemic are disease may acquire infection from contamination on the cow with subclinical bacteremia, through the navel from the environment and also as a congenital infection [21]. The encephalitic form of the disease results from infection of the terminals of the trigeminal nerve consequent to abrasion of the buccal mucosa from feed or browse or from infection of tooth cavities. Spinal myelitis is believed to result from growth to spinal nerves subsequent to body area infections [44].

Clinical Presentation and Course of the Disease in Human and Animals

The average incubation period is 3 weeks that is from 3 days to 3 months [19]. After ingestion of the contaminated food, the bacteria that survive the acidity of the stomach enter the small intestine, cross the epithelial barrier and then spread to the liver, spleen, central nervous system and the fetus in pregnant women [45]. The incidence of human listeriosis is low (0.1–11.3 cases per million inhabitants), although rare, the mortality rate of listeriosis is from 20 to 30 [46] 25% worldwide [47] but in those with underlying disease or receiving immunosuppressant patients without adequate treatment up to 70% [48] and with the highest hospitalization rate amongst known food borne pathogens > 95% [49] 91% [47]. Listeriosis affects domestic and wild animals, most often sheep and cattle, rarely goats, horses and poultry. Cows can excrete Listeria after miscarriage or during udder infections followed by mastitis, through milk. This was observed in some cases for several years. Contamination of milk by these bacteria can also be of faecal origin. Paralysis and circling movement was observed in sheep affected by listeriosis in late stage of the disease [50]. In animals, listeriosis usually occurs in five distinct clinical presentations, of which encephalitis is by far the most common form, followed by abortions, whilst neonatal septicemia, mastitis and kerato conjunctivitis/uveitis occur quite rarely. These syndromes seldom overlap within the same animal or the same flock. Some authors speculate that encephalitis occurs as a distinct syndrome and more frequently than other clinical syndromes in farm ruminants because immunity acquired through ingestion of contaminated silage protects against septicemia and abortion but is not fully effective in protection against encephalitis [51]. Furthermore, ruminants may commonly be asymptomatic intestinal carriers of the organism [52].

Occurrences of *Listeria monocytogenes* in Different Food of Animal Origin

In general, pathogenic microorganisms can contaminate raw milk in two ways. The first way is an endogenous contamination where the milk is contaminated by a direct transfer from the blood (Systemic infection) to the milk or via an infection in the udder called mastitis. The second way is an exogenous contamination, where the milk is contaminated during or after milking by the faeces, the exterior of the udder and teats, the skin, the environment, etc [53]. Based on documents [54-57] *Listeria monocytogenes* is considered as the main microbiological hazards associated with raw milk consumption among different pathogens. The EU summary report mentioned that non-compliance of *L. monocytogenes* primarily occurred in soft and semi-soft cheeses made from raw or low heat-treated cows' milk [58].

Some factors which are involved in contamination index between the region where the impermanent cattle confinement, poor hygienic milking conditions, no cleaning, low milk production, and milk storage regions [59] have introduce a procedure for the quick detection of *Listeria monocytogenes* in raw milk and soft cheese via merging of redox potential measurement methods for real time PCR and enrichment in which identification in an easy, time and the cost effective manner. Concerning butter, cream and butter milk, there is less information available in the scientific literature. But it is the main microbiological hazards in butter made from raw milk and it has been detected in butter. However, the risk of infection after consumption of raw milk butter is estimated to be relatively lower in comparison with certain cheeses. *L. monocytogenes* also the main microbiological hazards in and it has been detected in cream. Even though pasteurization of raw milk destroys L. monocytogenes, this process does not eliminate later risk of contamination of dairy products [60].

The detection of *Listeria monocytogenes* in food is of particular concern in terms of consumer safety as this organism is capable of growing on both raw and cooked meat at refrigeration temperature [61]. *L. monocytogenes* is commonly found in comminuted meats and is detected less often in tissues of freshly slaughtered pigs than in ground pork [62]. Contamination may also occur via tools or hands, footwear and gloves and aprons of the personnel involved in manufacturing [34]. Contamination of RTE poultry products can occur after cooking by cross-contamination environmentally or via workers, surfaces and equipments [63]. While processed meat and poultry products are cooked to destroy Listeria, these bacteria can recontaminate the product while it is being handled, packaged or distributed [64].

A more recent study reported 196 *L. monocytogenes* isolates and demonstrated 3 genotypes out of total of 144 liquid whole egg samples [65]. studies were done by [66] found 72.4% of fish and 44.4% of shellfish tested were found to be positive for Listeria species [67, 68] reported 37.8% of sea food samples collected from Mysore was positive for Listeria species. Listeria species in tropical sea food of Keralaand India found that L. innocua was the most prevalent species of Listeria with an incidence rate of 28.7% and *L. monocytogenes* with low prevalence i.e., 1.2% of the total samples tested [69]. Observed the higher incidence of *L. monocytogenes* (23.9%) in fish and fish swab samples from Kerala [70].

Table 1. Different study in Africa on the Occurrences of *L. monocytogenes* from food of animal origin.

No	Authors and year	Country	Number of samples	Diagnostic techniques	Overall prevalence	Prevalence of L.monocytogenes by food category by food category
1	[12]	Nigeria	255	Plating on compact DRY LS	63.5%	Beef (88%), Chicken (64.7%) and fish (37.7%)
2	[71]	Ethiopia	384	ISO 11290-1	6.25%	Raw meat (6.66%), Minced beef (12%), Fish (6%), Pasteurized milk (0%), Raw milk (4%), Cottage cheese (0%)
3	[72]	Egypt	80	plating on PALCAM	11.25%	Retail local and imported pork by-products (11.25%)
4	[73]	Ethiopia	240	ISDFM	4.1%	Raw meat (6.8%), Raw milk (3.4%), Cottage cheese (1.7%),
5	[74]	Morocco	288	ISO 11290-1	5.90%	Raw milk (8.33%),
6	[75]	Algeria	227	AFNOR V08055	2.6%	Heat treated dairy products (3%), meat product s (2.6%)
7	[76]	Ethiopia	391	USFDA method	5.4%	Raw milk (13%), liquid whole egg (4.3%), raw beef (2.6%), cottage cheese (1%)
8	[77]	Egypt	576	Oxoid and plated on OXFORD aga	14%	Meat products (16%), poultry products (9%), sea food products (8%), dairy products (14%)
9	[78]	Uganda	100	FDA-BAM	6.1%	Rawmilk (13%), yoghurt (3%), fermented dairy products (0%)

10	[79]	Ethiopia	711	ISO 11290-1	4.8%	Soft cheese (3.9%), meat products (3.7% to 5.1%)
11	[80]	Nigeria	115	ISO 11290-1	25%	Smoked fish (25%)
12	[81]	South Africa	99	plating on Oxford agar	9.2%	Fresh chicken (17%), frozen chicken (24%)

Listeria monocytogenes Status in Ethiopia

Although foods of animal origin such as milk, cheese, meat and poultry are consumed well in Ethiopia, published information on the status of food borne listeriosis caused by *L. monocytogenes* is very limited and incomplete in both in the veterinary and public health sectors [5]. But some study for example: by [82] showed that Listeria species exist in the Ethiopian food production system and his study detected *L. monocytogenes* and L. innocua were found. In Ethiopia, a study has shown the presence and distribution in a variety of raw and ready- to-eat food products in Addis Ababa with a prevalence of 5.1% [73, 83] described 4.1% of prevalence from raw meat and dairy products like raw milk, cheese and cream cake collected from the capital and five neighboring towns in Ethiopia. According to [13] research study the overall prevalence of Listeria species was 28.4% and specifically that of *L. monocytogenes* was 5.6%. Taking the prevalence of Listeria species into consideration, cheese was found to be highly contaminated at 60%, followed by pasteurized milk samples (40%), raw milk (18.9%) and yoghurt (5%). Considering the prevalence of *Listeria monocytogenes* only, raw milk had the lowest contamination while cheese had the highest, followed by pasteurized milk and yoghurt. The study shows as raw milk and milk products produced in urban and per urban areas of central Ethiopia were contaminated with pathogenic bacteria, L. monocytogenes. Finally the study concluded that milk and milk product contamination with *L. monocytogenes* in the central highlands of Ethiopia is an issue of public health significance. The presence of *L. monocytogenes* in raw bovine milk and especially milk products such as pasteurized milk warrants an urgent regulatory mechanism to be put in place. High public health concern as most of the milk and milk products in Ethiopia are consumed in raw forms without being treated with sufficient heat. Considering the high fatality rate for listeriosis, especially in the risk groups, this rate of prevalence is of high public health concern [13].

Outbreak of *Listeria monocytogenes*

The first confirmed role of food in the transmission of listeria infection was provided by the outbreak that occurred in 1977 in Boston (USA) in which 20 persons were reported to have developed listeriosis by eating contaminated raw celery of tomatoes and lettuce [21]. There have been recent outbreaks of food poisoning associated with *L. monocytogenes* [13]. In the year 1986, the WHO has recognized *L. monocytogenes* as an important emerging food-borne pathogen. There have been many recent high-profile outbreaks of listeriosis worldwide that have resulted in numerous fatalities [84] reviewed a particular issue of listeriosis-associated brain stem encephalitis [84].The listeriosis presence in human population is low in the percentages1%, but with high fatal outcome 30% [85]. Low incidence of human listeriosis is low annually causes 2500 of serious cases of illness with approximately 500 deaths [86]. [87] suggested that 250 death rate, 1600 invasive infection and 1500 hospitalizations per year by listeria species. Of those individuals with laboratory confirmed listeriosis, there is a 94% hospitalization rate and 15.9% death rate. Hazard of listeriosis in pregnant women is ten times greater than the common people and four times higher in individuals aged sixty-five years or older. Back ground information about outbreak of listeriosis can be found in ECDC, CDC and WHO disease fact sheets [88-90]. During the period 2012–2016, between 1 754 and 2 555 *Listeria monocytogenes* cases were reported annually to the European Surveillance System (TESSy) by 30 EU/EEA countries [88]. Event background information in 2017, Finland reported in EPIS-FWD an urgent inquiry describing a cluster of *L. monocytogenes* PCR sero group IVb, MLST ST6 confirmed by WGS (in-house cg MLST scheme), with 13 cases detected from different parts of Finland between 2016 - 2017. As of 2017, nine EU/EEA countries had replied to the urgent inquiry. Four countries reported cases that could be linked microbiologically to the Finnish cluster based on the WGS data using either cg MLST [91, 92] or SNP analysis (In-house pipelines).

2.9. Methods for detection and Analysis of *Listeria monocytogenes*

Methods of detection

L. monocytogenes contamination usually occurs in very low numbers both in foods and in the processing environment so it is vital that any analysis performed includes one or more enrichment steps which inhibit other microflora, and allow both the increase of *L. monocytogenes* in sufficient numbers to allow detection and the recovery of injured/stressed cells. The standard microbiological methods for identification of Listeria species are laborious and time consuming, requiring a minimum of five (5) days to recognize listeria spp and about 10 days to identify *L. monocytogenes* by confirmation test [93]. It is therefore necessary to use a chromogenic medium that will give results within 24 hrs [12]. Three methods of analysis are most commonly used: the International Standard (ISO-11290) method which uses a two-step enrichment in Fraser broth, the United States Department of Agriculture (USDA) method which uses a two-step enrichment in University of Vermont media (UVM) and the One-broth Listeria method which has been approved for use by the Association Française de Normalisation (AFNOR) and takes considerably less incubation time and yields results in 2 days as opposed to the 4-5 days needed for the other two methods [27]. All these methods involve plating on Listeria selective agar (Traditional or chromogenic agars) and require confirmation of isolates as *L. monocytogenes* by biochemical or molecular tests. The use of real-time PCR (RTi-PCR), in combination with traditional culture, to detect the presence or absence of Listeria has also been explored in recent years [94, 95].

By amplifying Listeria specific genes through PCR and quantifying them by the detection of a fluorescent probe attached to the DNA fragments, even low numbers of the bacteria can be detected within a few hours (After enrichment) as opposed to the several days it takes to complete traditional plating techniques. For best use, RTi-PCR should be combined with the traditional methods so that isolates can be obtained from the direct detection of *L. monocytogenes* in food as it lacks the required sensitivity, may be subject to inhibition by food ingredients and can detect the presence of DNA from live as well as dead cells [6, 96] and studied that the recent reports showed the immediate detection of *Listeria monocytogenes* by PCR curve analysis.

There is a wide range of different test methods for Listeria spp. and *L. monocytogenes* that have been reviewed by [97 these include antibody-based tests, enzyme linked immunosorbent assay (ELISA), immune-capture methods, molecular methods targeting different genes and biosensor methods.

Characterizations of isolates

In order to identify the source or route of contamination, it is necessary to identify the strain type of *L. monocytogenes* contaminating the food or the processing environment rather than just give a positive/negative result. Differentiation of *L. monocytogenes* strains by serotyping is one of the oldest methods of typing and is based on the somatic (O) and flagellar (H) antigen differences between strains. As more exacting typing techniques have since been developed, serotyping of strains now offers little in terms of strain identification but can be helpful in the characterization of strains [98]. However, to further differentiate strains into their serotype, testing with antisera needs to be performed, it can be prohibitively expensive. Some reactions in antisera testing can be variable, for instance, currently serotypes 4b and 4e cannot be separated by this method. The vast majority of listeriosis outbreaks, approximately 90%, are caused by 1/2b and 4b serotypes, both of which are commonly found in food and food processing facilities. In general, serotype 1/2a has been isolated most frequently from food and the food processing environment [99,100]. Although, it is thought that some serotypes may be generally more virulent than others, currently all *L. monocytogenes* strains must be treated as virulent. Therefore, the identification of certain serotypes in a food or a processing facility does not mean that they will or will not cause disease. The gold standard for *L. monocytogenes* sub-typing remains pulsed field gel electrophoresis (PFGE), although other methods do offer advantages. PFGE is quite expensive, takes several days and requires trained staff to perform. However, it offers better discriminatory power than most other methods and can be compared between labs if performed according to international standard practices [101]. After characterizing the molecular diversity of isolates in the environment in question, putative routes of transmission and/or sources of entry into the environment can be identified [102] identified three potential contamination scenarios that can increase the risk of food contamination, hot-spot contamination (Where a specific area is contaminated), widespread

contamination (Where contamination is spread throughout the facility) and sporadic contamination (where non-persistent contamination occurs on an irregular basis). Visualization of the contamination on a facility map can help identify the putative contamination routes [103]. Thus, control strategies can be adjusted/ targeted to remove the source of contamination and interrupt the route of transfer to the food. Analysis of such results can not only identify persistent strains, but can also identify an area which may be colonized by a particular strain, leading to possible recontamination events. It can also be used to prevent the spread of strains throughout the facility. Multilocus sequence typing (MLST) is also commonly used in strain typing, by sequencing a specific set of alleles of housekeeping genes and analyzing the variations in the sequences, which allows identification of strain differences.

Although less discriminatory than PFGE, the evolutionary distance between strains can be measured, by inspecting the number of alterations in the sequences, which cannot be performed by PFGE [104]. PCR to detect different genes present in *L. monocytogenes* strains is also commonly used for strain characterization. The presence/absence of different genes can be a good indication of whether or not a strain is virulent or whether it possesses genes which may help it to persist in a food processing facility. Several genes, such as the stress survival islet SSI-1 and the Tn6188transposon, which confers resistance to certain quaternary ammonium compounds, have been identified which appear to confer advantages to strains which may help them to survive in the seemingly inhospitable environment of a processing facility [105, 106].

Similarly, several genes which contribute to virulence have been identified, for example listeriolysin S (LLS) and act A, and the use of PCR to detect these genes can help to evaluate strains ability to cause disease [107]. Other options for characterization of *L. monocytogenes* isolates include Multiple-Locus Variable Tandem Repeat Analysis (MLVA), ribotyping, phenotypic or biochemical arrays and Fourier Transform infrared spectroscopy [108]. In recent years, the price of whole genome sequencing (WGS) has lowered significantly allowing the use of WGS in more routine applications. As opposed to PFGE or MLST, WGS examines the entire sequence of a genome, rather than just part of it and so gives a much higher strain differentiation [109]. Individual genes can also be examined through the use of WGS.

For example, in the Quargel cheese outbreak in Austria in 2009/2010, WGS was used to identify 2 distinct 1/2a *L. monocytogenes* strains (QOC1 and QOC2) which overlapped to form the outbreak [110]. Through whole genome sequencing (WGS), specific genes which contribute to invasion and survival were also identified including the presence of a vip homologue in QOC2 which encodes a surface protein, likely responsible for the higher invasion efficiency of QOC2 in comparison with QOC1. As costs continue to fall, WGS is increasingly being used in outbreak investigations as it offers a much more comprehensive overview of a strain and gives a significantly higher confidence in strain identification.

Challenge to determine the ability of food to support growth of *Listeria monocytogenes*

Certain foods are categorized in a higher risk category for contamination with L. monocytogenes. These are ready-to-eat (RTE) foods since the heat step of cooking, which would kill any *L. monocytogenes* present, is missing in these foods. Thus, if the food product is able to support the growth of L. monocytogenes, bacterial numbers can reach high levels, even at refrigeration temperatures, posing a health risk for consumers. Determining the ability of RTE foods to support the growth of *L. monocytogenes* is important, especially in those jurisdictions where there is no zero tolerance policy for *L. monocytogenes* (e.g. Europe, Canada and Australia). The ability of *L. monocytogenes* to grow in food products may be estimated based on specifications of the physico-chemical characteristics of the product, consultation of the available scientific literature, or predictive mathematical modeling. Such predictive models are useful, but for many reasons, including the possibility of overestimation/underestimation of growth in food products, in most cases growth assessment will involve laboratory -based studies, so-called challenge tests. From a public health perspective, overestimation of growth is a fail-safe' scenario, although such overestimation can be inaccurate from a food producer's perspective. For example, in 40% of cases Combase predicted growth in cheese when no growth was seen in growth experiments [111]. It was further shown that the growth characteristics of *L. monocytogenes* were different in liquid and solid matrices [112].

Regulations Relating to *L. monocytogenes*

In Africa, in general, there is little awareness or regulation relating to L. Monocytogenes. For example, a recent amendment to the South African Foodstuffs [85] referring to microbiological standards has nothing on Listeria spp. The Dairy Standard Agency (DSA) has guidelines in its Codes of Practice relating to *L. monocytogenes* in raw milk for final consumption, pasteurised milk, UHT milk, cream and salted butter [113]. In these products, the guidelines recommend the absence (in 25 g) of *L. monocytogenes* in raw milk for consumption and in other products. In general, companies that export, use the relevant regulation in the country they export to. One South African voluntary standard (South African National Standard [SANS] 885:2011) that specifically refers to the prevalence of *L. monocytogenes* in processed meat products, allows a maximum of 100 cfu/g at the end of shelf life. In Europe, Regulation (EC) No 2073/2005 sets the microbiological criteria for *L. monocytogenes* in foods that must be complied with. This regulation primarily covers RTE food products and requires that *L. monocytogenes* must be absent from foods (10 x 25 g) intended for infants and for special medical purposes , and allows different criteria depending on the ability of the food product to support growth of L. monocytogenes. In Canada and Australia/New-ZealandCriteria-for-Listeria-monocytogenes-in-ready-to-eatfoods.aspx, the regulations are in line with European regulations, allowing a differentiation between foods that can and cannot support growth. However, in the USA there is zero tolerance ‘of *L. monocytogenes* (Absence in 5 x 25 g of food is required at all times, and in the processing environment).

Treatment of *L. monocytogenes* Infections in Human and Animals

Listeria species resistant to penicillin G and tetracycline, but some are less resistant to chloramphenicol, amoxicillin acid, clindamycin, kanamycin and erythromycin [114]. Highest resistance detected against penicillin, nalidixic acid and erythromycin, with all 78 (100 %) tested for Listeria species presenting resistance [115].

Prevention and Control

As *L. monocytogenes* is a ubiquitous organism, its complete elimination is an unrealistic aim. Control is a more practical approach. Such control can be achieved by attention to detail in hygiene strategies, monitoring occurrence of the organism or using novel control methods such as bacteriocins (Are ribosomally-synthesised peptides that are pore-forming agents, which act by disrupting the integrity of the target cell membrane) and bacteriophage (Are viruses that infect and can kill) bacteria and are logical candidates for bio -control of *L. monocytogenes* in food [14]. The potentially long incubation time for L.monocytogenes to cause disease can also make it difficult to trace the disease to a specific food and source of contamination [3]. It is therefore important to remove as many sources of contamination as possible from the food processing environment to reduce the possibility of food contamination [14].

Non-food contact surfaces, especially floors and drains, can be a reservoir of *L. monocytogenes* in the meat industry [34]. Care has to be taken to clean and sanitize these sites, because they may contaminate other sites in the food processing facility. Cooking is effective methods to eliminate *L. monocytogenes* from meat. The ideal processing method would improve the shelf life and safety of the meat product, not compromise organoleptic or nutritional value is convenient and economical to apply, and not cause objections by consumers [116]. People at risk of infection such as pregnant woman or person with a weak immune system should take additional precautions with these types of foods. It is pertinent to mention that proper hygiene, and sanitation in food establishments, pasteurization of dairy products, cooking of meat, and fish and health education of high risk groups about the severity of disease, mode of transmission, and preventive measure will certainly help to reduce the incidence of listeriosis [117].

Conclusion

Listeria monocytogenes is the most important specie in the genus Listeria causing human and animal health threat. Its epidemiological studies would help in better understanding of the sources of infection and their risk assessment, routes of transmission, clinical forms and better management of the infection. Standard and hygienic operating methods in the farming, processing and marketing of foods are the way forward to reduce the incidence of listeriosis. *Listeria monocytogenes* and other Listeria species isolated from food of animal origin animal with significant percentage. The contamination sources of Ready-to-eat foods of animal origin are more likely to be associated with

insufficient hygienic practices and improper handling. The presence of this organism in these undercooked food products could be a potential risk for consumers. From the above conclusion the following recommendations are forwarded:

- Effective cleaning and sanitation programs and safe handling procedures are important
- Training for carrying out standard sanitary, hygiene and technical operations.
- Further studies on the occurrence of Listeria species in various food products should be carried out.

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