*South Asian Journal of Research in Microbiology*

***Volume 18, Issue 4, Page 26-34, 2024; Article no.SAJRM.112912***
***ISSN: 2582-1989***

# Phenotypic and Genotypic Virulence Potentials of *Listeria monocytogenes* Isolated from Different Food Samples in Yola

**Halima Isa [a*], Mohammed Bashir [a] and Nafisat Adamu Kachalla [a]**

*[a] Microbiology Department, Faculty of Life Sciences, Modibbo Adama University, Yola, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors HI and MB designed the study, author HI wrote the protocol, and wrote the first draft of the manuscript. Authors HI, MB and NAK carry out the laboratory work. Author HI managed the literature searches. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/SAJRM/2024/v18i4356

**Open Peer Review History:**
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/112912

***Original Research Article***

***Received: 23/12/2023***
***Accepted: 27/02/2024***
***Published: 04/04/2024***

## ABSTRACT

**Aim:** the aim of this work is to determine the Phenotypic and Genotypic Virulence Potentials of *Listeria monocytogenes* Isolated from Different Food Samples in Yola.
**Study Design:** the study was a randomized study; the samples (sources of organisms) were collected at random from different market and shops within the study area.
**Place and Duration of Study:** the study was carried out in Yola, the capital of Adamawa State, north eastern Nigeria.
**Methodology:** phenotypic virulence potentials of the isolates was determined by haemolytic activity on 5% sheep blood agar and biofilm formation ability, using Christensen's tube method and microtiter plate method for the qualitative and quantitative biofilm formation respectively. Genotypic

**Corresponding author: Email: halimaisa1446@gmail.com, halimaisa@mau.edu.ng;*

virulence determination was done by polymerase chain reaction amplification of *plcB, hlyA* and *actA* genes.
**Results:** All the isolates (100%) were beta haemolytic on 5% sheep blood agar and all the isolates were biofilm formers but with different degree of biofilm formation abilities. Genotypically all the isolates (100%) indicated the presence of all the three virulence genes amplified.
**Conclusion:** food samples isolates analyzed are potentially virulence pathogenic of *L. monocytogenes* that can pose food borne listeriosis to high risk individuals in the study area.

*Keywords: Foodborne; genes; genotypic; Listeriosis L.; monocytogenes; pathogenic; phenotypic; virulence.*

## 1. INTRODUCTION

*"Listeria monocytogenes* is an important, ubiquitous, foodborne bacterium that can contaminate food products during or after processing. It poses a significant risk to the food industry, particularly producers of ready-to-eat (RTE) foods (of vegetables and animal origin) due to its ability to proliferate over a vast range of adverse environmental conditions" [1]. Hence, it has become a major concern for the food industry.

*"Listeria* species has been associated with a wide variety of food sources particularly poultry, red meat, and meat products. In Egypt, *Listeria* contamination rate in meat and chicken products was reported to be 41%, lower than the 73.9% reported in Malaysia from imported frozen beef and 83.3% from raw minced meat in Turkey" [2,3]. "The bacteria can be endemic in food processing environments, because it survives food-processing technologies that rely on acidic or salty conditions and, unlike many pathogens, can continue to multiply slowly at low temperatures, allowing for growth even in properly refrigerated foods. Hence, their presence may be indicative of poor hygiene or cross contamination, which is considered a possible source of *Listeria* contamination in processed meat" [4].

*"L. monocytogenes* is the main cause of food borne listeriosis in humans. Rarely, food borne infections were reported by *L. ivanovii* and *L. seeligeri*. Strains of *L. monocytogenes* have different pathogenic potential, as some strains are very virulent, whereas some of them are non-infectious agents" [5]. "Determination of the pathogenic potential of *L. monocytogenes* is important from food safety and public health perspective" [6]. "Listeriosis has emerged as an atypical food borne illness of major public health concern because of the severity of the disease (meningitis, septicemia, and abortion), the high case fatality rate (20–30% of cases), the long incubation period, and the predilection for individuals who have an underlying condition which leads to impairment of T-cell-mediated immunity" [7,8].

*"Listeria monocytogenes* has been recovered from several foods such as meat, milk, and fish products; ice cream; vegetables; and several ready-to-eat foods" [9,10]. "*Listeria monocytogenes* is responsible for cases and outbreaks of febrile gastrointestinal disease in otherwise healthy people and invasive listeriosis, which usually affects pregnant women, newborns, the elderly and immunocompromised individuals. The genomic epidemiology of *L. monocytogenes* has been investigated and genetic differentiation of the organism within and between food categories was calculated based on allele frequencies in each food category. It has been found that some clonal complexes (CCs) are overrepresented but could not identify any epidemiological risk factors. This suggested persistent contamination in food production settings, and producers that process a wide variety of raw food produce, could significantly contribute to lowering the *L. monocytogenes* disease burden" [11].

"The scientific interest in *Listeria* derives from the fact that the virulent species are facultative intracellular pathogens, enabling them to survive within host cells, similar to pathogenic *Mycobacteria, Salmonella, Shigella, Legionella*, and several other important intracellular bacterial pathogens" [12]. "Virulence factors in the infectious cycle of pathogenic Listeria are encoded on a 9-kb pathogenicity island known as *PrfA*-dependent virulence gene cluster or LIPI-1 (Listeria pathogenicity island 1)" [13]. "This chromosomal locus comprises the genes : (i) *hly*, encoding a cholesterol-binding, pore-forming toxin (listeriolysin O, LLO) essential for the bacterial escape from phagosome;(ii) *plcA* and *plcB,* encoding two phospholipases C with different substrate specificities (*PlcA* is

phosphatidyl inositol specific and *PlcB* is a lecithinase with a broad substrate range) and which co-operate with LLO in the escape from phagocytic vacuoles; (iii) *mpl*, encoding a metalloprotease involved in the maturation of pro-*PlcB*; (iv) *actA,* coding for the ActA protein which mediates actin-based intra- and intercellular motility of the bacteria; and (v) *prfA,* which encodes PrfA (positive regulatory factor A). This protein activates transcription of all genes of *LIPI-1*, including its own" [14].

"In the cytoplasm, *L. monocytogenes* replicates rapidly and produces the surface actin assembly-inducing (ActA) protein" [15]. "*ActA* induce actin formation creating a comet tail, ultimately pushing the bacterium towards the host cell surface to invade neighboring cells. In this manner, *L. monocytogenes* replicates and spread within the host avoiding the extracellular space and evading the immune system" [14].

"Pathogenic Listeria spp. produces three different enzymes with phospholipase C (PLC) activity that is involved in virulence. Two, *PlcA* and *PlcB*, are present in *L. monocytogenes* and *L. ivanovii*; the third, SmcL, is specific to *L. ivanovii*. Key virulence factor essential for pathogenicity, having a vital role not only in intracellular parasitism but also in several other functions in the interaction of Listeria with their vertebrate host" [14].

"Haemolysin of *L. monocytogenes* is an SLO-related cytolysin belonging to the family of cholesterol-dependent, pore-forming toxins (CDTX). This toxin was given the name listeriolysin O (LLO), and one of its key characteristics was determined, its low optimum pH (5.5) and the narrow pH range at which it is active (4.5 to 6.5)". [43] "LLO not only mediates lysis of the primary phagosomes formed after the uptake of extracellular bacteria but is also required for the efficient escape of *L. monocytogenes* from the double-membrane vacuole that forms upon cell-to-cell spread. The pores or membrane lesions caused by LLO probably facilitate the access of Listeria phospholipases to their substrates, leading to total dissolution of the physical barrier that delimits the phagosomal compartment" [14].

## 2. MATERIALS AND METHODS

### 2.1 Source of Organisms

The organisms (*Listeria monocytogenes)* were previously isolated from different food samples obtained from Yola, the Capital of Adamawa state. The samples are cabbage, frozen chicken, fresh fish, raw meat, ice-cream, fruit salad and yoghurt.

### 2.2 Determination of Phenotypic Virulence Potentials of *L. monocytogenes*

#### 2.2.1 Qualitative determination of biofilm formation

Biofilm formation was determined using Christensen's tube method, where 1ml of the standardized inoculum was introduced into test tubes containing 10ml of nutrient broth and incubated for 24 hours at 37 °C without shaking. After 24 hours of incubation, the tubes were decanted and washed with PBS (pH 7.3) and dried. The dried tubes were stained with 0.1 % crystal violet, the excess stain was removed, and the tubes were washed again with deionized water. The tubes were dried in an inverted position and observed for slime layer formation. Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface was not considered biofilm formation. All the isolates were tested in triplicate. Tubes were examined for crystal violet retention. The amount of biofilm formation was recorded as 0-absent, 1-weak, 2-moderate, or 3-strong based on the intensity of crystal violet retention in the tubes [15,6]

#### 2.2.2 Quantitative determination of biofilm formation

The microtiter plate method was used for the quantitative measurement of biofilm. "Overnight grown colonies were diluted to obtain an $OD_{600}$ of 0.1 in each growth medium and 200 µl of bacterial solution was transferred in triplicate wells in 96-well microplates. Microplates were incubated without shaking for 24 h at 37˚C. Plates were inverted and the media and planktonic cells were removed by gentle tapping. To remove loosely attached bacteria, wells were washed twice with 300 ml of sterile saline solution (8.5 g NaCl per liter). Then biofilms were fixed with 300 µl of 96% v/v ethanol for 20 min and air-dried completely at room temperature after removal of the ethanol. For staining bacterial biomass, 220 µl of 0.1% w/v crystal violet (CV) solution was added per well, and plates were incubated also without shaking for 30 min. Then the solution was removed by sharply tapping the plates upside down. Wells were washed 3 times with 300 µl of saline and air dried completely before filling with 150 µl of 33% v/v acetic acid. Plates were placed on a plate

**Table 1. Primers used for PCR amplifications of virulence genes**

| Gene | Primers | Gene size (bp) | Reference |
|---|---|---|---|
| *hly*A | (for.) GCA GTT GCA AGC GCT TGG AGT GAA<br>(rev.) GCA ACG TAT CCT CCA GAG TGA TCG | 456 | [19,10] |
| *plcB* | (for.) CTG CTT GAG CGT TCA TGT CTC ATC CCC C<br>(rev.) ATG GGT TTC ACT CTC CTT CTA C | 1484 | [20,11] |
| *act*A | (for.) CGC CGC GGA AAT TAA AAA AAG A<br>(rev.) ACG AAG GAA CCG GGC TGC TAG | 839 | [21,12] |

shaker with slight agitation for 10 min to completely dissolve CV and get homogenized solutions. The amount of destained CV was determined by reading $OD_{600}$ in a microplate reader" [16,7].Optical Density (O.D) $_{600}$< (0.1AU = Weak); (0.1-0.4AU = moderate); (>0.4- 1AU= Strong); (> 1AU = Very Strong).

#### 2.2.3Haemolysis test

"Standardized inoculums of *L. monocytogenes* isolates were streaked on 5% sheep blood agar and incubated at 37˚C for 24 hours after which zone of β-haemolysis on the blood agar was observed" [17,8].

### 2.3 Determination of Genotypic Virulence Potentials of *L. monocytogenes*

#### 2.3.1 DNA extraction

Freshly grown *L. monocytogenes* colonies collected from the surfaces of 24-hour nutrient agar culture plates were used for DNA extraction [18,9]. Qiagen QIAamp DNA mini kit was used and the DNA extraction procedure was according to the manufacturer's instructions.

#### 2.3.2 PCR Amplification and detection of virulence genes

The isolates were screened by multiplex PCR for the presence of some of the virulence genes (*hlyA, plcB*, and *actA*) using the primers in Table 1 above.

Amplification was performed in 50µL reaction volume as described by [20] containing 10 µL (30–50 ng) of extracted DNA template from the identified *L. monocytogenes* cultures, 5 µL 10×PCR buffer, 5 µL $MgCl_2$ (25 mM), 4 µL dNTPs (25 mM), 1.54 µL (2.5 U/ µL) Ampli Taq DNA polymerase, 2 µL (0.5 µM) from each primer pairs. The volume of the reaction mixture was completed to 50 µL using Double Distilled Water (DDW) and the tubes were placed in a DNA thermal cycler (SelectCycler II). The samples were subjected to an initial denaturation step of 95°C for 2 min, followed by 35 amplification cycles of 15s at 95°C (denaturation), 30s at 60°C (annealing), and 90s at 72°C (primer extension) followed by a final extension step of 72°C for 10 min.

#### 2.3.3 Separation of the PCR product

Electrophoresis' separation of the PCR product was performed on 1.5 % agarose gel, which was stained with ethidium bromide. The sizes of the PCR products were also compared with a standard 100bp DNA marker. A reaction mixture with no DNA template was incorporated as a negative control. The process was performed at 110 V and 1.81 A for 1 hour and 15 minutes, and the gel was observed under ultraviolet light [20,11].

## 3. RESULTS AND DISCUSSION

### 3.1 Phenotypic Virulence Potentials of *Listeria monocytogenes* Isolates

#### 3.1.1 Biofilm Formed by *L. monocytogenes* isolates

Both Christensen's tube method and microtiter plate methods for the determination of biofilm formation ability revealed that all the isolates are biofilm formers but with different degree of biofilm formation abilities as shown in Table 2.

The *L. monocytogenes* isolates in this work showed different degrees of biofilm formation ability at room temperature (25-30°C); from weak, to moderate and strong. Most of the isolates (45.5%) showed moderate biofilm formation ability, some (18.1%) showed strong ability, while others (36.3%) had weak biofilm formation ability. However, there are discrepancies in the qualitative and quantitative values of biofilm observed in some isolates. This may be because the two methods, measured biofilm formed on different media (glass and polystyrene) and the organism have different adhering ability to the different media. They adhere preferentially, to glass which is less

**Table 2. Biofilm Formation Ability of *L. monocytogenes* isolates**

| s/n | Isolates Code | Degree of Biofilm Formation | |
|---|---|---|---|
| | | Microtiter Plate Assay($OD_{600}$) | Christensen's tube Method |
| 1 | Lm 2C | Moderate | Moderate |
| 2 | Lm 3C | Strong | Strong |
| 3 | Lm 4C | Moderate | Moderate |
| 4 | Lm 8Fc | Moderate | Moderate |
| 5 | Lm 9Fc | Moderate | Strong |
| 6 | Lm 10Fc | Moderate | Moderate |
| 7 | Lm 11Y | Strong | Moderate |

**Microtiter Plate Assay; Optical Density ($OD_{600}$)< (0.1AU = Weak); (0.1-0.4AU = moderate); (>0.4- 1AU= Strong); (> 1AU = Very Strong). A.U =Absorbance Unit. Christensen's tube Method; base on the intensity of crystal violet retention in the tubes, 0-absent, 1-weak, 2-moderate or 3-strong*

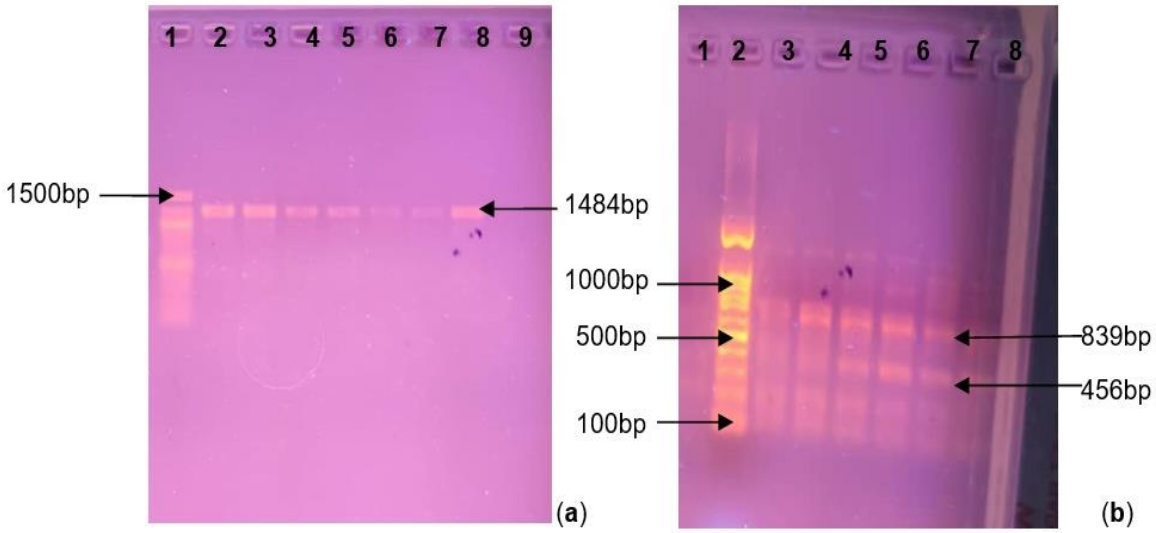


**Fig. 1(a). Gel picture of an amplified *plcB* gene (1484 bp). Lane 1: Molecular weight Marker (100 bp); Lanes 2-8: positive isolates and Lane 9: Negative control Fig. 1(b). Multiplex PCR products of an amplified *hlyA* gene (456 bp) and *actA* gene (839 bp). Lane 3-8 positive isolates for *actA* and *hlyA*; Lane 2: Molecular weight Marker (100 bp); Lane 1: Negative control**

hydrophobic than polystyrene. More so, the qualitative method (Christensen's tube method) is prone to human error as no machine is involved in reading the result. Therefore, the quantitative method (microtiter plate method) was used to identify the biofilm formers in this study. Similarly, [22] analyzed biofilm formation of *L. monocytogenes* on different surfaces at different temperatures and observed complex organization of *L. monocytogenes* biofilms at 22 and 37°C in terms of cell number and extracellular polymeric substance (EPSs) produced. Whereas, a rudimentary biofilm consisting of a sparse cluster of cells and few EPSs were observed at 4 and 12°C with higher levels on glass compared to the more hydrophobic stainless steel and polystyrene. [23,14] also reported that *L. monocytogenes* significantly formed biofilm at 25, 37°C.

This finding indicates that "*L. monocytogenes* can form biofilm on several surfaces used in the food industry at different temperature, representing a serious alarm for food safety because it could serve as a source of contamination. *L. monocytogenes* can attach to many food-contact surfaces, such as stainless steel, polystyrene and glass" [22]. "It has been found to persist in food industries for several years, where it could cause recurrent cross-contamination of food products" [24]. "When organized as biofilm, the self-produced extracellular polymeric matrix

gives extra protection to bacteria from harsh environmental conditions such as desiccation, nutrient deprivation, or disinfectant treatment" [25]. "As a consequence, it is challenging to control bacterial contaminations in the Food Processing Environment (FPE)" [18]. "The ability of foodborne pathogens, including *L. monocytogenes*, Yersinia enterocolitica, Campylobacter jejuni, and Escherichia coli O157:H7 to attach to food and food-contact surfaces is well known. Some authors have found that *L. monocytogenes* has become one of the major causes of food product contamination and disease transmission" [26,27]. "In 2011, there was an outbreak linked to whole cantaloupe contaminated with *L. monocytogenes*. It was speculated that the root cause of the outbreak was the unsanitary condition of the packing shed, but the microorganisms were also found in other places, including on the conveyor belt, and in the drying area and the floor drain. In 2018, a rock melon *L. monocytogenes* outbreak in Australia killed four people" [28,29]. Latorre et al. [30] conducted "a study on the presence of *L. monocytogenes*–containing biofilm in milking equipment as a potential source of contamination on a dairy farm". "After obtaining positive results, electron microscopy scanning of the equipment showed the presence of individual cells and clusters of bacteria and it was postulated that they were mainly associated with scratched surfaces" [29].

#### 3.1.2 Haemolytic characteristics of *L. monocytogenes* isolates

Zone of β-haemolysis on 5% sheep blood agar was produced by all the isolates identified as *L. monocytogenes* indicating that all the isolates are haemolytic.

Haemolysis is one of the determining factors of virulence and pathogenicity of many haemolytic bacteria. All isolates in this study showed β-haemolytic activity; this indicates that they are pathogenic. This is not surprising because, previously, food isolates were tested with a tissue culture model and shown to be cytopathogenic indicating their pathogenicity [31,32]. Based on that, the absence or presence of this virulence factor could be a tool to assess the risks related to food product consumption [33].

### 3.2 Genotypic Virulence of *L. monocytogenes* Isolates

#### 3.2.1 Virulence genes detected from *L. monocytogenes* isolates

The molecular detection of virulence genes *hlyA*, *plcB,* and *actA* from the *L. Monocytogenes* isolates with more phenotypic virulence potentials (biofilm formation ability and haemolysis), indicated the presence of all the three virulence genes in all the isolates. Fig. 1 (a) and (b) present gel pictures of the amplified virulence genes in all the isolates.

Previous studies conducted on several types of food isolates have documented similar results of incidence of virulence genes in all examined *L. monocytogenes* isolates [34,35,36]and [37]. Also [38] reported variable rates of virulence gene detection in *L. monocytogenes* isolates. In addition, other studies also confirmed similar findings in samples isolated from diverse types of food, raw milk, milking machines, workers' hands and clinical specimens [39,40,41].

Although, some polymorphisms and punctual mutations may be present in certain virulence genes and their presence may contribute in favour of attenuated virulence from *L. monocytogenes* strains [42]. The presence of the virulence genes *hlyA* in all of the isolates, justified the phenotypic haemolytic activity observed in all the isolates. Therefore, the presence of these virulence genes (*hlyA, ActA* and *plcB*) and other virulence factors such as biofilm formation and haemolysis in all the isolates, demonstrated the potential pathogenicity of these isolates.

## 4. CONCLUSION

The food isolates are virulents and potentially pathogenic *L. monocytogenes* that can pose food borne listeriosis to high risk individuals in the study area. The finding provides evidence of a serious public health issue since this organism represents a potential threat to consumers of all the *L. monocytogenes*-positive food samples studied, especially those from the risk groups (pregnant women, elderly and immunocompromised individuals).

## ACKNOWLEDGEMENTS

We appreciate Dr. Ja'afar N. Ja'afar, Director; Chevron Forensic Laboratory Modibbo Adama

University Yola, Mal. Ibrahim Ahmed Raji also of Chevron Forensic Laboratory, and Hayatu Raji of Biochemistry Department MAU, Yola for their support during the molecular aspect of this work.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Khan KM, Pao W, Kendler J. Epidural abscess and vertebral osteomyelitis caused by *Listeria monocytogenes*: Case report and literature review. Scand Journal of Infectious Diseases. 2001;33:714–716.
2. Falomri M, Gazalbo D, Rico H. Coliform bacteria in fresh vegetable: From cultivated lands to consumers. Current research in technology education top applied microbiology and microbial beotechnology. 2010;2:1175-1181.
3. Erkan ME, Vural A. Investigation on microbial quality of some leafy green vegetables. Journal of Food Technology. 2008;6:2.
4. Karamoko Y, Ibenyassine K, Ennaji MM, Anajjar BA, Mhand R. and Chouibani M. Bacterial pathogens recovered from vegetables irrigated by waste water In Morocco. Journal of Environmental Health. 2007;17:221–230.
5. Liu D, Lawrence M, Austin FW, Ainsworth AJ. Comparative assessment of acid, alkali and salt tolerance in *Listeria monocytogenes* virulent and avirulent strains. FEMS Microbiology Letter. 2007; 243:373–378.
6. Zeinali T, Jamshidi A., Bassami M, Rad M. Sero groupidentifcation and virulence gene characterization of *Listeria monocytogenes* isolated from chicken carcasses. Iranian Journal of Veterinary Science and Technology. 2015;7(2):9–19.
7. World Health Organization, WHO. Listeriosis; 2018. Available:https://www.who.int/news-room/fact-sheets/detail/listeriosis
8. Allerberger F, Wagner M. Listeriosis: A resurgent foodborne infection. Clinical Microbiology and Infectious Diseases. 2010;16:16–23.
9. Kwiatek K. Occurrence of *Listeria monocytogenes* in selected food of animal origin. Bulletin of the Veterinary Institute in Pulawy. 2004;48:269–272.
10. Garrido V, Vitas AI, Garcıa-Jalon I.. Survey of *Listeria monocytogenes* in ready-to-eat products: Prevalence by brands and retail establishments for exposure assessment of listeriosis in Northern Spain. Food Control. 2009;20(11):986–991.
11. Coipan CE, Friesema, IM, van Hoek AH, van den Bosch T, van den Beld M, Kuiling, S. et al. New insights into the epidemiology of *Listeria monocytogenes* – A cross-sectoral retrospective genomic analysis in the netherlands (2010–2020). Frontiers in microbiology. 2023;14:1147137.
12. Kashisha D, Dharmendra KS, Sunil KM, Rajiv P, Suresh KD. Label-free impedimetric detection of *Listeria monocytogenes* based onpoly-5-carboxy indole modified ssDNA probe. Journal of Biotechnology. 2015;200:70–76.
13. Vazquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Dominguez-Bernal G, Goebel W, et al. *Listeria pathogenesis* and molecular virulence determinants. Clinical Microbiology Review. 2001;14:584–640.
14. Jose´ AV, Michael K, Patrick B, Trinad C, Gustavo D, Werner Goebel, et al. *Listeria Pathogenesis* and molecular virulence determinants. Clinical Microbiology Reviews. 2001;14(3):584-640. DOI: 10.1128/CMR.14.3.584–640.2001
15. Lee EY, Choi DY, Kim DK, Kim JW, Park JO, Kim S, et al. Gram-positive bacteria produce membrane vesicles: Proteomics based characterization of Staphylococcus aureus-derived membrane vesicles. Proteomics. 2009;9:5425–5436.
16. Harika K, Shenoy VP, Narasimhaswamy N,Chawla K. Detection of biofilm production and Its impact on antibiotic resistance profile of bacterial isolates from chronic wound infections. Journal of global infectious diseases. 2020;12(3):129–134.
17. Lee B-H, Cole S, Badel-Berchoux S, Guillier L, Felix B, Krezdorn N. et al. Biofilm formation of *Listeria monocytogenes* strains under food processing environments and pan-genome-wide association study. Frontiers in Microbiology. 2019;10:2698.
18. Franz Allerberger. Listeria: Growth, phenotypic differentiation and molecular microbiology. FEMS Immunology and Medical Microbiology. 2003;35:183-189.
19. Maria A, Eliana G, Alzira MMB, Elaine CP. Quantification of *Listeria monocytogenes* in minimally processed leafy vegetables using a combined method based on

enrichment and 16S rRNA real-time PCR. Food Microbiology. 2010;27:19–23.

20. Paziak-Domanska B, Bogulawska E, Wiekowska-Szakiel M, Kotlowski R, Rozalska B, Chmiela M. Evaluation of the API test, phosphatidylinositol-specific phospholipase C activity and PCR method in identification of *Listeria monocytogenes* in meat foods. FEMS Microbiology Letter. 1991;171:209–14.
21. Osman KM, Samir A, Abo-Shama UH, Mohamed EH, Orabi A. and Zolnikov T. Determination of virulence and antibiotic resistance pattern of biofilm producing *Listeria species* isolated from retail raw milk. BMC Microbiology. 2016;16:263.
22. Suarez M, Vazquez-Boland JA. The bacterial actin nucleter protein ActA is involved in epithelial cell invasion by *Listeria monocytogenes*; 2001. Available:www.ncbi.nlm. nih.gov/pubmed [Accession No. AF103807].
23. Di Bonaventura G, Piccolomini R, Paludi D, D'Orio V, Vergara A, Conter M. et al. Influence of temperature on biofilm formation by *Listeria monocytogenes* on various food-contact surfaces: Relationship with motility and cell surface hydrophobicity. Journal of Applied Microbiology. 2008;104:1552–1561.
24. Tomicic RM, Cabarkapa IS, Vukmirovic DM, Levic JD. Tomicic, ZM. Influence of growth conditions on biofilm formation of *Listeria monocytogenes*. Food and Feed Research, 2016;43: 19–24.
25. Ferreira V, Wiedmann M, Teixeira P, Stasiewicz MJ. *Listeria monocytogenes* Persistence in food-associated environments: Epidemiology, strain characteristics, and implications for public health. J. Food Prot. 2014;77:150–170.
26. Chmielewski RAN, Frank JF. Biofilm formation and control in food processing facilities. Comprehensive Reviews in Food Science and Food Safety. 2003;2(1):22–32. Available:https://doi.org/10.1111/j.1541-4337.2003.tb00012.x
27. Shi X, Zhu X. Biofilm formation and food safety in food industries. Trends in food science and technology. 2009;20(9):407–413. Available:https://doi.org/10.1016/j.tifs.2009.01.054
28. Neuman W. Listeria outbreak traced to Colorado cantaloupe packing shed; 2011. Available:https://www.nytimes.com/2022/10/20/business/listeria-outbreaktraced-
29. Srey S, Jahid IK, Ha SD. Biofilm formation in food industries: A food safety concern. Food Control. 2013;31(2):572–585. Available:https://doi.org/10.1016/j.foodcont.2012.12.001
30. Latorre AA, Van Kessel JS, Karns, JS, Zurakowski, MJ, Pradhan AK., e al. Biofilm in milking equipment on a dairy farm as a potential source of bulk tank milk contamination with *Listeria monocytogenes*. Journal of Dairy Science. 2010;93(6):2792–2802. Available:https://doi.org/10.3168/jds.2009-2717
31. Esbelin J, Santos T, and Hébraud M. Desiccation: An environmental and food industry stress that bacteria commonly face. Food Microbiology. 2018;69:82–88.
32. Amoril JG, Bhunia AK. Immunologica l and cytopathogenic properties of *Listeria monocytogenes* isolated from naturally contaminated meats. Journal of Food Safety. 1999;19: 195– 207.
33. Jacquet C, Doumith M, Gordon JI, Martin PMV, Cossar TP, Lecuit MA.Molecular marker for evaluating the pathogenic potential of foodborne *Listeria monocytogenes*. Journal of Infectious Diseases. 2004;189:2094-2100.
34. Almeida RM, Barbosa AV, Lisbôa RC, Santos AFM, Hofer E, Vallim DC, et al. Virulence genes and genetic relationship of *L. monocytogenes* isolated from human and food sources in Brazil. Brazilian Journal of Infectious Diseases. 2017;21:282-289.
35. Iglesias MA, Kroning IS, Decol LT, Franco BD, and Silva WP. Occurrence and phenotypic and molecular characterization of *Listeriamonocytogenes* and *Salmonella spp*. in slaughterhouses in southern Brazil. Food Research Internaional. 2017;100:96-101.
36. Jamali H, Radmehr B, and Thong KL. Prevalence, characterization, and antimicrobial resistance of Listeria species and *Listeriamonocytogenes* isolates from raw milk in farm bulk tanks. Food Control. 2013;34:121-125.
37. Oliveira TS, Varjão LM, Silva LNN, Pereira RCB, Hofer E, Vallim, DC, et al. *Listeria monocytogenes* at chicken slaughterhouse: Occurrence, genetic relationship among isolates and evaluation

of antimicrobial susceptibility. Food Control. 2018;88:131-138.
38. Coroneo V, Carraro V, Aissani N, Sanna A, Ruggeri A, Succa S, et al. Detection of virulence genes and growth potential in *Listeria monocytogenes* strains isolated from Ricotta Salata cheese. Journal of Food Science. 2016;8:20-26.
39. Du X, Zhang X, Wang X, Su Y, Li P, Wang S. Isolation and characterization of *Listeria monocytogenes* in chinese food obtained from the central area of China. Food Control. 2017; 74:9-16.
40. Su X, Zhang J, Shi W, Yang X, Li Y, Pan H, et al. Molecular characterization and antimicrobial susceptibility of *Listeria monocytogenes* isolated from foods and humans. Food Control. 2016; 70:96-102.
41. 41.Tahoun MB, Abouelez RMM, Abdelfatah EN, Elsohaby I, El-gedawy AA, Elmoslemany AM. *Listeria monocytogenes* in raw milk, milking equipment and dairy workers: Molecular characterization and antimicrobial resistance patterns. Journal of Global Antimicrobial Resistance. 2017;10:264-270.
42. Orsi RH, Den bakker HC, and Wiedmann M. *Listeria monocytogenes* lineages: Genomics, evolution, ecology, and phenotypic characteristics. International Journal of Medical Microbiology. 2011;301:79-96.
43. Vázquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Domínguez-Bernal G, Goebel W, González-Zorn B, Wehland J, Kreft J. *Listeria pathogenesis* and molecular virulence determinants. Clinical microbiology reviews. 2001 Jul 1;14(3):584-640.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*https://www.sdiarticle5.com/review-history/112912*