



Integrated Surveillance of *Listeria monocytogenes* in Food Products of Dakahlia, Egypt: Prevalence, Risk Factors, and One-Health Implications

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Abstract

Listeria monocytogenes (*L. monocytogenes*) represents a critical foodborne pathogen with extraordinary environmental resilience and severe clinical impact, particularly affecting immunocompromised individuals, pregnant women, neonates, and the elderly person. This study aimed to elucidate the prevalence, molecular characteristics, and antimicrobial resistance profiles of *L. monocytogenes* across diverse food supply products in Dakahlia Governorate, Egypt, while evaluating the efficacy of advanced cold enrichment protocols for pathogen recovery. This study was conducted with 500 samples systematically collected from retail markets (n=150), supermarkets (n=150), slaughterhouses (n=100), and dairy farms (n=100). Isolation and identification, incorporating dual enrichment and advanced cold enrichment protocol were performed. Biochemical, phenotypic, and molecular (PCR-based) assays targeted key virulence genes (*prfA*, *hlyA*) were conducted. Antimicrobial susceptibility was assessed using CLSI M45 guidelines, and statistical analyses explored contamination dynamics and risk factors. *L. monocytogenes* was isolated from 4.20% of samples (95% CI: 2.76-6.34%), with the highest prevalence in dairy farms (7.00%) and high-risk food categories including minced meat (7.94%), cream (7.89%), yogurt (7.69%), and milk (7.00%). The cold enrichment protocol enhanced detection sensitivity significantly. All isolates harbored both *prfA* and *hlyA* virulence genes, confirming high pathogenic potential. Alarming, isolates exhibited near-universal resistance to β -lactams and high multidrug resistance rates (85.7-90.5%), while maintaining complete susceptibility to gentamicin, imipenem, and linezolid. This study highlights the urgent public health threat posed by multidrug-resistant *L. monocytogenes* in Egyptian food supply products. The findings underscore the necessity for advanced molecular surveillance, stringent antimicrobial stewardship, and integrated One Health interventions to safeguard food safety and public health.

Keywords: *Listeria monocytogenes*, antimicrobial resistance, virulence genes, food safety, One Health.

Introduction

Listeria monocytogenes, a Gram-positive, facultative intracellular bacterium, represents one of the most formidable foodborne pathogens due to its extraordinary environmental resilience [1], high fatality rates (20–30%), and capacity to cause severe disease in vulnerable populations [2]. This psychrotrophic pathogen thrives across a broad ecological range, growing at temperatures from 30°C to 37°C, tolerating pH levels of 4.6–9.5, surviving in high-salt environments (up to 20% NaCl), and maintaining viability under low water activity conditions [3].

Globally, *L. monocytogenes* causes significant public health burden despite relatively low incidence rates compared to other foodborne pathogens. The bacterium's virulence arsenal—including listeriolysin O (LLO, encoded by *hlyA*), actin assembly protein (*ActA*), internalins (InlA/B), and the transcriptional regulator PrfA—facilitates host cell invasion, intracellular replication, systemic dissemination, and crossing of the blood-brain and placental barriers [4]. Fresh produce, dairy products, ready-to-eat meats, and processed foods serve as primary transmission vehicles, with major outbreaks documented globally highlighting the pathogen's devastating potential when contamination occurs in food supply chains

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[5]. The persistence of *L. monocytogenes* in food production environments is exacerbated by its capacity to form biofilms on stainless steel, rubber, and polymer surfaces, enhancing resistance to desiccation, sanitizers, and disinfectants through extracellular polymeric substance (EPS) production [6]. These biofilms facilitate long-term colonization of processing facilities, creating recurrent contamination reservoirs that are difficult to eradicate through conventional cleaning protocols [7].

Furthermore, the pathogen employs sophisticated molecular adaptations to refrigeration temperatures, including membrane lipid composition modification, accumulation of cryoprotective osmolytes, and cold-shock protein expression, which collectively maintain metabolic function at temperatures as low as -0.4°C . Temperature downshifts to 7°C induce co-activation of the stress-response sigma factor σB and the virulence regulator PrfA, upregulating hlyA and prfA expression while simultaneously enhancing biofilm formation—a phenomenon termed the "refrigeration paradox" [8,9].

Antibiotic resistance in *L. monocytogenes* has emerged as a critical public health crisis, particularly in regions with unregulated agricultural antimicrobial use [10]. Egyptian isolates exhibit concerning resistance patterns, with studies reporting high multidrug resistance rates that compromise first-line therapies for invasive listeriosis. In Egypt specifically, the food supply products (milk, cheese, yogurt, meat, etc) faces heightened vulnerability due to infrastructure limitations, regulatory gaps, and climatic conditions that promote pathogen persistence. The purpose of this study is to use sophisticated detection techniques, for detection of *L. monocytogenes* in diverse food products in the Dakahlia Governorate. Another goal is to clarify the pathogenic potential of isolated strains and guide focused public health and veterinary actions within a One Health framework, it also aims to carry out molecular characterization of virulence genes.

Material and Methods

Study Design and Sample Collection

A cross-sectional study was undertaken in Dakahlia Governorate, Egypt over a 12 month period (January to December) 2023, to assess the prevalence of *L. monocytogenes* across various segments of the food supply chain. A total of 500 samples were systematically collected from four distinct sources—retail markets (n=150), supermarkets (n=150), slaughterhouses (n=100), and dairy farms (n=100)—encompassing diverse specimen types including raw milk, vegetables, seafood, poultry, beef, and processed meat products. Specimens were aseptically collected in sterile, lockable plastic bags, maintained under refrigeration ($4\pm0.5^{\circ}\text{C}$), and delivered within 2 hours to the Laboratory of Bacteriology, Immunology, and Mycology department, faculty of Veterinary

Medicine, Mansoura University. To ensure statistical robustness and representativeness, sampling was stratified by source type, geographic distribution (urban, peri-urban, and rural zones), and seasonality with monthly collections spanning the study period.

Bacteriological analysis

To enhance the recovery of *L. monocytogenes* and other psychrotrophic pathogens, a cold enrichment strategy was implemented. Samples were stored at 4°C for 1–4 weeks prior to cultivation [11] and incubated in *L. monocytogenes* Enrichment Broth (NEOGEN) to suppress competing mesophilic microbiota while promoting the proliferation of cold-tolerant pathogens [12]. The isolation of *L. monocytogenes* was conducted following a modified ISO 11290-1:2017 protocol, incorporating a dual enrichment and selective plating strategy. Primary enrichment was performed in Tryptic Soy Broth (TSB) (HiMedia) for 24 h at 30°C to resuscitate stressed *L. monocytogenes* cells. Secondary enrichment utilized Fraser Broth (Oxoid™) supplemented with Fraser selective supplement (acriflavine, nalidixic acid, and cycloheximide) for 48 h at 37°C to amplify *L. monocytogenes* populations under selective pressure [13]. Enriched cultures were streaked onto Oxford Agar (Oxoid™) and PALCAM Agar (HiMedia), both supplemented with selective antimicrobial agents (Oxford: colistin, ceftazidime, and fosfomycin; PALCAM: polymyxin B, acriflavine, and lithium chloride). Presumptive *L. monocytogenes* colonies were identified based on morphological criteria: black colonies with esculin hydrolysis halos on Oxford Agar and gray-green colonies with black centers on PALCAM Agar. colonies are small (≈ 1 mm), shiny and smooth; after 48 h colonies may enlarge (≈ 1 – 2 mm), often appear slightly sunken or embedded with a central depression. Colonies were subcultured onto Tryptic Soy Agar with 0.6% yeast extract (TSAYE) for purity and incubated at 37°C for 24–48 h [14].

Biochemical and Phenotypic Characterization

The biochemical and phenotypic characterization of *L. monocytogenes* isolates was conducted using standardized assays to confirm their identity and distinguish them from closely related bacterial species [15]. On Gram staining, it appears as short rods (coccobacilli), often occurring singly, in short chains and Cells stain deep purple due to the thick peptidoglycan cell wall typical of Gram-positive bacteria. The catalase and oxidase assays were performed to differentiate *L. monocytogenes* from other bacteria, such as *Enterococcus* spp. and *Aeromonas* spp. The isolates exhibited catalase-positive and oxidase-negative reactions, aligning with the expected biochemical profile of *L. monocytogenes*. The characteristic umbrella-like motility of *L. monocytogenes* was evaluated using semi-solid motility agar (0.4% agar, tryptic soy broth [TSB] base). Freshly grown cultures were stab-

inoculated into the medium and incubated at 25°C for 24 hours. Radial spreading from the stab line was observed, confirming the motility pattern associated with *L. monocytogenes* [16]. A reference strain of *L. monocytogenes* serotype 1/2a, obtained from the department's bacterial culture repository, was included as a positive control to validate the biochemical and hemolytic profiles of the isolates. Isolates demonstrating concordance with the reference strain were presumptively identified as *L. monocytogenes* and subsequently cryopreserved at –80°C in TSB supplemented with 40% glycerol for future genomic and virulence analyses.

Molecular Identification of *Listeria monocytogenes*

The molecular identification of *L. monocytogenes* isolates was conducted through the detection of two key virulence-associated genes: *prfA* (217 bp), which encodes the transcriptional activator PrfA, and *hlyA* (388 bp), responsible for encoding listeriolysin O (LLO) (Table1). DNA was extracted from bacterial colonies by heat lysis at 92 °C for 20 min using heat block (Thermo Fisher Scientific, USA) then the supernatant was taken after centrifugation. Polymerase chain reaction (PCR) amplifications were performed using a thermocycler (Biomtra, Göttingen, Germany). Reaction mixtures (20 µL) contained 10 µL of 2X TOPsimple™ DyeMIX-nTaq PCR Master Mix (Enzynomics, South Korea), 1 µL each of forward and reverse primers (Metabion, Planegg, Germany), 3 µL of template DNA, and nuclease-free water to a final volume of 20 µL. Thermal cycling consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for *prfA* or 58 °C for *hlyA* for 30 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 7 min. Each sample well was filled with 8-10 µl of PCR product. Ethidium bromide was added to both agarose gel and TBE buffer to stain the PCR products. Amplicon separation which was achieved using 1.2% agarose gel electrophoresis, and bands were visualized under ultraviolet (UV) light to confirm successful gene amplification [17]

Antimicrobial susceptibility testing

Antimicrobial susceptibility of *L. monocytogenes* isolates was assessed by disk diffusion on Mueller–Hinton agar, following CLSI M45 guidelines, with inoculum standardized to 0.5 McFarland from subcultures on TSAYE incubated at 35 ± 2 °C. Disks purchased from Tulip included ampicillin (10 µg), amoxicillin (10 µg), trimethoprim–sulfamethoxazole (25 µg), gentamicin (10 µg), imipenem (10 µg), doxycycline (30 µg), linezolid (30 µg), and carbenicillin (100 µg). Plates were incubated at 35 ± 2 °C for 18–24 h, and zones measured by two readers; any discrepancy >2 mm triggered re-measurement. *L. monocytogenes* serotype 1/2a served as QC in each batch, with zone ranges checked against CLSI or published data. Interpretive

criteria were taken from CLSI M45 where available; for antibiotics lacking species-specific breakpoints [20], EUCAST (European Committee on Antimicrobial Susceptibility Testing) non–species-specific for BSAC (British Society for Antimicrobial Chemotherapy) were applied with justification based on typical MIC distributions for *L. monocytogenes* [21].

Statistical analysis

Data analysis was performed using Python (v3.x) with libraries including Pandas, NumPy, and SciPy for data manipulation and descriptive statistics. Chi-square tests ($\alpha=0.05$) were used to compare contamination rates across sampling strata, and Fisher's exact test was employed for small sample analyses [22]. Confidence intervals for prevalence estimates were calculated using the Wilson score method, which provides better coverage rates for small samples. Odds ratios with 95% confidence intervals were calculated using continuity correction for categorical risk factor analysis. Multivariate analyses accommodated spatial and temporal variations in the data.

Results and Discussion

Overall Prevalence and Distribution

The comprehensive cross-sectional study revealed that 21 out of 500 samples (4.20%) tested positive for *L. monocytogenes*, with a 95% confidence interval of 2.76-6.34% (Fig. 1). This prevalence rate aligns with global surveys of *L. monocytogenes* in diverse food matrices, which typically report contamination rates in the low single-digit percentages. The prevalence estimates and risk patterns observed in this study align with global surveillance data while highlighting region-specific characteristics [23]. The 4.2% overall positivity is comparable to moderate contamination rates reported globally, though significantly lower than some developing regions where contamination rates can exceed 10-20%. European studies consistently report similar prevalence ranges in dairy products and ready-to-eat foods, supporting the validity of our methodological approach [24].

Molecular Characterization and Virulence Potential

All 21 *L. monocytogenes* isolates underwent comprehensive molecular characterization through PCR amplification of key virulence-associated genes, demonstrating universal presence of both *prfA* (217 bp) and *hlyA* (388 bp) genes (Fig. 2) across all isolates (100% positivity). The *prfA* gene encodes the transcriptional activator *prfA*, which serves as the central regulator of virulence in *L. monocytogenes*, orchestrating expression of essential pathogenicity determinants. The *hlyA* gene encodes listeriolysin O (LLO), a critical virulence factor that facilitates pore formation and enables escape from phagolysosomes during intracellular infection. The universal detection

of both virulence genes indicates that all recovered isolates possess the molecular machinery necessary for invasive disease, supporting their classification as potentially pathogenic strains capable of causing severe human listeriosis [25]. This finding contrasts with some reports where partial gene presence has been documented, emphasizing the pathogenic potential of the local *L. monocytogenes* population. The universal presence of virulence genes across all isolates confirms their pathogenic potential, indicating that even low-level contamination could pose serious health risks to vulnerable populations including pregnant women, immunocompromised individuals, and elderly consumers [26]. This finding underscores the critical importance of comprehensive surveillance and control measures across the entire food production chain.

Analysis by sample source revealed significant variations in contamination rates, with dairy farms exhibiting the highest prevalence at 7.00% (7/100 samples), followed by supermarkets at 4.00% (6/150), retail markets at 3.33% (5/150), and slaughterhouses at 3.00% (3/100). These findings are remarkably consistent with epidemiological studies reporting similar prevalence rates in Egyptian dairy cattle farms [27]. Chi-square analysis indicated no statistically significant association between source type and contamination ($\chi^2=2.601$, $p=0.457$, $df=3$), suggesting that contamination risk is distributed across multiple sectors of the food supply chain (Fig. 3). This finding supports the concept that *L. monocytogenes* represents a ubiquitous threat requiring comprehensive surveillance across all food production sectors.

High-Risk Food Categories and Statistical Significance

Among sample types with sufficient representation (≥ 10 samples), minced meat demonstrated the highest contamination rate at 7.94% (5/63), followed by cream at 7.89% (3/38), yogurt at 7.69% (2/26), and milk at 7.00% (7/100). Risk factor analysis using Fisher's exact test revealed that high-risk sample types (minced meat, cream, yogurt, and milk) showed significantly higher odds of contamination compared to other food categories (OR=4.98, 95% CI: 1.74-14.25, $p=0.001$) (Fig. 4). This finding is particularly concerning from a public health perspective, as these products are often consumed by vulnerable populations and may undergo minimal thermal processing. The high prevalence in minced meat aligns with global reports indicating that ready-to-eat meat products serve as primary transmission vehicles for *L. monocytogenes*. Several sample categories including aged cheese, chicken, Fesikh, pickles, Rumi cheese, smoked herring, and sardines showed no detectable contamination [28]. The identification of high-risk

food categories provides actionable intelligence for regulatory authorities to implement risk-based surveillance programs [29]. These findings support targeted intervention strategies focused on products with the highest contamination potential and consumption patterns among vulnerable populations.

Geographic Distribution and Spatial Clustering

Geographic analysis revealed marked spatial variation in contamination patterns across the study region, with Dikirnis location exhibiting the highest prevalence at 5.90% (17/288 samples), significantly exceeding other locations including Aga at 3.23% (1/31), Menyet El Nasr at 2.94% (3/102), and Mansoura where no positive samples were detected (0/79) (Fig. 5). This spatial clustering suggests localized environmental reservoirs or shared contamination sources that warrant targeted investigation and intervention. The phenomenon may be attributed to differences in local infrastructure, regulatory enforcement, processing practices, or environmental factors that influence pathogen persistence and transmission [30]. The geographic clustering observed in Dikirnis and seasonal peaks during cooler months suggest opportunities for targeted intervention strategies.

Seasonal Variation and Temporal Patterns

Temporal analysis revealed distinct seasonal patterns in *L. monocytogenes* prevalence, with notable peaks during October (9.09%, 2/22 samples) and January (8.00%, 4/50 samples).

When grouped by season, autumn showed the highest prevalence (8.00%, 6/75), followed by winter (5.93%, 7/118), spring (4.07%, 5/123), and summer (1.63%, 3/184) (Fig. 6). This seasonal pattern aligns with previous studies where winter months consistently showed higher *L. monocytogenes* detection rates compared to warmer seasons [31]. The phenomenon may be attributed to enhanced survival and proliferation of the psychrotrophic pathogen under cooler temperature conditions, coupled with seasonal variations in food production and storage practices. Chi-square analysis of seasonal distribution indicated no statistically significant association ($\chi^2=6.597$, $p=0.086$, $df=3$), though the observed trends suggest ecological factors influencing pathogen persistence and transmission dynamics. The seasonal patterns observed, with peaks during cooler months, align with established ecological principles governing psychrotrophic pathogen behavior [32]. However, local climatic conditions, production cycles, and sampling schemes strongly influence seasonal trends, explaining some discrepancies with global patterns.

Antimicrobial Resistance Profile and Clinical Implications

Comprehensive antimicrobial susceptibility testing performed on all 21 *L. monocytogenes* isolates revealed concerning multifaceted resistance patterns highlighting both intrinsic and potentially acquired resistance mechanisms. Resistance (100%) was observed to ampicillin, carbenicillin, and doxycycline, with all isolates demonstrating inhibition zones below established susceptibility thresholds (Table 2). Additionally, 95.2% of isolates (20/21) showed resistance to amoxicillin, with only a single isolate demonstrating susceptibility [33]. Also, there are high percentages of *L. monocytogenes* strains that were multidrug-resistant, with especially high resistance to tetracyclines and β -lactams. Based on resistance patterns to multiple antibiotic classes [34], an estimated 85.7-90.5% of isolates demonstrated multidrug resistance (MDR) phenotypes, substantially exceeding global averages and reflecting regional patterns of antimicrobial use in agricultural settings. Trimethoprim-sulfamethoxazole exhibited heterogeneous response patterns, with 81.0% resistance (17/21 isolates). In contrast, gentamicin, imipenem, and linezolid maintained complete susceptibility (100%) across all isolates, supporting their continued role in combination therapies for severe invasive infections. The retained efficacy of gentamicin is particularly significant as it remains the cornerstone of combination therapy with β -lactams for invasive listeriosis [35]. The universal resistance to β -lactam antibiotics primarily occurs through penicillin-binding protein (PBP) modifications [36]. *L. monocytogenes* possesses multiple PBPs that serve as targets for β -lactam antibiotics, with recent studies identifying a critical W428R substitution in PBP B1 that confers resistance to ampicillin, amoxicillin, and meropenem in clinical isolates [37]. PBP3 serves as the primary lethal target for β -lactams, and alterations in its affinity significantly impact resistance levels [38]. The intrinsic resistance to cephalosporins like carbenicillin results from low affinity of these drugs for PBP3 [39, 40]. Universal doxycycline resistance is mediated through mobile genetic elements carrying tetracycline resistance genes, predominantly involving the *tet(M)* gene on Tn916-like transposons [41, 42]. These transposons facilitate horizontal gene transfer from Enterococcus species, explaining the widespread dissemination of tetracycline resistance [43]. Additionally, efflux pump mechanisms involving *tet(A)*, *tet(K)*, and *tet(L)* genes contribute to resistance by actively removing tetracycline from bacterial cells [44,45]. The high resistance rate (81.0%) to trimethoprim-sulfamethoxazole involves plasmid-mediated resistance genes, particularly the *dhfrD* gene encoding trimethoprim-resistant dihydrofolate reductase [46, 47]. These genes are often carried on mobile genetic elements including Tn3-like transposons, facilitating their spread among bacterial populations [48]. *L.*

monocytogenes possesses several intrinsic resistance mechanisms through core chromosomal genes including the *fosX* gene conferring intrinsic fosfomycin resistance through enzymatic hydrolysis, the *norB* gene providing resistance to fluoroquinolones through efflux mechanisms, and the *lin* gene mediating lincomycin resistance via ribosomal protection mechanisms [49,50]. These intrinsic resistance determinants are ubiquitously present in *L. monocytogenes* populations and contribute to the baseline resistance profile [51]). The high prevalence of MDR strains is particularly concerning given the organisms' intrinsic pathogenic potential and the limited therapeutic options for invasive listeriosis. The concerning multidrug resistance patterns observed, with 85.7-90.5% of isolates demonstrating MDR phenotypes, substantially exceed global averages and reflect regional patterns of antimicrobial use in agricultural settings. Universal resistance to ampicillin (100%), carbenicillin (100%), and doxycycline (100%), coupled with high resistance to amoxicillin (95.2%) and trimethoprim-sulfamethoxazole (81.0%), significantly limits first-line therapeutic options [52]. These resistance mechanisms reflect complex molecular pathways involving altered penicillin-binding proteins, mobile genetic elements, and efflux systems, representing a concerning public health threat that requires enhanced surveillance and antimicrobial stewardship interventions [53].

Finally, the implementation of enhanced surveillance systems incorporating whole-genome sequencing and rapid diagnostic technologies, represents a strategic advancement toward real-time pathogen detection and source attribution. A One Health approach integrating human, animal, and environmental surveillance systems is essential for addressing *L. monocytogenes* contamination at its source. Future research priorities should focus on molecular epidemiological investigations to characterize strain diversity and transmission pathways, development of cost-effective point-of-care diagnostic platforms suitable for resource-limited settings, and evaluation of intervention strategies targeting identified risk factors in dairy production systems.

Conclusion

This study demonstrates that *L. monocytogenes* contamination presents a moderate but significant public health challenge within Egypt's food safety framework. The overall prevalence of 4.20% observed in this investigation aligns with regional surveillance data, while the elevated contamination rate of 7.00% in dairy farm environments reflects established epidemiological patterns linking ruminant livestock to *L. monocytogenes* reservoirs. The universal presence of virulence genes across

isolates, combined with high antimicrobial resistance rates, underscores the pathogenic potential of circulating strains. Geographic clustering in Dikrnis and seasonal variation with peaks during cooler months provide actionable intelligence for targeted public health interventions. The retained susceptibility to gentamicin, imipenem, and linezolid offers viable therapeutic alternatives for clinical management, though continuous antimicrobial susceptibility monitoring remains critical given the documented emergence of resistance patterns in *L. monocytogenes* globally.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

Research Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt, ethically approved this study.

TABLE1. Primers used for PCR reaction

Target Gene	Primer Name	Sequence (5'→3')	Amplicon Size	References
<i>hlyA</i>	PCRGO PCRDO	F:GAA TGT AAA CTT CGG CGC R:AAT CAG GCC GTC GAT GAT TTG AAC TTC ATC	388 bp	[18]
<i>prfA</i>	LIS-F LIS-R	F: TCA TCG ACG GCA ACC R: TCG G TGA GCA ACG TAT CCT CCA GAG T	217 bp	[19]

TABLE 2. Antimicrobial Susceptibility Testing Results for *L. monocytogenes*

Antibiotic (Disk Content)	Reference Criteria	Susceptible (n, %)	Resistant (n, %)
Ampicillin (10 µg)	≥21 mm = S; ≤20 mm = R (CLSI M45Ed3)	0 (0%)	21 (100%)
Amoxicillin (10 µg)	≥12 mm = S; ≤11 mm = R (EUCAST		
non-species breakpoint)	1 (4.8%)	20	
(95.2%)			
Trimethoprim-Sulfamethoxazole (25 µg)	≥16 mm = S; ≤15 mm = R (CLSI M45Ed3)	4 (19.0%)	17
(81.0%)			
Gentamicin (10 µg)	≥13 mm = S; ≤12 mm = R (CLSI M45Ed3)	21 (100%)	0 (0%)
Imipenem (10 µg)	≥16 mm = S; ≤13 mm = R (CLSI M45Ed3 + SFM 2023)	21 (100%)	0 (0%)
Doxycycline (30 µg)	≥14 mm = S; ≤13 mm = R (BSAC 2023 / J.		
Antimicrob. Chemother.)	0 (0%)	21 (100%)	
Linezolid (30 µg)	≥21 mm = S; ≤20 mm = R (BSAC		
2023 / Antimicrob. Agents	21 (100%)	0 (0%)	
Chemother.)			
Carbenicillin (100 µg)	≥18 mm = S; ≤17 mm = R (CLSI		
M02-A8, historical			
criteria)	0 (0%)	21 (100%)	

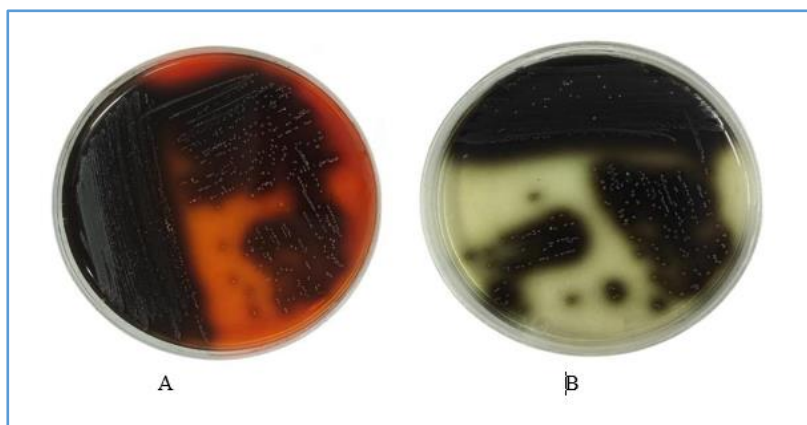


Fig. 1. Culture characters of *L. monocytogenes*, A: Grayish-green colony with black center and a black halo on Palcam media, B: Brown-green colored colonies with a black halo on Oxford Media

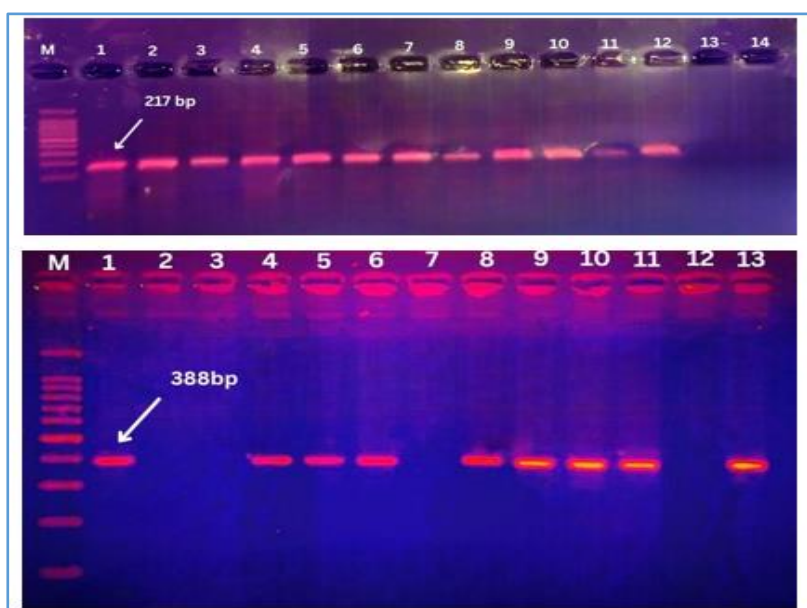


Fig. 2. Agarose gel electrophoresis showing amplification of *prfA* gene at 217bp and *hlyA* gene at 388 bp. Lane M, 100 bp DNA ladder; lane 1, positive control (*L. monocytogenes* serotype 1/2a)

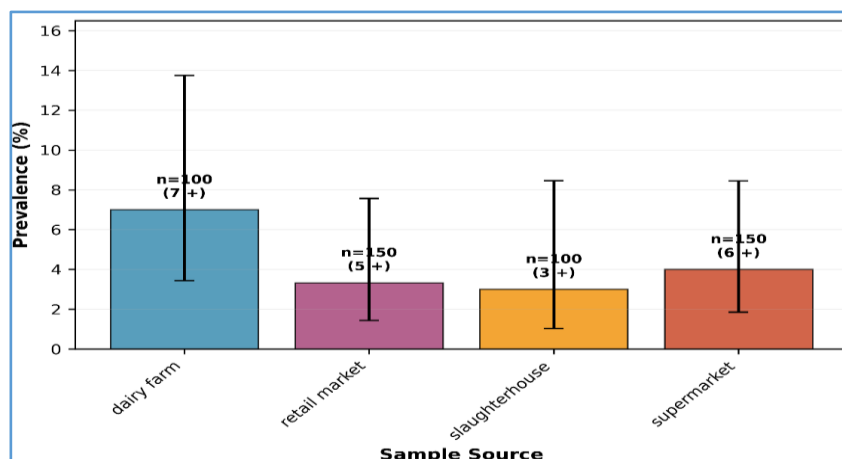


Fig. 3. Prevalence by source

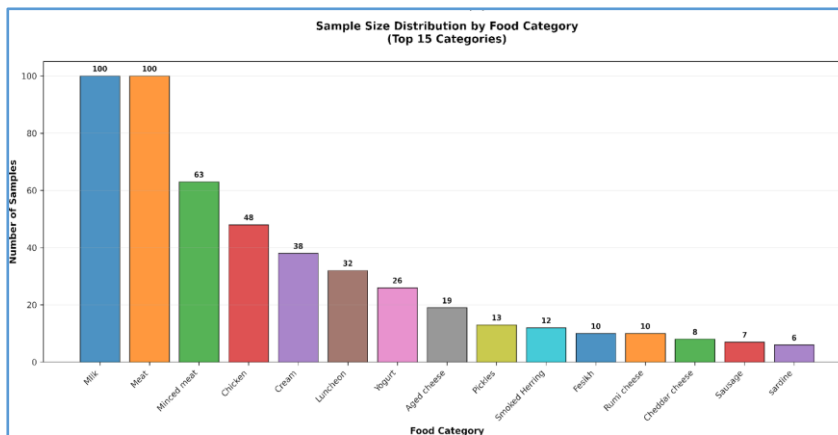


Fig. 4. Distribution of samples by food categories

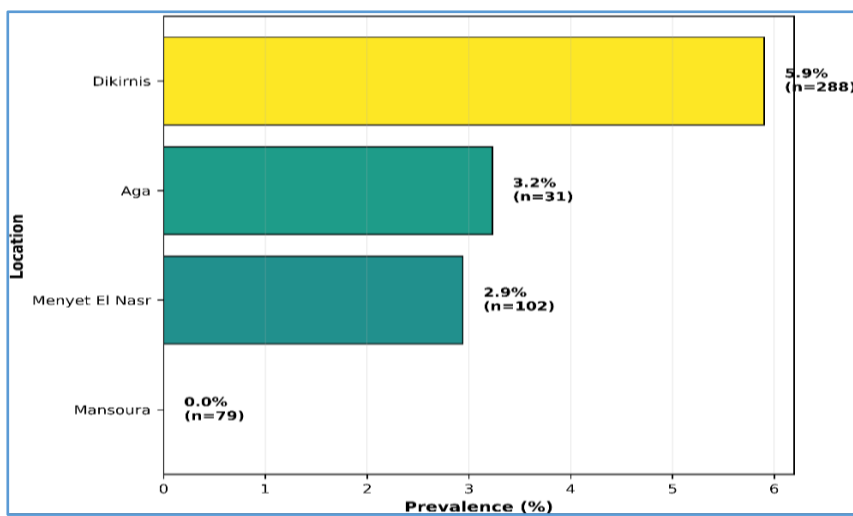


Fig. 5. Distribution of *L. monocytogenes* across locations

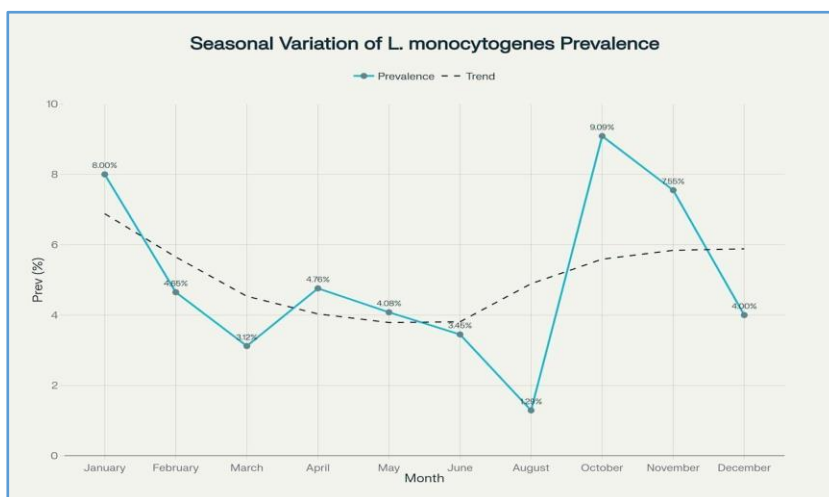


Fig. 6. Seasonal variation of *L. monocytogenes* vs global trend

References

1. Radoshevich, L. and Cossart, P. *Listeria monocytogenes*: Towards a complete picture of its physiology and pathogenesis. *Nature Reviews Microbiology*, **16**(1), 32–46 (2018).
2. Moura, A., Criscuolo, A., Pouseele, H., Maury, M. M., Leclercq, A., Tarr, C. and Brisse, S. Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. *Nature Microbiology*, **2**(2), 16185 (2016).
3. Quereda, J. J., Morón-García, A., Palacios-Gorba, C., Dessaux, C., García-del Portillo, F., Pucciarelli Morrone, M. G. and Ortega, Á. D. Pathogenicity and virulence of *Listeria monocytogenes*: From Environmental to Medical Microbiology. *Virulence*, **12**(1), 2509–2545 (2021).
4. Kayode, A. J. and Okoh, A. I. Antibiotic resistance profile of *Listeria monocytogenes* recovered from ready-to-eat foods surveyed in South Africa. *Journal of Food Protection*, **85**(12), 1807-1814 (2022).
5. Dykes, G. A. and Moorhead, S. M. Effects of osmotic pressure, acid, or cold stresses on antibiotic susceptibility of *Listeria monocytogenes*. *International Journal of Food Microbiology*, **56**(2–3), 167–173 (2000).
6. Hingston, P., Chen, J., Allen, K., Hansen, L. T. and Wang, S. Strand specific RNA-sequencing and membrane lipid profiling reveals growth phase-dependent cold stress response mechanisms in *Listeria monocytogenes*. *PLoS One*, **12**(6), e0180123 (2017).
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*. ISO (2017).
8. Dussurget, O., Pizarro-Cerdá, J. and Cossart, P. Molecular determinants of *Listeria monocytogenes* virulence. *Annual Review of Microbiology*, **58**, 587–610 (2004).
9. Walker, S. J., Archer, P. and Banks, J. G. Growth of *Listeria monocytogenes* at refrigeration temperatures. *Journal of Applied Bacteriology*, **68**(2), 157–162 (1990).
10. Ferreira, V., Wiedmann, M., Teixeira, P. and Stasiewicz, M. J. *Listeria monocytogenes* persistence in food-associated environments: Epidemiology, strain characteristics, and implications for public health. *Journal of Food Protection*, **77**(1), 150–170 (2014).
11. Erdogan, H. M., Cripps, P. J. and Morgan, K. L. Optimization of a culture technique for the isolation of *Listeria monocytogenes* from faecal samples. *Journal of Veterinary Medicine, Series B: Infectious Diseases and Veterinary Public Health*, **49**(10), 502–506 (2002).
12. Schmid, B., Klumpp, J., Raimann, E., Loessner, M. J., Stephan, R. and Tasara, T. Role of cold shock proteins in growth of *Listeria monocytogenes* under cold and osmotic stress conditions. *Applied and Environmental Microbiology*, **75**(6), 1621–1627 (2009).
13. Jansen, W. T. and Kessel, M. A. *Listeria monocytogenes* ready-to-eat seafood: prevalence, subtypes, and antibiotic resistance. *International Journal of Food Microbiology*, **259**, 91–98 (2017).
14. Doyle, M. P. and Schoeni, J. L. Selective-enrichment procedure for isolation of *Listeria monocytogenes* from fecal and biologic specimens. *Applied and Environmental Microbiology*, **51**(5), 1127-1129 (1986).
15. Hanes, R. M. and Huang, Z. Investigation of antimicrobial resistance genes in *Listeria monocytogenes* from 2010 through to 2021. *International Journal of Environmental Research and Public Health*, **19**(9), 5506 (2022).
16. Ahmed, M. S. Taha, Z. M. A. and Omer, L. T. Isolation and molecular identification with resistant profile determination of *Listeria monocytogenes* from imported chicken carcasses in Duhok, Kurdistan Region, Iraq. *Journal of Pure and Applied Microbiology*, **9**, 97–103 (2015).
17. Silva, A., Almeida, G., Sousa, M., Teixeira, P. and Rodrigues, M. Antimicrobial resistance and virulence characterization of *Listeria monocytogenes* isolated from ready- to- eat foods. *BioMed Research International*, **2015**, Article 851712 (2015).
18. Kevenk, T. O. and Terzi, G. Prevalence, antimicrobial resistance and serotype distribution of *Listeria monocytogenes* isolated from raw milk and dairy products. *Journal of Food Safety*, **36**(1), 12208 (2016). DOI:10.1111/jfs.12208
19. Germini, A., Masola, A., Carnevali, P. and Others.. Simultaneous detection of *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* by multiplex PCR. *Food Control*, **20**(8), 733–738 (2009)
20. Clinical and Laboratory Standards Institute. *Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria* (3rd ed., CLSI guideline M45). Wayne, PA: CLSI (2015).
21. European Committee on Antimicrobial Susceptibility Testing. *EUCAST breakpoint tables for interpretation of MICs and zone diameters* (Version 14.0) (2023).

22. Fisher, R. A. On the interpretation of χ^2 from contingency tables, and the calculation of P. *Journal of the Royal Statistical Society*, **85**(1), 87–94 (1922).
23. Sergelidis, D., Iossifidou, E., Soultos, N., Lazou, T., Drakopoulos, G., Konstantelis, I. and Tzikas, Z. Prevalence of *Listeria monocytogenes* in ready-to-eat seafood marketed in Thessaloniki (Northern Greece). *Veterinary World*, **7**(11), 1004–1009 (2014).
24. Filiouis, G., Johansson, A., Frey, J. and Perreten, V. Prevalence, genetic diversity and antimicrobial susceptibility of *Listeria monocytogenes* isolated from open-air food markets in Greece. *Food Control*, **20**(3), 314–317 (2009).
25. Chen, M., Cheng, J., Wu, Q., Zhang, J., Chen, Y., Xue, L., Lei, T., Zeng, H., Ye, Q., Wu, S., Bai, J. and Wang, J. Occurrence, antibiotic resistance, and population diversity of *Listeria monocytogenes* isolated from fresh aquatic products in China. *Frontiers in Microbiology*, **9**, 2215 (2018).
26. Van de Merwe, C., Simpson, D. J., Qiao, N., Otto, S. J. G., Kovacevic, J., Gänzle, M. G. and McMullen, L. M. Is the persistence of *Listeria monocytogenes* in food processing facilities and its resistance to pathogen intervention linked to its phylogeny? *Applied and Environmental Microbiology*, **90**(6), e00861–24 (2024).
27. Elsayed, M. M., Elkenany, R. M., Zakaria, A. I., and Badawy, B. M. *Epidemiological study on Listeria monocytogenes* in Egyptian dairy cattle farms: Insights into genetic diversity of multi-antibiotic-resistant strains by ERIC-PCR. *Environmental Science and Pollution Research*, **29**(36), 54359–54377 (2022).
28. Nüesch-Inderbilen, M., Bloemberg, G. V., Müller, A., Stevens, M. J. A., Cernela, N., Kollöffel, B. and Stephan, R. Listeriosis caused by persistence of *Listeria monocytogenes* serotype 4b sequence type 6 in cheese production environment. *Emerging Infectious Diseases*, **27**(1), 284–288 (2021).
29. Hayes, P. S., Graves, L. M., Ajello, G. W., Swaminathan, B., Weaver, R. E., Wenger, J. D. and Broome, C. V. Comparison of cold enrichment and U.S. Department of Agriculture methods for isolating *Listeria monocytogenes* from naturally contaminated foods. *Applied and Environmental Microbiology*, **57**(8), 2109–2113 (1991).
30. Lewis, S. J. and Corry, J. E. Comparison of a cold enrichment and the FDA method for isolating *Listeria monocytogenes* and other *Listeria* spp. from ready-to-eat food on retail sale in the U.K. *International Journal of Food Microbiology*, **12**(2–3), 281–286 (1991).
31. Dykes, G. A. and Moorhead, S. M. Survival of osmotic and acid stress by *Listeria monocytogenes* strains of clinical or meat origin. *International Journal of Food Microbiology*, **56**(2–3), 161–166 (2000).
32. Kathariou, S., Gray, M. J., Hallier-Smith, V., Jones, T. R. and Wiedmann, M. *Listeria monocytogenes* in ready-to-eat foods: Emerging evidence linking to human disease clusters. *Frontiers in Microbiology*, **11**, 570420 (2020).
33. Patrick, M. E., Jackson, B. R., Winthrop, K. L., Shutt, K., Zink, D., Mahon, B. E. and Silk, B. J. Listeriosis outbreaks in the United States, 1998–2008. *Emerging Infectious Diseases*, **16**(1), 1–7 (2010).
34. Vázquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W. and Kreft, J. *Listeria monocytogenes*: Epidemiology, human disease, and control. *Microbiological Reviews*, **65**(3), 555–562 (2001).
35. Leoni, F., Leong, D., Sheppard, K., Pitt, T. and Stringer, S. C. Characterization of *Listeria monocytogenes* in the food-processing environment: Prevalence, antimicrobial resistance, and biofilm formation. *AIMS Microbiology*, **8**(2), 2085 (2022).
36. Ruggeri J, Saá Ibusquiza P, López-Cabo M, Pietzka A, Allerberger F, Rodríguez-Herrera JJ. Exploring the antibiotic resistance of *Listeria monocytogenes* in food environments - a review. *Critical Reviews in Microbiology* **50**(6), 1035–1067 (2024).
37. Vicente MF, Perez-Daz JC, Baquero F, Angel de Pedro M, Berenguer J. Penicillin-binding protein 3 of *Listeria monocytogenes* as the primary lethal target for beta-lactams. *Antimicrobial Agents and Chemotherapy* **34**(4), 539–542 (1990).
38. Wamp, Sabrina, Rosalyn Wagner, Franziska Schuler, Alexander Krüttgen, Antje Flieger, and Sven Halbedel. "A pbpB1 mutation causing β -lactam resistance in clinical *Listeria monocytogenes* isolates. *bioRxiv* 2025-04 (2025).
39. Goffin C and Ghuysen JM. Multimodular penicillin-binding proteins: an enigmatic family of orthologs and paralogs. *Microbiology and Molecular Biology Reviews* **62**(4), 1079–1093 (1998).
40. Poyart-Salmeron C, Trieu-Cuot P, Carlier C, MacGowan A, McLauchlin J and Courvalin P. Genetic basis of tetracycline resistance in clinical isolates of *Listeria monocytogenes*. *Antimicrobial Agents and Chemotherapy* **36**(2), 463–466 (1992).
41. Brown, P., Hernandez, K., Parsons, C., Chen, Y., Gould, N., DePerno, C.S., Niedermeyer, J. and Kathariou, S. Tetracycline resistance in *Listeria monocytogenes* and *L. innocua* from wild black bears (*Ursus americanus*) in the United States is mediated by novel transposable elements. *Applied and Environmental Microbiology* **89**(11), e01205–23 (2023).
42. Charpentier E, Courvalin P. Antibiotic resistance in *Listeria* spp. *Antimicrobial Agents and Chemotherapy* **43**(9), 2103–2108 (1999).
43. Romanova, N.A., Wolffs, P.F.G., Brovko, L.Y. and Griffiths, M.W. Role of efflux pumps in adaptation and resistance of *Listeria monocytogenes* to benzalkonium chloride. *Applied and environmental microbiology*, **72**(5), 3498–3503 (2006).

44. Conter M, Zanardi E, Ghidini S and Ianieri A. Investigation of antimicrobial resistance genes in *Listeria monocytogenes* from food and feed. *Microorganisms*. **9**(5), 1074 (2021).
45. Charpentier E and Courvalin P. Emergence of the trimethoprim resistance gene *dhfrD* in *Listeria monocytogenes* BM4293. *Antimicrobial Agents and Chemotherapy* **41**(5), 1134-1136 (1997).
46. Haubert L, Kroning IS, Iglesias MA, Silva WP and Motta AS. Characterization of a *Listeria monocytogenes* plasmid with antibiotic and stress resistance genes. *Microbial Genomics* **11**(1), mgen001445 (2025).
47. Yan H, Yu R, Li D, Shi L, Schwarz S, Du XD, et al. A novel multi-resistance gene cluster located on a plasmid-borne transposon in *Listeria monocytogenes*. *Journal of Antimicrobial Chemotherapy* **75**(4), 868-872 (2020).
48. Scotti, M., Han, L., Alvarez, S., Leclercq, A., Moura, A., Lecuit, M. and Vazquez-Boland, J. Epistatic control of intrinsic resistance by virulence genes in *Listeria*. *PLoS Genetics*, **14**(9), p.e1007525 (2018).
49. Parra-Flores, J., Holý, O., Bustamante, F., Lepuschitz, S., Pietzka, A., Contreras-Fernández, A., Castillo, C., Ovalle, C., Alarcón-Lavín, M.P., Cruz-Córdova, A. and Xicohtencatl-Cortes, J. Virulence and Antibiotic Resistance Genes in *Listeria monocytogenes* Strains Isolated From Ready-to-Eat Foods in Poland. *Frontiers in Microbiology* **12**, p.796040 (2022).
50. Hanes, R.M. and Huang, Z. Investigation of Antimicrobial Resistance Genes in *Listeria monocytogenes* from 2010 through to 2021. *International Journal of Environmental Research and Public Health*, **19**(9), p.5506 (2022).
51. Torres-Moitinho L, Lopes E, Silva J, Pereira JN, Gonzalez-Barron U, Pena-Rodriguez S, et al. Antimicrobial resistance of *Listeria monocytogenes* from animal foods to first- and second-line drugs in the treatment of listeriosis from 2008 to 2021: A systematic review and meta-analysis. *Computational and Mathematical Methods in Medicine*.**2022**, 1351983 (2022).
52. McCollum, J. T., Cronquist, A. B., Silk, B. J., Jackson, K. A., O'Connor, K. A., Cosgrove, S.... Jackson, B. R. Multistate outbreak of listeriosis associated with cantaloupe — United States, August–September 2011. *New England Journal of Medicine*, **369**(11), 944–953 (2013).
53. Jackson, B. R., Tarr, C., Strain, E., Jackson, K. A., Conrad, A., Carleton, H. A. and Zink, D. (2016). Implementation of nationwide real-time whole-genome sequencing to enhance listeriosis outbreak detection and investigation. *Clinical Infectious Diseases*, **63**(6), 380–386 (2016).

الرصد الشامل لبكتيريا لستيريا مونوسيتوجينيس في منتجات غذائية بمحافظة الدقهلية، مصر: الانتشار، عوامل الخطر، وآثارها على الصحة العامة

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الملخص

تُعد بكتيريا لستيريا مونوسيتوجينيس (*L. monocytogenes*) من أخطر مسببات الأمراض المنقولة عن طريق الأغذية، نظراً لصلابتها العالية في البيئة الخارجية وتأثيرها السريري الخطير، خاصة على الفئات المعرضة للخطر مثل الحوامل والأطفال حديثي الولادة وكبار السن. هدفت هذه الدراسة إلى تحديد مدى انتشار بكتيريا *L. monocytogenes* في مختلف أنواع الأغذية في محافظة الدقهلية، ومعرفة خصائصها الجزيئية ونمط مقاومتها للمضادات الحيوية، بالإضافة إلى تقييم فعالية بروتوكولات التثري البارد المتقدمة لاستخلاص البكتيريا. شملت الدراسة 500 عينة تم جمعها بشكل منهجي من الأسواق (150 عينة)، والمحلات التجارية الكبرى (150 عينة)، ومجازر الأبقار (100 عينة)، والمزارع الحليبية (100 عينة). تم عزل البكتيريا وتحديد استخدامها باستخدام بروتوكول التثري المزدوج وبروتوكول التثري البارد المتقدم. أجريت اختبارات كيميائية ونمطية وجزيئية (PCR) تستهدف جيني الخطرة الرئيسيين (*hlyA* و *prfA*). تقيمت حساسية البكتيريا للمضادات الحيوية وفقاً لبروتوكول CLSI M45، وتحللت بيانات الدراسة إحصائياً لتحديد عوامل الخطر. تم عزل *L. monocytogenes* من 4.20% من العينات (95% فاصل الثقة: 2.76-6.34%)، وكان أعلى معدل انتشار في المزارع الحليبية (7.00%) وفي فئات الأغذية عالية الخطورة مثل اللحوم المفرومة (7.94%)، والكريم (7.89%)، والزبادي (7.69%)، والحليب (7.00%). أدى استخدام بروتوكول التثري البارد إلى تحسن ملحوظ في حساسية الكشف. احتوت جميع العينات على جيني الخطرة *hlyA* و *prfA*، مما يؤكد قدرتها العالية على التسبب في المرض. ومن المثير للقلق أن العينات أظهرت مقاومة شبيهة تامة لمضادات بيتا-لاكتام، ومعدلات عالية من مقاومة متعددة الأدوية (85.7-90.5%)، مع الحفاظ على حساسية تامة لجنتايميسين وإيميبينيم و لينزوليد. تُسلط هذه الدراسة الضوء على التهديد المُلح للصحة العامة الذي تُشكله بكتيريا الليستيريا المستوحدة المقاومة للأدوية المتعددة في المنتجات الغذائية المصرية. وتؤكد النتائج على ضرورة المراقبة الجزيئية المتقدمة، والإدارة الصارمة لمضادات الميكروبات، وتدخلات الصحة الواحدة المُتكاملة لحماية سلامة الغذاء والصحة العامة.

الكلمات الدالة: الليستيريا المستوحدة، مقاومة مضادات الميكروبات، جينات الضراوة، سلامة الغذاء، الصحة الواحدة.