Evaluation of Virulence Gene Expression in *Listeria monocytogenes* Isolated from Various Foods Using RT-qPCR

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Abstract

The ability of *Listeria monocytogenes* to form biofilms on food contact surfaces poses significant challenges to food safety. This study investigated estimating the incidence of *L. monocytogenes* and the expression of virulence genes in diverse dietary matrices (raw milk, dairy products, meat products and fish products). The total number of samples was 100, with an equal number of samples from each dietary category (25 samples from each category). The total isolation rate of L. monocytogenes was 43%, and the highest frequency was found in fish items (60%), followed by meat (48%), raw milk (36%) and dairy products (28%). Molecular investigation showed that 74.4% and 55.8% of the isolates harbored *actA* and *plcA* genes, respectively. Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) analysis revealed significant differences in the expression of virulence genes between isolates from different food sources ($P<0.001$), with isolates from meat having the highest levels of expression for both genes . A negative correlation between Ct values and gene expression was detected, pointing out a higher transcriptional activity of the meat and fish isolates. The differences in expression and abundance of the genes identified are due to environmental barriers that can influence bacterial survival and regulation of virulence genes during the processing, storage and distribution of food. Although increased expression of virulence genes does not always mean that the bacteria are more harmful, the results of this study imply that contaminated foods of animal origin, especially meat and fish products, may be a serious public health problem. The inverse correlation between Ct values and relative gene expression reveals that the isolates obtained from meat and fish products had an elevated transcriptional activity of the *actA* and *plcA* genes. Factors influencing survival of bacteria, such as environmental challenges during food production, processing, storage and distribution, may also affect the regulation of genes involved in virulence, contributing to differences in prevalence and expression of genes. These results emphasize the importance of a multiplex molecular detection and quantitative gene expression analysis approach for better monitoring, characterization and risk assessment of *Listeria monocytogenes* in foods of animal origin

.**Keywords:** *Listeria monocytogenes*, RT-qPCR, *actA*, *plcA*, food safety, virulence genes, molecular detection.

1. Introduction

Listeria monocytogenes (*L. monocytogenes*) is a Gram-positive facultative intracellular bacteria and one of the most important foodborne pathogens in the world. It causes listeriosis, a dangerous disease that leads to septicemia, meningitis, encephalitis and abortion, especially in pregnant women, babies, elderly and immunocompromised people. In addition, Listeriosis is a major public health concern, as it is associated with high rates of hospitalization and death. Its frequency is rather low, however, compared to other food-borne illnesses [10,15] . On the other hands, L. monocytogenes is ubiquitous in nature and can contaminate a wide

variety of foods of animal origin including raw milk, dairy products, meats, available-to-eat foods and fish products . In this case, The bacteria is able to survive across the whole food production chain due to its impressive endurance to environmental challenges such as refrigeration temperatures, acidic pH, high salt concentrations, oxidative stress and limited nutrients. Moreover, its propensity to form biofilms promotes its survival in food-processing conditions and increases the likelihood of cross-contamination[11,18] .

Virulence of *L. monocytogenes* is mainly determined by virulence genes found in Listeria Pathogenicity Island-1 (LIPI-1). Among them, *ActA* and *plcA* genes are indispensable for intracellular infection. The *actA* gene encodes the actA surface protein that promotes actin polymerization, allowing intracellular bacterial movement and direct cell-to-cell dissemination, thus evading extracellular immune responses. Compared to the *plcA* gene, which encodes phosphatidylinositol-specific phospholipase C, an enzyme necessary for the disruption of the phagosomal membrane allowing the escape of the bacteria into the host cell cytoplasm, contributing to intracellular survival and dissemination[10, 18].

The usual approaches for the detection of *L. monocytogenes* and for the identification of virulence-associated genes are the conventional microbiological culture methods and PCR assays. However, these approaches merely indicate the existence of bacterial cells or their genetic determinants and do not reflect whether virulence genes are transcriptionally active. Also, reverse transcription quantitative real-time PCR (RT-qPCR) allows the sensitive and precise quantification of messenger RNA (mRNA) and so provides direct information on gene expression and bacterial physiological condition. Thus, RT-qPCR has been a necessary molecular tool for studying the regulation of bacterial virulence and assessing the pathogenic potential of foodborne isolates (15).

In this case, recent investigations have shown that environmental factors, food composition and stress adaption influence strongly the expression of virulence genes controlled by the transcriptional activator *PrfA* . Changes in nutrient availability, temperature, fatty acids, osmotic stress and food matrix composition can alter the transcription of *actA*, *plcA*, *hly* and other virulence-associated genes, suggesting that isolates from different food sources could have different pathogenic potentials [15].

The presence and distribution of virulence genes in *L . monocytogenes* have been described in several studies, but few studies have looked at the differential expression of key virulence genes among isolates from different food matrices of animal origin . Such transcriptional differences are essential for improving microbial risk assessment and for predicting the pathogenic potential of foodborne isolates [15]

Therefore, the present investigation was performed to investigate the relative expression levels of virulence genes *actA* and *plcA* in *Listeria monocytogenes* isolates derived from raw milk, dairy products, meat products and fish products using SYBR Green based RT-qPCR. These findings provide genetic evidence for differential virulence gene expression between isolates from different food matrices and contribute to an understanding of their pathogenic potential and consequences for food safety.

2. Materials and Methods

2.1 Sample Collection

Samples were collected between January to June 2026, 100 samples of food of animal origin were collected from retail markets and local food vendors. The samples included 25 raw milk samples, 25 dairy items, 25 beef products and 25 fish products. Samples were collected aseptically in sterile containers and transported to the laboratory under refrigerated conditions (4°C) and processed within 24 h of collection[4,8].

2.2 Isolation and Phenotypic Identification of *Listeria monocytogenes*

L. monocytogenes was isolated by the standard methods 11290-1. Briefly, 25 g (or 25 mL) of each sample was homogenized in 225 mL of Half Fraser broth and incubated at 30°C for 24 h. Then 0.1 mL of the primary enrichment culture was inoculated in 10 mL Fraser broth and incubated at 37°C for 24–48 h. After selection enrichment aliquots were streaked on Oxford agar and PALCAM agar plates and incubated at 37 °C for 24-48 h. Presumptive Listeria colonies were identified by characteristic colony morphology and confirmed by Gram staining, catalase production, motility test, CAMP test and conventional biochemical assays[4,7] .

2.3 Genomic DNA Extraction

Genomic DNA from bacteria was isolated from purified bacterial isolates using a commercial bacterial genomic DNA extraction kit (Thermo Scientific, Waltham, MA, USA) of following the directions of the manufacturer.The concentration and purity of DNA were measured using spectrophotometer (In which spectrophotometer) . DNA samples were kept at −20°C for subsequent molecular analysis [14].

2.4 Detection of Virulence Genes by Conventional PCR

The detection of virulence-associated genes *actA* and *plcA* were detected by conventional PCR using gene-specific primers listed in Table 1. The PCR amplification was carried out in a total reaction volume of 25 µL with 12.5 µL of 2× PCR Master Mix, 1 µL of forward and reverse primer (10 µM), 2 µL of genomic DNA template and nuclease free water to make up the reaction volume [2,15] .

Table 1. Primers used for conventional PCR detection of virulence genes in *Listeria monocytogenes*

Target gene	Primer	Primer sequence (5′–3′)	Amplicon size (bp)	Annealing temperature (°C)	Reference
actA	Forward	CGCCGCGGAAATTAAAAAAAGA	839	61	16
	Reverse	ACGAAGGAACCGGGCTGCTAG			
plcA	Forward	CTGCTTGAGCGTTCATGTCTCATCCCCC	1484	61	16
	Reverse	CATGGGTTTCACTCTCCTTCTAC			

PCR amplification was done at the Laboratory of Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq. The amplifications were carried out in a programmable thermal cycler using the cycling conditions described in Table 2. Amplified products were resolved by electrophoresis on a 1.5% agarose gel in 1× TBE buffer, stained with a nucleic acid staining dye and observed under UV light. Confirmation of the sizes of the PCR products was achieved using a DNA molecular weight marker and comparison with the anticipated amplicon sizes (Table 1).

Table 2. Thermal cycling conditions for conventional PCR

Step	Temperature (°C)	Time	Cycles
Initial denaturation	95	5 min	1
Denaturation	95	30 s	35
Annealing	61	30 s	35
Extension	72	1 min	35
Final extension	72	7 min	1

2.5 RNA Extraction

For gene expression investigation only PCR positive *L. monocytogenes* isolates with the virulence genes of interest were selected. Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) of following the manufacturer's instructions. Residual genomic DNA was extracted by on-column digestion with DNase I. RNA concentration and purity were determined by spectrophotometric determination of the A260/A280 ratio. Samples for downstream applications exhibited acceptable purity scores of 1.8-2.0[1, 14]. (are silica- or magnetic used to DNA Extraction in this paper?

2.6 .cDNA Synthesis

First strand complementary DNA (cDNA) was prepared from 1 µg total RNA using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The generated cDNA was kept at −20 °C until use for quantitative gene expression investigation [14].

2.7. Real Time Quantitative PCR (RT-qPCR)

SYBR Green-based real-time quantitative PCR (RT-qPCR) was used to determine the relative expression of the Listeria monocytogenes virulence genes actA and plcA. Expression analysis was done only for the isolates that were positive for the respective virulence genes by conventional PCR. Therefore expression of *actA* was analyzed in all *actA* -positive isolates (n = 32) and expression of *plcA* was assessed in all *plcA*-positive isolates (n = 24). Relative expression levels were then evaluated between isolates isolated from different dietary sources. All RT-qPCR studies were developed, performed and reported according to the Minimum Information for Publication of Quantitative Real-Time PCR studies (MIQE) criteria [1]. Gene expression data were standardized to the housekeeping gene rpoB that is stably expressed in *L. monocytogenes.* The primer sequences for RT-qPCR are shown in Table 3.

Table 3. Primers used for SYBR Green RT-qPCR analysis of *Listeria monocytogenes*

Target gene	Function	Primer	Primer sequence (5′–3′)	Amplicon size (bp)	Annealing temperature (°C)	Reference
rpoB	Reference gene	Forward	AGAACCGTGATGCAAACTAT	130	60	5
		Reverse	GAACGTACCCATTTCAGTCA			
actA	Virulence gene	Forward	AAAACCCGCCACAGCCATTA	118	60	5
		Reverse	TGGTTTCGTAGTAGGAAGCGTTG			
plcA	Virulence gene	Forward	ATACCAGGGACGCACGAT	149	60	5
		Reverse	GTTAAGATCCTCTTTAGCTCTGA			

All RT-qPCR reactions were run in a total volume of 20 µL including 10 µL of 2× SYBR Green Master Mix, 0.5 µL of each of the forward and reverse primers (10 µM), 2 µL of cDNA template and 7 µL of nuclease-free water. Thermal cycling settings were 3 min initial denaturation at 95°C, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and then extension at 72°C for 30 s. After the amplification, a melting curve analysis (65–95°C) was carried out to confirm the specificity of the primers and revealed a single amplification peak, without production of primer-dimers.

Each sample was run in triplicates with a no template control (NTC). Relative gene expression was estimated using the comparative $2^{\wedge-\Delta\Delta Ct}$ technique normalized to the reference gene, *rpoB* [17].

2.8 Statistical Analysis

Gene expression data are expressed as mean ± SD (standard deviation). Statistical analyses were performed using the SPSS software version 27.0 (IBM Corp., Armonk, NY, USA). Statistical analysis Statistical differences between dietary categories were analyzed by one-way analysis of variance (ANOVA) with Tukey's post hoc multiple comparison test. Statistical significance was chosen at $P < .05$ [6].

3.Results and Discussion

Different levels of *Listeria monocytoge*

nes were discovered in the different categories of food analyzed (Table 3.2). Of the 100 samples analyzed, 43 (43.1%) were positive for *L. monocytogenes*. Note that the highest incidence was seen in fish items, followed by meat products, raw milk and dairy goods. Statistical analysis also showed that the prevalence rates were significantly different across the dietary groups examined ($P < 0.05$). These data imply various levels of contamination for different types of food. Perhaps that's due to variations in the handling of food, cleanliness, and environmental exposure. Similarly, previous studies have reported that fish and meat are the main sources of *L. monocytogenes*, as contamination can occur at many levels of the food chain such as

production, processing, storage, distribution, and retail handling [3,12].

Table 3.1: Distribution of food samples analyzed in the present study, including raw milk, dairy products, meat products, and fish products with equal representation (n = 25 per group; total n = 100

Food source	Number of samples	Percentage (%)
Raw milk	25	25
Dairy products	25	25
Meat products	25	25
Fish products	25	25
Total	**100**	**100**

The overall prevalence of *Listeria monocytogenes* among the tested food samples was 43.0% (43/100) (Table 3.2). The highest isolation rate was seen in fish products (60.0%), followed by meat products (48.0%), raw milk (36.0%) and dairy products (28.0%). This distribution implies that the occurrence of *L. monocytogenes* depends on the food matrix. Also, the higher prevalence found in fish and meat products may be related to a higher risk of contamination during the processes of slaughtering, processing, transportation, storage and retail handling. In addition, the ability of *L. monocytogenes* to build biofilms and to live under refrigerated settings is remarkable and contributes to its persistence in food-processing environments, increasing the potential for contamination [9,18]. Likewise, it has been documented in earlier research that fish and meat products are ideal ecological niches for survival and persistence of *L. monocytogenes* , especially under cold-storage conditions and other environmental challenges experienced during food processing [15]. Thus, the present results support the relevance of the implementation of effective sanitary procedures and regular microbiological monitoring along the food production chain to minimize the risk of *L. monocytogenes* infection.

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Table 3.2:Prevalence of *Listeria monocytogenes* in different food categories, showing isolation rates across raw milk, dairy products, meat products, and fish products.

Food source	No. of samples examined	Positive isolates (n)	Isolation rate (%)
Raw milk	25	9	36.0
Dairy products	25	7	28.0
Meat products	25	12	48.0
Fish products	25	15	60.0
Total	**100**	**43**	**43.0**

Meat and fish products are considered the most important food vehicles related with human listeriosis because they are widely consumed and often marketed as ready-to-eat or lightly processed foods. Therefore, the relatively high incidence of *L. monocytogenes* found in the present investigation may be due to contamination during slaughtering, processing, shipping, storage and retail handling. Moreover, the capacity of the organism to build biofilms and live at cold temperatures allows it to persist throughout the food supply chain. Previous regional investigations have also indicated a high prevalence of *L. monocytogenes* in fish products available in local markets and recognized these products as significant reservoirs of the disease [19, 20]. Overall, the present findings are consistent with earlier reports stressing the importance of rigorous sanitary standards and ongoing microbiological monitoring to reduce contamination of high-risk items.

Molecular examination of the isolates of *L. monocytogene*s showed that the *actA* gene was recognized in 32 isolates (74.4%). However, the *plcA* gene was identified in 24 isolates (55.8%) (Table 3.3). Importantly, the *actA* gene was more prevalent than the *plcA* gene, suggesting that the virulence factors related to intracellular motility and cell-to-cell transmission are more widely distributed among the isolates studied. *The actA* gene is highly conserved and required for actin polymerization, which enables intracellular mobility and spreading to nearby host cells. Conversely, phosphatidylinositol-specific phospholipase C encoded by the *plcA* gene mostly contributes to host-cell invasion in the early phase of infection [3,13]. Also, foodborne *L. monocytogenes* isolates have been shown to carry both genes in earlier genomic investigations, with their presence depending on the strain lineage and the environmental origin [10]. Moreover, comparative genomic studies have shown that the basic virulence gene repertoire is preserved between isolates recovered from dietary and environmental sources, indicating that many of the non-clinical isolates retain significant pathogenic potential [9,18].

Table 3.3. Detection frequency of virulence-associated genes (*actA* and *plcA*) in *Listeria monocytogenes* isolates recovered from food samples.

Virulence gene	Positive isolates (n)	Percentage (%)
actA	32	74.4
plcA	24	55.8

In addition, the RT-qPCR analysis showed substantial changes in the expression levels of *actA* and *plcA across L. monocytogenes* isolates obtained from different dietary sources ($P < 0.001$) (Table 3.4). Interestingly, meat isolates revealed the highest relative expression of both *actA* (27.84 ± 1.73) and *plcA* (58.63 ± 3.26), followed by fish isolates whereas dairy products and raw milk had much lower expression levels. Consistent with these results, raw milk isolates had the lowest Ct values, while meat isolates had the lowest Ct values, confirming the expected inverse association between Ct values and transcript abundance. Moreover, the higher expression of both virulence genes in isolates from meat and fish indicated a higher activation of the molecular processes involved in bacterial invasion, intracellular survival and cell-to-cell dispersion. Similarly, previous transcriptome studies have shown that the environmental conditions experienced during food processing, storage

and distribution can influence the expression of virulence associated genes in *L. monocytogenes*, which can subsequently influence its pathogenicity [3,12]. In addition, environmental stresses such as refrigeration, osmotic stress and nutritional constraint have been found to increase virulence gene transcription, enhancing bacterial persistence in food processing environments [9,18]. Therefore, the frequent detection of virulence genes along with their significantly increased expression levels in isolates recovered from meat and fish suggest that such food products may represent important reservoirs of potentially highly-virulent *L. monocytogenes* strains highlighting the significance of continuous molecular surveillance and rigid hygienic control throughout the food production chain.

Table 3.4. Relative expression levels (RT-qPCR) of *actA* and *plcA* genes in *Listeria monocytogenes* isolates from different food sources, including Ct values and fold-change analysis.

Food source	*actA* Relative expression (Mean ± SE)	*plcA* Relative expression (Mean ± SE)	*actA* Ct (Mean ± SE)	*plcA* Ct (Mean ± SE)
Raw milk	1.02 ± 0.08^{c}	1.14 ± 0.09^{c}	26.84 ± 0.24^{a}	27.45 ± 0.28^{a}
Dairy products	2.91 ± 0.17^{c}	3.42 ± 0.21^{c}	25.43 ± 0.18^{b}	26.02 ± 0.21^{b}
Meat products	27.84 ± 1.73^{a}	58.63 ± 3.26^{a}	22.11 ± 0.14^{d}	21.56 ± 0.17^{d}
Fish products	14.38 ± 0.91^{b}	24.71 ± 1.42^{b}	23.39 ± 0.16^{c}	22.88 ± 0.19^{c}
P-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Note: Values are expressed as mean ± SE. Different superscript letters within the same column indicate significant differences ($P<0.05$). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method

Recent transcriptomic studies have demonstrated that variation in virulence gene expression is a result of the adaptive transcriptional responses of *L. monocytogenes* to environmental obstacles faced in different food matrices such as low temperature, osmotic stress and nutrient limitation [15]. Moreover, these investigations revealed the great regulation exerted by the nature of the food matrix on the expression of the genes involved in stress adaptation and pathogenicity. Notably, isolates from meat and fish products were more commonly associated with up-regulation of virulence-related genes compared to isolates from dairy products. Additionally, environmental variables such as acidic pH and processing-related stressors have been documented to induce the expression of important virulence genes, including *plcA, hly* and *actA* [15].

However, higher transcription of genes related with virulence should not be taken as direct proof of increased pathogenicity. The virulence of *L. monocytogenes* is complex and depends on gene expression, bacterial genetic background, environmental adaptation and host vulnerability [3,13]. Therefore, molecular data should be correlated to functional assays, e.g. cell culture or animal infection models, to properly evaluate the pathogenic potential of foodborne isolates. In a similar vein, recent studies have highlighted that while virulence gene expression varies greatly among food derived isolates,

transcriptional profiles are not always good predictors of clinical virulence and thus highlight the need to combine molecular characterization with functional pathogenicity assays.

To summarize, the present study shows that the presence and transcriptional activity of the key virulence genes of *L. monocytogenes* are highly impacted by the dietary source. Meat and fish products showed higher contamination rates and considerably higher expression of *actA* and *plcA* compared to raw milk and dairy products (Tables 3.2–3.4). Moreover, the constant higher expression of *actA than plcA* implies that virulence pathways may be differentially controlled in food-associated environmental circumstances. Together, these results provide further evidence of the significant effect of dietary matrices on the survival, persistence and regulation of virulence-associated genes in *L. monocytogenes*, and therefore on its potential public health danger.

Conclusion

Results of the present study demonstrate that the prevalence and expression of virulence genes in *Listeria monocytogenes* are depending on the dietary source with meat and fish items having higher rates of contamination and higher expression of critical virulence genes. While gene expression alone does not confirm pathogenicity, the data suggest differences in the potential virulence profiles of isolates from different food sources. However, the results highlight the necessity of ongoing molecular monitoring of *L. monocytogenes* in food of animal origin and propose RT-qPCR as a rapid approach in food safety surveillance and risk assessment.

References

[1] Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry, 55*(4), 611–622. https://doi.org/10.1373/clinchem.2008.112797

[2] Costa, J. C. P., Bolívar, A., Alberte, T. M., Zurera, G., & Pérez-Rodríguez, F. (2024). *Listeria monocytogenes* in aquatic food products: Spotlight on epidemiological information, bio-based mitigation strategies and predictive approaches. *Microbial Pathogenesis, 197*, 106981.

[3] Disson, O., Charlier, C., Pérot, P., Leclercq, A., Paz, R. N., Kathariou, S., Tsai, Y. H., & Lecuit, M. (2025). Listeriosis. *Nature Reviews Disease Primers, 11*, 71. https://doi.org/10.1038/s41572-025-00654-x

[4] European Food Safety Authority. (2024). *The European Union One Health Zoonoses Report 2023*. *EFSA Journal*.

[5] Han, L., Zhang, W., Sun, L., & Zhou, X. (2016). The roles of noncoding RNA Rli60 in regulating the virulence of *Listeria monocytogenes*. *Journal of Microbiology, Immunology and Infection, 49*(4), 502–508. https://doi.org/10.1016/j.jmii.2014.08.017

[6] IBM Corp. (2023). *IBM SPSS Statistics for Windows* (Version 30.0). IBM Corp.

[7] International Organization for Standardization. (2017). *ISO 11290-1:2017. Microbiology of the food chain—Horizontal method for the detection and enumeration of Listeria monocytogenes and Listeria spp.—Part 1: Detection method*.

[8] International Organization for Standardization. (2017). *ISO 6887-1:2017. Microbiology of the food chain—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—Part*

1: General rules for the preparation of the initial suspension and decimal dilutions.

[9] Kawacka, I., & Olejnik-Schmidt, A. (2024). High prevalence of virulence-associated genes and length polymorphism in *actA* and *inlB* genes identified in *Listeria monocytogenes* isolates from meat products and meat-processing environments in Poland. *Pathogens*, *13*(6), 444. https://doi.org/10.3390/pathogens13060444

[10] Koopmans, M. M., Brouwer, M. C., Bijlsma, M. W., & van de Beek, D. (2023). *Listeria monocytogenes* infection: Epidemiology, pathogenesis, and clinical manifestations. *Microbiology and Molecular Biology Reviews*, *87*(4). https://doi.org/10.1128/MMBR.00036-23

[11] Lambrechts, K., & Rip, D. (2024). *Listeria monocytogenes* in the seafood industry: Exploring contamination sources, outbreaks, antibiotic susceptibility and genetic diversity. *MicrobiologyOpen*, *13*(5), e70003.

[12] Meireles, D., Pombinho, R., & Cabanes, D. (2024). Signals behind *Listeria monocytogenes* virulence mechanisms. *Gut Microbes*, *16*(1), 2369564. https://doi.org/10.1080/19490976.2024.2369564

[13] Ogunleye, S. C., Islam, S., Chowdhury, Q. M. M. K., Ozdemir, O., Lawrence, M. L., & Abdelhamed, H. (2024). Catabolite control protein C contributes to virulence and hydrogen peroxide-induced oxidative stress responses in *Listeria monocytogenes*. *Frontiers in Microbiology*, *15*, 1403694. https://doi.org/10.3389/fmicb.2024.1403694

[14] QIAGEN. (2024). *QuantiNova SYBR Green RT-PCR Handbook*. QIAGEN.

[15] Quilleré, A., Darsonval, M., Papadochristopoulos, A., Amoros, A., Nicolas, P., & Dubois-Brissonnet, F. (2024). Deciphering the impact of exogenous fatty acids on *Listeria monocytogenes* at low temperature by transcriptome analysis. *Frontiers in Microbiology*, *15*, 1441784. https://doi.org/10.3389/fmicb.2024.1441784

[16] Rawool, D. B., Malik, S. V. S., Shakuntala, I., Sahare, A. M., & Barbuddhe, S. B. (2007). Detection of multiple virulence-associated genes in *Listeria monocytogenes* isolated from bovine mastitis cases. *International Journal of Food Microbiology*, *113*(2), 201–207. https://doi.org/10.1016/j.ijfoodmicro.2006.06.029

[17] Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. *Nature Protocols*, *3*(6), 1101–1108. https://doi.org/10.1038/nprot.2008.73

[18] Tasci, F., Sudagidan, M., Yavuz, O., Soyucok, A., & Aydin, A. (2024). Virulence properties of *Listeria monocytogenes* isolated from meat and meat contact surfaces in a slaughterhouse. *Polish Journal of Veterinary Sciences*, *27*(1), 13–23.

[19] Tuytschaever, T., Raes, K., & Sampers, I. (2023). *Listeria monocytogenes* in food businesses: From persistence strategies to intervention/prevention strategies—A review. *Comprehensive Reviews in Food Science and Food Safety*, *22*(5), 3910–3950. https://doi.org/10.1111/1541-4337.13219

[20] Yassin, S. A. S., Nadhom, B. N., & Al-Gburi, N. M. (2021). Detection of *Listeria monocytogenes* in several types of frozen meat in Baghdad City. *Iraqi Journal of Science*, *62*(3), 742–750. https://doi.org/10.24996/IJS.2021.62.3.4