

SURVIVAL OF LISTERIA MONOCYTOGENES IN A FOOD PROCESSING ENVIRONMENT

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Abstract

Gram-positive, intracellular, broad, and facultative, *Listeria monocytogenes* is a human and creature foodborne microbe. They may be found naturally in environmental sources such as soils, human and animal feces, and digestive systems. The pathogen causes listeriosis, which may result in meningitis, abortion, diarrhea, and, in severe instances, death. The most vulnerable groups to listeria infections are newborns, young children, old people, those with impaired immune systems, and pregnant women. *L. monocytogenes* tainting of food has as of late raised serious worries among all gatherings engaged with the food business and the wellbeing business. Their contamination has been connected to numerous foodborne flare-ups, especially those welcomed on by the ingestion of arranged and refrigerated prepared-to-eat foods. To build familiarity with the need to diminish their colonization, transmission, cross-pollutions, and diseases, a survey of *L. monocytogenes* and its relationship with foods is vital.

Keywords: *Listeria monocytogenes*, listeriosis, food safety, food processing

1. Introduction

Moderate listeriosis, a *Listeria monocytogenes* contamination of the gastrointestinal framework, frequently appears as regular "food harming" side effects such as torments in the mid-region, queasiness, and the runs. *L. monocytogenes* may, be that as it may, enter the gastrointestinal epithelial hindrance to spread more serious contaminations all through the body, causing bacteremia. The microbes may likewise go through the blood-tissue boundary, which empowers them to taint the mind or uterus, where they can prompt serious hazardous illnesses including meningitis, encephalitis, unexpected early termination, or premature delivery. Despite the generally low incidence of listeriosis in humans, it has a case fatality rate of up to 30% (EFSA, 2014), making it the third deadliest of all foodborne diseases and immunosuppressive. Those who lack skills are especially helpless (Vazquez-Boland et al., 2001; Cartwright et al., 2013). The latest data from the EU show that in 2012, there were 1642 cases, and the death rate was 12.1% (EFSA, 2014).

Listeria monocytogenes is assumed to be a significant pathway of food contamination due to its widespread distribution in the area where food is prepared (Pérez-Rodríguez et al., 2008). Food processors must be vigilant when caring for *Listeria monocytogenes*. *L. monocytogenes* may survive for long periods of time in places like food processing plants because to its capacity to endure a wide range of stressors and develop biofilms (Moorhead and Dykes, 2004; Zhang et al., 2011). (Latorre et al., 2011; Cruz and Fletcher, 2011) Throughout time, the consistent identification of certain strains of *Listeria monocytogenes* in workplaces suggests that the use of strains in food processing may be warranted. Bongamjang, etc. (2013) used ribotyping to demonstrate 11 years of diligence in the smoked fish processing office for *Listeria monocytogenes* strains. Horch et al. (2013) utilizing genome sequencing, he demonstrated that two distinct strains survived at two separate fish processing factories over the course of more than six years (Vongkamjan et al., 2013).

Prepared to eat (RTE) meals have a higher risk categorization than other entrees because they do not undergo an intensity phase of cooking that would kill any *L. monocytogenes* present (Luber et

al., 2011; EFSA, 2013). *L. monocytogenes* poses a threat since it may replicate even when stored in the refrigerator, making it an issue for products with a long shelf life. Recent outbreaks of foodborne listeriosis have been linked to a wide range of ready-to-eat (RTE) goods, including cheddar (Choi et al., 2014; Rychli et al., 2014), melon (McCollum et al., 2013), and cooked gammon (Hachler et al., 2013). According to a benchmark examination on RTE feasts adopted by the European Association in 2010 and 2011, the prevalence rates of *L. monocytogenes* were 2.07% in meat products, 0.47% in cheddar things, and a very concerning occurrence of 10.4% in seafood commodities. (EFSA, 2013) Current European Commission (EC) guidelines expect RTE foods that cannot uphold its development to have less than 100 CFU/g during their usability period, and a zero resistance strategy has been established for *L. monocytogenes* in RTE foods that are expected for newborn child utilization or as a restorative food (EC, 2005).

Listeria monocytogenes has been identified as a significant foodborne pathogen by many sporadic instances of listeriosis and significant illness outbreaks that have occurred all over the globe. The latest pandemic in the US was brought about by eating sausages, and 14 states revealed around 101 cases and 16 fatalities to the Communities for Infectious prevention and Avoidance (CDC). In excess of 500 000 pounds of food from lunch get-together meat and sausage makers have been reviewed because of plausible *Listeria* tainting (1). As per P. Mead (CDC; individual correspondence), *L. monocytogenes* serotype 4b was found in 4 wiener tests developed from this occurrence at convergences of under 0.3 settlement shaping units (CFU)/g. This plague underlines the requirement for expanded *Listeria* responsiveness in food testing. Target cell levels in examples should be raised to recognizable levels, and test improvement strategies should consider the likelihood that these cells might be available in food items at low levels or in a sub-mortally injured or harmed structure. *Listeria* levels are diminished and sub-deadly damage might come about because of processing difficulties including the expansion of sodium nitrite (NaNO₂), sodium chloride (NaCl), lactic corrosive, warming, freezing, and contact with sanitizers.

There is a requirement for detachment strategies in the food processing area with further developed ability to recognize both low level and injured *Listeria* populaces. Many methodologies rely upon the utilization of profoundly specific enhancement strategies, which permit *Listeria* to flourish

specially in contrast with other food poisons. As confirmation that processing conditions are being kept liberated from this microorganism, the U.S. Division of Agribusiness' Food Safety Investigation Administration (USDA-FSIS; 2) is presently prescribing that offices that produce prepared to-eat meat and poultry items be expected to test food contact surfaces for the presence of *L. monocytogenes*. This guideline, whenever carried out, would underscore the need for incredibly delicate and dependable *Listeria* scientific methodology.

1.1.Detection of Sub lethally Injured Listeria

Most conventional and rapid methods for detecting *Listeria monocytogenes* in foods and natural examples use highly specific boosting media to promote growth over competing base greens. The recuperation of sub-mortally harmed *Listeria* that could be available in a scope of warmed frozen, fermented foods or warmed, frozen, and disinfected areas inside food processing settings isn't thought about by these exceptionally specific enhancement procedures. It is generally known that *Listeria* may be harmed by exposure to a range of processing procedures, including as drying, irradiation, sub lethal heating, and freezing, as well as chemical exposure (sanitizers, preservatives, acids; 14). Injury is reversible in food systems under the right circumstances, and damaged *Listeria* may heal sub-lethal damage. In whole and 2% milk kept at 4°C, *L. monocytogenes* that have been damaged by heat are repaired.

By acknowledging that *Listeria* may live in a damaged condition in food items and food processing settings, many researchers have tried to increase the sensitivity of the present detection techniques. Except for cold enrichment, all existing detection methods include selective enrichment and/or selective plating. For regular sample analysis, cold enrichment is not practical since it might take many months to produce positive findings. Current techniques overestimate the real incidence of *Listeria* by neglecting to account for the recovery of wounded *Listeria*. The capacity of frequently used plating medium to restore wounded *Listeria* has been documented in a number of earlier investigations. Phenylethanol, acriflavin, polymixin-acriflavin, and sodium chloride were among the substances that forestalled both thermally pushed and non-focused on *Listeria* from recuperating.

1.2.Sources of *Listeria Monocytogenes*

Gram-positive *Listeria monocytogenes* is an avascular, intracellular, spiny, G+C-fixation-poor, facultative anaerobe (VazquezBoland et al., 2001; Sukhadeo et al. ., 2009). It is catalase-positive, L-rhamnose-positive, and oxidase-negative, and it is able to move about at temperatures between 10 and 25 degrees Celsius (Arun, 2008; Sukhadeo et al., 2009). Microorganisms are single- or multi-celled organisms that grow to a length of 1-2 m as they mature (Arun, 2008). Growth is optimal between 30 and 37 degrees Celsius, yet it can tolerate temperatures from 1 to 45 degrees C. (Swaminathan et al., 1995). *Listeria monocytogenes* can survive in a variety of hostile environments, including those with a broad pH range (4.1 to 9.6), high salt content (10%), and the presence of antibiotic experts (Liu et al. al., 2005; Arun, 2008). *Bacillus*, *Clostridium*, *Enterococci*, *Streptococcus*, and *Staphylococcus* are only the tip of the iceberg when it comes to *Listeria monocytogenes* (Vazquez-Boland et al., 2001; Sukhadeo et al., 2009). *L. Monocytogenes*, *L. ivanovii*, *L. ivanovii* subsp. London, *L. seeligeri*, *L. innocua*, *L. welshimeri*, and *L. grayi* are only some of the six unique species of *Listeria*. Researchers have portrayed *monocytogenes* as a disease in both humans and animals. However, studies have revealed that *L. ivanovii* poses a threat to livestock, especially sheep and cattle. While *L. ivanovii* and *L. celigeria* have very seldom been linked to human illness (Rocourt and Cossart, 1997)

1.3.Food Safety Strategies to Minimize *Listeria Monocytogenes*

Aseptic and clean packing and processing methods are being intensively evaluated in an effort to reduce the prevalence of *Listeria monocytogenes* in food and, by extension, the prevalence of listeriosis. This decreases *Listeria monocytogenes* colonisation, transmission, and cross-contamination of the environment and its food supplies. The ranch, the processing offices, and the biological systems should be in every way the focal point of a proficient control technique for this infection. Severe adherence to standard working systems should be kept at each level. Domesticated animals ought to be brought up in spotless, dry environmental factors in agribusiness. Especially, soils ought not to be wet or sodden since such circumstances will advance the improvement of this disease. Cows lodging offices should be occasionally cleaned and

sanitized. Keep wild animals from attacking the ranch (which could go about as repositories), especially where feeds are kept

Every business should have L. monocytogenes processing and natural checking procedures in processing offices. Such techniques should be remembered for the association's HACCP plan. Accentuation ought to be put on disinfection techniques, processing and pressing methods, representative cleanliness, and standard L. monocytogenes testing programs in checking plans. To stop extra transmissions, examinations should be done just after to recognize the source assuming the sickness is found during reconnaissance. To guarantee that specialists comprehend the worth of good clean practices, the executives should lay out clear guidelines and give staff preparing. Keep away from activities including moving individuals and hardware from unrefined substance to finished item areas, without utilizing clean gloves, utilizing messy gear, and taking care of unsanitary articles prior to reaching completed merchandise. To keep this space dry, cooling frameworks ought to have dehumidifying capacities. Palletizing and covering the pressing supplies until utilization are likewise suggested. Fridge temperatures ought to be routinely checked in retail shows, things ought not be blended from different providers, and showcases ought to be all around bundled. Items that have turned sour ought to be discarded out right. It is exhorted that clients get further preparation on food safety issues. Likewise, feasts ought to be appropriately ready or warmed (on account of prepared to-eat dinners) before utilization.

2. Literature Review

Listeria monocytogenes has been found in a wide variety of foods including cooked meats, reconstituted meats, smoked salmon, soft cheddar cheese, vegetables, minced batches, detergents, meat grinders, sheep, goat and milk. (Rahimi et al., 2010) , prepared foods (Aurora et al., 2008), raw and washed egg testing, poultry meat and meat products (Mahmood et al. (Rivoal et al., 2010)). Regulations for the presence of Listeria monocytogenes in foods Canada maintains a tolerance limit of less than 100/g for certain foods and intolerance to others (Farber et al. al., 1996), and the United States has not proposed resistance to L. monocytogenes. Monocytogenes in 25 grams of food (Knife et al., 1996)

About Europe, it's expressed moreover prompted that *L. monocytogenes* should not be available in that frame of mind than 100 cfu/g for the span of an item's timeframe of realistic usability, and items in which the development of the bacterium is conceivable should not contain in excess of 25 g of the microbe when they leave the creation office except if the producer can demonstrate as per the general inclination of the important power that the item won't surpass the 100 cfu/g limit for the term of the timeframe of realistic usability (ESFA, 2010).

Both conventional culture methods and various PCR-based approaches have been used to easily and rapidly isolate and detect *Listeria monocytogenes* (Johansson, 1998; Choi and Hong, 2003; Becker et al. , 2005; Loncarevic et al., 2008; Aurora et al., 2009). Such techniques and strategies are important for epidemiological investigations, clinical applications, and corrective actions. Purchasers' interests about food safety are developing, and listeria disease is a huge general medical problem. In this outline, *L. monocytogenes*, events, seclusion techniques, and potential anticipation systems are momentarily covered. *L. monocytogenes* is alluded to in this writing both uniquely and plurally.

The most weak gatherings to listeriosis incorporate babies, youngsters, pregnant ladies, those with debilitated insusceptible frameworks (like those with Helps, malignant growth, or organ relocate beneficiaries), and the older (Schlech, 2000; Liu, 2006). Albeit however listeriosis is exceptionally extraordinary (under 0.1% of all food-borne illnesses), it has extremely high casualty rates (20 to 30%) and may have death rates as high as 75% in high-risk people (Mead et al., 1999; Ireton, 2006). (Khelef et al. 2006) The most run of the mill indications of listeriosis incorporate fever, watery looseness of the bowels, sickness, migraines, and a throbbing painfulness in the muscles and joints (Arun, 2008). Early termination, sepsis, meningoencephalitis, neuro-encephalitis, chorioamnionitis, gastroenteritis, and bacteremia are among the serious clinical side effects of intrusive listeriosis (Khelef et al., 2006; Sukhadeo et al., 2009).

According to Gillespie et al. (2006) Sandwiches and margarine from emergency rooms resulted in a total of 48 cases of listeriosis. According to de Valk et al. (2001) reported that 42 of her people in France contracted listeriosis after eating pork rillettes and jellied pork tongues. In the United

States, 13 cases of Mexican-style tender cheddar (MacDonald et al., 2005) and 93 cases of cooked turkey meat consumption (Olsen et al., 2005; Gottlieb et al., 2006) have been reported. , 16 cases from eating cooked sliced turkey (Frye et al., 2002) and 108 cases from eating hot dogs (Mead et al., 2006). Makino et al. (2005) found a total of 38 cases in Japan associated with eating cheddar.

The introduction of *L. rhamnosus* E-97800, *L. rhamnosus* LC-705, and *L. plantarum* ALC01 in the biodefense meat production community may reduce the prevalence of *L. monocytogenes* in certain frankfurters (Työppönen et al., 2003). These societies exhibit a clear antilisterial activity by *Listeria monocytogenes*. In addition, joint treatment of food specimens such as fungi with ozone and natural caustics has been shown to reduce the underlying population levels of infection (Yuk et al., 2007). As reported by Nyachuba et al. (2007) Nitrite damages *Listeria*, advising that nitrite may reduce listerial cell development.

Films or coatings made from whey protein isolate (WPI) that were conjugated to the lactoperoxidase structure (LPOS) stopped *Listeria monocytogenes* from growing in smoked salmon, as reported by Min et al. (2005).. At the point when grape juice was exposed to super high strain homogenisation (UHPH), *L. monocytogenes* practical counts couldn't be found (Velázquez-Estrada et al., 2010). *L. monocytogenes* might be controlled very well by utilizing a steam treatment framework (Bremer et al., 2002).

3. Research methodology

3.1.Cultures

It is *Listeria monocytogenes* serotype 1, pathogenic to mice, hemolytic, and distinct from soft cheddar. In order to make an active inoculum, they were cultivated at 35 degrees Celsius and kept on tryptose agar (TA; Difco). Tuscia College, Department of Agrobiological and Agrochemistry, Viterbo, Italy, provided the initial assemblages (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). The yoghurt was made with fresh skim milk, and the accompanying was brought the day before.

3.2.Preparation of Yogurt

After heating in a 90° C. water shower for 10 minutes with mixing, the milk was cold at 33 C. and the inoculum (4 percent v/v) was added. From this point onwards, appropriate amounts of activation cultures were included into triplicate 600mL milk tests to obtain *Listeria* spp. provided the underlying inoculum for monocytogenes. 10³ CFU/ml was 6. For the purpose of incubation, milk with inoculum at 33 C. til hard clots formed (5 hours). Controls of contaminated yogurt and no *Listeria* cells were then introduced and aged at 4 °C.

3.3. Checking for Monocytogenic *Listeria*

Cell counts were appropriate at days 9, 63, 78, 2, 9, 1 and 15 surface plating on McBride *Listeria* agar was used to count *L. monocytogenes* (MLA, Bio life and Milan, Italy). According to the previously reported method, co-colony development was evaluated by counting colonies after 48 hours of air incubation at 35 degrees Celsius (Massa et al. 1990) not one case of *L. monocytogenes* was discovered. Cryoprotected samples (0.1 ml total) were uniformly plated out on MLA clone plates and incubated as specified.

3.4. Determination of pH

While testing for *L. monocytogenes*, the not entirely settled by quickly putting the cathode (a pH meter made by Beckman) into very much blended examples.

4. Results

At 12 hours, the amount of suitable cells in milk infused with 2.3 x 10⁴, 6.4 x 10⁵, and 3.4 x 10³ cfu/ml of *Listeria monocytogenes* decreased by 1 log compared to normal (Table 1). *Listeria monocytogenes* should be harvested after cooling has progressed after volume addition at 4 °C. Direct plating or cold-enhancement strategies couldn't confine live *Listeria* following 5 days. The beginning pH of the examples with low inoculum was 4.9 at the hour of chilling, and the last pH was 4.2 at the last examining when live cells were found. Following 48 hours, a huge reduction in reasonable cells was found in yogurts Y4, Y5, and Y6 (Table 2). Further decreases in the quantity of enduring life forms made their recuperation by cold enrichment just practicable following 5 days (Y4) and 7 days (Y5 and Y6). The pH dropped from 5.0 to 4.2 in the examples

with critical inoculum. No examples of vaccinated yogurt containing *Listeria monocytogenes* were found.

Table 1: *Listeria monocytogenes* persistence during yogurt production and storage at 3°C following low dose vaccination

Time after inoculation	Temperature (C°)	pH*	Y1 ^t	Y2 ^t	Y3 ^t
0	32	7.6	4.36	4.38	4.96
4 hour	3	5.6	4.56	4.87	4.98
11 hour	3	5.4	3.56	3.15	3.54
23 hour	3	5.8	E ¹	E	3.15
3 day	3	5.7	E	3.56	E
6 day	3	5.2	ND§	ND	ND

* Mean of three values.

^t *L. monocytogenes* (log₁₀cfu/g) recovered.

< 10 cfu/g recovered after cold enrichment

§ Not detected.

Table 2: After high level inoculation, *Listeria monocytogenes* survive during yogurt preparation and storage at 3°C.

Time after inoculation	Temperature (C°)	pH*	Y1 ^t	Y2 ^t	Y3 ^t
0	32	7.6	8.36	8.38	8.96
4 hour	3	6.6	6.56	8.87	8.98
11 hour	3	5.4	5.56	6.15	8.54
23 hour	3	5.8	4.25	5.62	6.25
3 day	3	5.7	3.25	4.56	5.62
6 day	3	5.2	E ¹	3.25	3.15
8 day	3	5.3	3.25	E	E

16 day	3	5.6	ND§	ND	ND
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* Mean of three values.

t *L. monocytogenes* (\log_{10} cfu/g) recovered.

< 10 cfu/g recovered after cold enrichment

§ Not detected.

Listeria is known to have the unfortunate ability to tolerate acidic pH levels. According to Dim and Killinger (1966), mortality was common at pH values below 5.6. Our findings are in line with those of various analysts, as ongoing research shows how this animal can survive at pH levels much lower than recently thought. As trustworthy As my patience allows! The pH of sterile cabbage juice containing *monocytogenes* was calculated to be between 4 and 5.6 by Conner et al. (1986). As shown by Area and Higgins (1989), *L. monocytogenes* can survive days to weeks at refrigerator temperatures and very low pH. For example, suitable cells were reduced from 6 cfu/ml of clean-squeezed oranges, pH-altered using HCl, to 25 cfu/ml in 43 days at .

While there haven't been any instances of *L. monocytogenes* being detached from yogurt as yet, it is conceivable. Assuming milk is once again debased with *L. monocytogenes* following intensity therapy in little plants involving rough strategies for yogurt make, there might be a gamble to general wellbeing. Sufficient degrees of cleanliness should be followed to forestall the presence of this microbe in yogurt, considering the requirement for more review to decide a potential safety edge for *L. monocytogenes* (Lacey and Kerr 1989).

5. Conclusion

The far and wide, shrewd, and huge food-borne microorganism *L. monocytogenes* keeps on stressing the food area and wellbeing specialists. High gamble individuals experience the ill effects of their ailment. Eating defiled food is the significant method for fostering an ailment. Most of study has zeroed in on prepared to-eat feasts, meat and meat items, and endlessly milk items as the primary drivers of flare-ups. The trouble of laying out nothing or least resistance of *L. monocytogenes* in foods is exacerbated by their ability to make due under refrigeration and various climatic circumstances. Procedures for *L. monocytogenes* and listeriosis disconnection and

recognition should be trustworthy and exact. The eventual fate of listeriosis anticipation lies on norm and clean working techniques in food creation, processing, and advertising.

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