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Comprehensive phenotypic, MALDI-TOF, and molecular characterization of *Listeria monocytogenes* isolated from sheep with reproductive and neurological disorders in Egypt

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ABSTRACT

Listeria monocytogenes (*L. monocytogenes*) is a significant cause of abortion and neurological disorders in sheep, leading to substantial economic losses in livestock production. This study aimed to characterize *L. monocytogenes* isolated from sheep with reproductive and neurological disorders by assessing their antimicrobial resistance and virulence gene profiles using phenotypic, proteomic, and molecular approaches. Seventy clinical specimens (n=70) were obtained from sheep, including 40 placental tissues/uterine fluids and 30 brain abscesses. The overall prevalence was 14.28%, with slightly higher rates in placental tissues (15%) and brain abscesses (13.33%). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) detected ten isolates as *L. monocytogenes*. Polymerase chain reaction (PCR) confirmed that all strains carried the *hly*A and *inl*A genes linked to virulence and the ability to invade host cells. Antimicrobial susceptibility testing showed complete resistance to cefotaxime (100%) and varying levels of resistance to fosfomycin (60%), gentamicin (40%), ciprofloxacin (30%), and meropenem (10%). However, all strains were susceptible to amoxicillin-clavulanic acid, levofloxacin, amikacin, doxycycline, and linezolid. No multidrug-resistant (MDR) strains were detected for the used antibiotics. These findings emphasize the importance of continued surveillance of *L. monocytogenes* and prudent antibiotic use in sheep farming systems.

1. INTRODUCTION

Listeria monocytogenes poses a serious One Health threat due to its zoonotic potential, environmental persistence, and significant burden on both public health and veterinary medicine (Jibo et al., 2022; Ravindhiran et al., 2023; Končurat and Sukalić, 2024). *L. monocytogenes* is a Gram-positive, facultative intracellular, non-capsulated, non-spore-forming, and rod-shaped bacterium. It is motile at 24–28 °C but becomes non-motile above 30 °C. (Ravindhiran et al., 2023). It belongs to the genus *Listeria,* which comprises 31 species and eight subspecies (Wareth and Neubauer, 2025). It is well adapted to harsh environmental conditions, such as growing at refrigeration temperatures, in acidic pH, and in high salt concentrations. Moreover, its ability to form biofilms enables persistent colonization in diverse environments such as silage, soil, feces, water, and food-processing facilities. (Osek et al., 2022)

In sheep, listeriosis is commonly caused by consuming poorly fermented silage with a pH >5.0, known as "silage disease." The disease is more prevalent during colder weather, especially in winter or early spring (WOAH, 2023; Mahmood and Al-Gburi, 2024). Sheep can either remain asymptomatic carriers or develop clinical listeriosis. As silent reservoirs, they eliminate it via feces and milk, which aids the survival of the pathogen on the farm and increases the risk of indirect human infection (Palacios-Gorba et al., 2023; Tola, 2024). When clinical disease develops, it is usually presented as abortion, encephalitis, and septicemia. It also causes high mortality rates ranging from 20% to 100%, depending on the severity (Chalenko et al., 2022). Abortion affects up to 20% of ewes, and meningoencephalitis occurs in 10–30% of cases, causing significant economic losses due to reduced productivity (Končurat and Sukalić, 2024).

Listeriosis may occur sporadically or as outbreaks (Schoder et al., 2023). Such outbreaks have been reported in various regions, including Uruguay, where three incidents affected 14 sheep (Matto et al., 2023), and Punjab, India, where a neurological outbreak involved 90 sheep and 16 goats (Mahajan et al., 2020). Human outbreaks have also been linked to consuming contaminated animal products. (Wareth and Neubauer, 2025)

Furthermore, the past few decades have shown a growing antimicrobial resistance trend in *L. monocytogenes* worldwide. Although the bacterium remains sensitive to most Gram-positive antibiotics, such as ampicillin and amoxicillin, resistance has increased due to the uncontrolled use of antibiotics in agriculture, veterinary medicine, and human healthcare. (Elbar et al., 2020; Karakaya et al., 2025) The rise of multidrug resistance is evident in regional data. In Iran, 59.09% of isolates were reported (Rezai et al., 2018). In Egypt, rates were 37.5% in Mansoura (Elbar et al., 2020), 64.9% in Sharkia, Giza, and Qaliubia (Elsayed et al., 2022), and 86.9% in the New Valley and Beheira Governorates (Sotohy et al., 2024).

Listeria monocytogenes in clinical samples can be diagnosed by culturing or molecular detection using PCR. In contrast to conventional phenotypic methodologies employed for microbial identification, MALDI-TOF-MS decreases the time by an average of 1.45 days. MALDI-TOF MS has proven to be a reliable, rapid, and cost-effective tool in routine clinical microbiology laboratories. However, it is limited in its ability to detect virulence or resistance genes.

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(Calderaro and Chezzi, 2024; Hosoda et al., 2025) These virulence genes are commonly detected using PCR (Chalenko et al., 2022).

Listeria monocytogenes survives and multiplies within host cells to induce listeriosis. It is driven by key virulence factors including *hly*A, *inl*A, *iap, ars*D, and *SSI*-1 (Karakaya et al., 2025) The *hly*A gene encodes listeriolysin O, a protein that facilitates intracellular survival and dissemination. The *inl*A and *inl*B genes are responsible for host cell invasion and brain colonization. Genes like *iap*, *arsD*, and *SSI*-1 contribute to adhesion, biofilm formation, and resistance (Končurat and Sukalić, 2024). Despite extensive global research on *L. monocytogenes*, there is limited data on its presence in Egyptian livestock. Therefore, this study aimed to perform a comprehensive phenotypic, MALDI-TOF MS, and molecular characterization of *L. monocytogenes* isolated from sheep exhibiting reproductive and neurological disorders in Sharkia and Qalyubia Governorates, Egypt.

2.MATERIAL AND METHODS

Ethical approval

The Ethical Committee of the Faculty of Veterinary Medicine at Benha University granted ethics approval for this investigation, with reference number (BUFVTM 05-09-24).

2.1. Sampling

Between May 2023 and December 2024, 70 clinical samples were collected from clinically suspected sheep in smallholder flocks and farms. Samples were selected based on recent outbreak reports and accessibility in Sharkia and Qalyubia, two major livestock-producing governorates in Egypt. They included reproductive tissues (n = 40; uterine fluid and placental tissues) from aborted ewes and brain abscesses (n = 30) from sheep exhibiting neurological symptoms. All samples were aseptically collected and transported at 4 °C to the Microbiology Laboratory, Faculty of Veterinary Medicine, Benha University, for bacteriological and molecular analyses.

2.2. Isolation and phenotypic confirmation of L. monocytogenes

2.2.1. Bacteriological isolation of *L. monocytogenes*

Listeria species were isolated following the described protocols (Sotohy et al., 2024). Primary enrichment involved inoculating 25 g or 25 mL of each sample in 225 mL of Listeria Enrichment Broth (HiMedia, India). The samples were homogenized for 30 seconds using a stomacher and then incubated at 30°C for 48 hours. After the enrichment step, aliquots were streaked onto Listeria-selective Oxford agar plates (Oxoid, England) and further incubated at 37°C for 24–48 hours. Colonies suspected to be *Listeria* from Oxford agar were further grown on HiCrome Listeria Agar (HiMedia, India) under the same conditions. The suspected colonies were purified and cultured in tryptone soya agar (HiMedia, India) supplemented with 6% yeast extract. This medium was incubated at 37°C for 24 hours, after which the colonies were maintained at 4°C for further morphological and biochemical analysis.

2.2.2. Morphological and biochemical identification of L. monocytogenes isolates

Phenotypic identification of *L. monocytogenes* was confirmed using Gram staining, catalase, oxidase testing, motility assays (wet mount and semi-solid agar at 25 °C and 37 °C), sugar fermentation (mannitol, rhamnose, xylose), the CAMP(Christie-Atkins-Munch-Petersen) test, and hemolysis on 5% sheep blood agar, following protocols established by WOAH (2023).

2.3. Proteomic identification of L. monocytogenes isolates using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS)

For species-level identification using MALDI-TOF MS, ten pure bacterial isolates were collected using transport swabs (VWR® M40 Transystem, Italy) and transferred under strict temperature-controlled conditions to the Friedrich–Loeffler-Institute (Jena, Germany). As soon as the transport swabs arrived, the samples were inoculated onto blood agar plates (Oxoid, UK) containing 7.5% defibrinated blood. Then, these plates were incubated at 37°C in a 5% CO_2 atmosphere for 24-48 hours to recover and isolate viable colonies. Fresh colonies from each isolate were individually suspended in 300 µL HPLC-grade water in 1.5 mL microcentrifuge tubes, which were then vortexed to create a homogeneous suspension. To inactivate the bacterial cell's enzymes while keeping the proteins intact, 900 µL of 100% ethanol was added, which also required further vortexing to mix well. The suspension underwent centrifugation at 11,000 × g for 2 min, and the resultant pellet was air-dried to remove any leftover ethanol. Each pellet was resuspended in 50 µL of a mixture of 70% formic acid and 50 µL acetonitrile aimed at lysing the cells and solubilizing proteins. The suspension was sonicated on ice for 1 minute at 100% amplitude and a 1.0 duty cycle and then centrifuged at 11,290 × g for 5 minutes. The obtained clear supernatant containing soluble proteins was used for MALDI-TOF MS analysis. One microliter was applied in duplicate from each protein extract to a polished steel MSP 96 target plate (Bruker Daltonik, Bremen, Germany), which was then air-dried. Each dried spot received 1 µL of saturated α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution containing 50% acetonitrile and 25% trifluoroacetic acid, which was allowed to dry. Spectra were acquired using a Microflex LT mass spectrometer (Bruker Daltonics, Bremen, Germany) and processed with MBT Compass Explorer software version 4.1. Identification used the manufacturer's log(score) algorithm. The spectral reference database used for species-level identification is internally generated by the Friedrich–Loeffler-Institute. Only isolates with a score of ≥2.300 were deemed confidently identified at the species level. Isolates with lower scores were excluded from further analysis per the protocol established by Khater et al. (2021).

2.4. Antimicrobial susceptibility testing for L. monocytogenes strains

The Kirby–Bauer disk diffusion method was employed to determine the antimicrobial susceptibility of the ten isolates of *L. monocytogenes* (n = 10) obtained in this study. Following the Clinical and Laboratory Standards Institute (CLSI) guidelines, isolate suspensions were prepared to meet the 0.5 McFarland turbidity standard (~$1.5 \times 10^{\wedge 8}$ CFU/ml) and streaked onto Mueller-Hinton agar plates (HiMedia, India). Twelve antibiotic disks (Biogram, India) representing ten different antimicrobial classes were used, including amoxicillin-clavulanic acid (AMC, 20/10 µg), levofloxacin (LEVO, 5 µg), clarithromycin (CLR, 15 µg), vancomycin (VA, 30 µg), amikacin (AK, 30 µg), gentamicin (GEN, 10 µg), ciprofloxacin (CIP, 5 µg), doxycycline (DO, 30 µg), fosfomycin (F, 50 µg), cefotaxime (CTX, 30 µg), meropenem (MEM, 10 µg), and linezolid (LZD, 30 µg). Incubation of the plates was conducted at 37 °C for 24 hours. In the absence of established CLSI breakpoints for *L. monocytogenes*, zone diameter interpretations were based on the criteria for

Staphylococcus spp., as recommended in the CLSI Performance Standards for Antimicrobial Susceptibility Testing, 2025, and Tsitsos et al. (2025).

2.5. Molecular identification of L. monocytogenes virulence genes

2.5.1. Genomic DNA extraction

Genomic DNA was extracted from *L. monocytogenes* strains using the GF-1 Bacterial DNA Extraction Kit (Vivantis Technologies, Malaysia) according to the manufacturer's protocol for Gram-positive organisms. In addition, the *L. monocytogenes* reference strain (Accession No. KR812472) was used as a positive control, while nuclease-free water was used as a negative control.

*2.5.2. PCR-based detection and visualization of hly*A *and inl*A *genes*

To assess virulence potential, all strains were screened for the presence of the *hly*A and *inl*A genes using polymerase chain reaction (PCR) following Mohamed et al. (2016). Amplification was performed using a GS-96 gradient thermocycler (Hercuvan, Malaysia), with a 25 µL reaction mixture containing 0.75 µL (10.00 µM) of each primer (Vivantis Technologies, Malaysia), 9.00 µL of deionized distilled water, 12.50 µL of Master Mix (Willofort Co. Ltd., London, UK), and 2.00 µL of extracted DNA. Each PCR run included both positive and negative controls, and the primer sequences and thermal cycling conditions are detailed in Tables 1 and 2. The resulting amplicons were separated by electrophoresis on a 1.5% agarose gel in 1X TBE buffer containing ethidium bromide (0.5 µg/mL), followed by electrophoresis for 30 minutes at 130 volts. DNA bands were visualized under UV light and imaged using a gel documentation system (Bio-Rad, USA).

Table 1. Primer sequences and expected amplicon sizes for PCR amplification of *L. monocytogenes hly*A and *inl*A genes.

Gene	Primer sequence (5´-3´)	Amplicon size (bp)	Reference
*hly*A	F: CGGAGG TTCC GCAAAAGATG R: CCTCCAGAGTGATCGATGTT	234 bp	(Furrer et al., 1991)
*inl*A	F: ACG AGT AAC GGG ACA AAT GC R: CCC GAC AGT GGT GCTAGA TT	800 bp	(Liu et al., 2007)

Table 2. PCR cycling parameters for amplification of *L. monocytogenes hly*A and *inl*A genes

Gene	Initial Denat.	Denaturation	Annealing	Extension	Final Exten.	Cycles
*hly*A	94˚C 4 min	94˚C 30 sec	55˚C 45 sec	72˚C 45 sec	72˚C 10 min	35
*inl*A	94˚C 4 min	94˚C 30 sec	52˚C 1 min	72˚C 1 min	72˚C 10 min	35

2.6. Statistical Analysis

Fisher's exact test was used to compare the detection rates of *L. monocytogenes* among the various sample types. This test was selected due to the moderate overall sample size and the relatively low number of positive cases. The analysis used the free GraphPad QuickCalcs tool (GraphPad Software, San Diego, CA; https://www.graphpad.com/quickcalcs/contingency1.

A statistically significant p-value was defined as a value less than 0.05.

3. RESULTS

3.1. Phenotypic and biochemical characterization of *L. monocytogenes* isolates

Colonies of *L. monocytogenes* appeared as gray to black with black halos on Oxford agar due to esculin hydrolysis and bluish-green with halo zones on HiCrome Listeria agar, indicative of lecithinase activity. All isolates were Gram-positive short rods arranged in characteristic "Chinese letter" patterns and were catalase positive and oxidase negative. Motility testing revealed positive tumbling motility at 25°C, while no motility was observed at 37°C. In semi-solid motility agar incubated at 25°C, all isolates demonstrated diffuse growth with a distinct umbrella-shaped pattern.

On 5% sheep blood agar, colonies exhibited narrow zones of β-hemolysis. The CAMP test showed enhanced β-hemolysis with *Staphylococcus aureus*. Carbohydrate fermentation tests indicated acid production from rhamnose, with no fermentation of mannitol or xylose. This fermentation profile supports species-level identification of *L. monocytogenes*, distinguishing it from other *Listeria* species.

3.2. Proteomic identification of *L. monocytogenes* using MALDI-TOF MS

All Ten isolates were identified as *L. monocytogenes*. each with an identification score of ≥2.300, indicating high-confidence species-level matches.

3.3. Prevalence of *L. monocytogenes* in sheep samples

Listeria monocytogenes was detected in 6 of 40 uterine/placental samples (15.0%) and 4 of 30 brain abscess samples (13.3%). Overall, 10 of 70 samples (14.3%) were positive (Table 3). In addition, Fisher's exact test revealed no statistically significant difference in prevalence between the two sample types (p = 1.00).

Table 3: Isolation and prevalence of *L. monocytogenes* from different clinical sheep samples.

Sample type	Collected Samples (n)	Positive Isolates (n)	Prevalence (%)
Uterine fluid/Placenta	40	6	15.00%
Brain Abscess	30	4	13.33%
Total	70	10	14.28%

3.4. Antimicrobial resistance profile of *L. monocytogenes* strains.

All ten *L. monocytogenes* strains were 100% susceptible to levofloxacin, linezolid, amikacin, doxycycline, amoxicillin-clavulanic acid, clarithromycin, and vancomycin. The resistance was observed to cefotaxime (100%), fosfomycin (60%), gentamicin (40%), ciprofloxacin (30%), and meropenem (10%) (Figure 1). Antimicrobial resistance was detected in 4/6 (66.7%) strains from farm-reared sheep and 2/4 (50%) from street-raised sheep. Nevertheless, no multidrug-resistant (MDR) strains were found for these antibiotics.

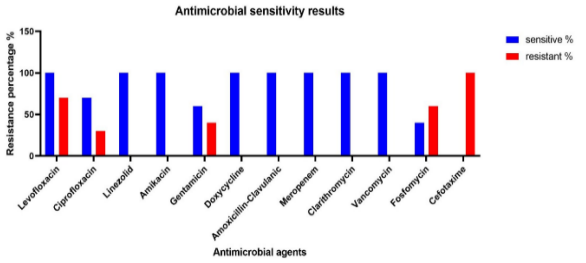

Figure 1: The antibiogram susceptibility of *L. monocytogenes* strains (n=10) showing sensitivity and resistance to the 12 tested antimicrobials.

3.4. Detection of *L. monocytogenes* virulence genes by PCR

All *L. monocytogenes* strains (n=10) were positive for both *hly*A and *inl*A virulence genes. (Figures 2a, b)

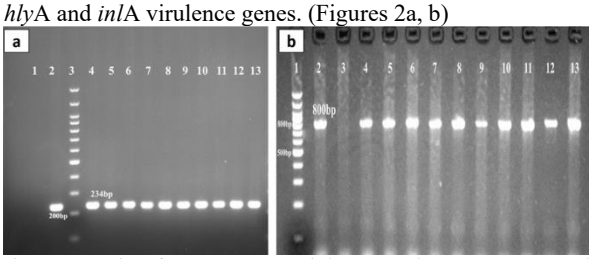

Figure 2: Detection of *L. monocytogenes* virulence genes by PCR: a. Agarose gel electrophoresis of the *hly*A gene (234 bp). Lane 1: negative control; Lane 2: positive control strain (*L. monocytogenes*, accession number: KR812472); Lane 3: 100 bp DNA ladder; Lanes 4–13: isolates showing specific *hly*A amplification; b. Agarose gel electrophoresis of the *inl*A gene (800 bp). Lane 1: 100 bp DNA ladder; Lane 2: positive control strain (*L. monocytogenes*, accession number: KR812472); Lane 3: negative control; Lanes 4–13: strains yielding specific *inl*A amplicons.

4. DISCUSSION

Listeria monocytogenes is a zoonotic pathogen with serious public health implications for animals and humans. This pathogen in sheep is known to cause significant reproductive and neurological disorders with high mortality, which leads to substantial economic losses. Moreover, *L. monocytogenes* can cause invasive listeriosis in humans after consuming the contaminated animal products (Samir et al., 2021; Wareth and Neubauer, 2025). The pathogen's survival in silage, refrigeration, and acidic environments leads to several global outbreaks (Elsayed et al., 2022). As global listeriosis cases rise, pressing priorities within the One Health framework include determining the prevalence of *L. monocytogenes* and enhancing rapid diagnostics in small ruminants. (Wareth and Neubauer, 2025).In this study, *L. monocytogenes* recovered from 10 out of 70, including 6 of 40 uterine/placental samples (15.0%) and 4 of 30 brain abscess samples (13.3%) from clinically suspected cases in sheep. While the detection rates were slightly different, statistical analysis showed no significant difference between them ($p = 1.00$), indicating the importance of the *L. monocytogenes* bacterium in both clinical conditions. The isolates demonstrated features characteristic of *L. monocytogenes*, which included colony morphology on Oxford and HiChrome *L. monocytogenes* agars, Gram-positive short rods on microscopy, and umbrella-shaped motility at 25°C. All isolates were positive for the CAMP reaction and showed the expected fermentation pattern of glucose- and rhamnose-positive and xylose-negative (Elbar et al., 2020; Samir et al., 2021). Both MALDI-TOF MS and PCR further confirmed identification. While MALDI-TOF MS is a faster and more affordable method for initial detection of *L. monocytogenes*, its accuracy can be limited by database quality, low biomass, mixed cultures, and difficulty distinguishing closely related species (Rychert, 2019; Han, Jeong, and Choi, 2021; Calderaro and Chezzi, 2024; Hosoda et al., 2025). The overall prevalence of *L. monocytogenes* in clinically affected sheep in Sharqia and Qalyubia governorates was 14.28%. It is higher than previously documented in Egypt, which was 13.5% in mixed samples from Sharqia, Qalyubia, and Giza (Elsayed et al., 2022); 12.9% in Zagazig (Ezzat et al., 2022); 8% in Damanhour (Farag et al., 2021); and 3.39% in Dakahlia (Elbar et al., 2020). Nevertheless, it was less than 36.5% in Dakahlia (El Sawaak et al., 2016). In reproductive tissues, the prevalence rate was 15%, which exceeds the previously documented rates in Egypt of 13% (Elsayed et al., 2022), 8% (Samir et al., 2021), and 2.8% in Turkey (Gülaydın et al., 2023). This increased prevalence might be explained by the immunosuppressive effects of pregnancy and the placental tropism of *L. monocytogenes* (Eallonardo and Freitag, 2024). In brain tissues, the prevalence was 13.33%, which is lower than in Iran, 57% (Esmaeili and Joghataei, 2024), and India, 42% (Mahajan et al., 2020), but higher than that in Mansoura, 5.56% (Elbar et al., 2020). Variations in prevalence rates may be attributable to the animals' immune status, the strain's virulence, the use of silage, the season, the geographical region, and the sample size. (Mahajan et al., 2020; Wareth and Neubauer, 2025). Antimicrobial susceptibility testing revealed a concerning resistance pattern. Strains showed complete resistance (100%) to cefotaxime, moderate resistance to fosfomycin (60%), gentamicin (40%), ciprofloxacin (30%), and meropenem (10%). However, all strains remained sensitive to levofloxacin, linezolid, amikacin, doxycycline, amoxicillin-clavulanic acid, clarithromycin, and vancomycin. Antimicrobial resistance was identified in 66.7% of strains from farm-reared sheep and 50% from street-raised sheep. This higher prevalence among farm animals may be associated with more frequent or prolonged antimicrobial use in farm management practices. (Elbar et al., 2020; Končurat and Sukalić, 2024). The intrinsic resistance of *L. monocytogenes* to third-generation cephalosporins like cefotaxime is caused by low-affinity penicillin-binding proteins (PBPs) and efflux mechanisms (Andriyanov et al., 2021; Kim et al., 2023). The 30% ciprofloxacin resistance observed in this study is consistent with findings from Korea (Kim et al., 2023). Still, it exceeds rates reported in Mansoura (0%) (Elbar et al., 2020), Italy (24%) (Caruso et al., 2020), Russia (2.5%) (Andriyanov et al., 2021), and Iraq (0%) (Mahmood and Al-Gburi, 2024). However, it remains lower than reported rates in Iran (38.37%) (Rezai et al., 2018) and Egypt (67.5%) (Elsayed et al., 2022). Ciprofloxacin resistance is often associated with overexpression of efflux pumps or mutations in *gyrA/parC* genes, which can vary based on regional antimicrobial use practices (Kim et al., 2023). Gentamicin resistance (40%) was higher than that observed in previous studies (Rezai et al., 2018; Andriyanov et al., 2021; Mahmood and Al-Gburi, 2024), but lower than the 81% resistance reported in Egypt (Elsayed et al., 2022), which reveals the excessive use of gentamicin in veterinary practice. Gentamicin is frequently employed with penicillin or ampicillin for its synergistic activity in listeriosis treatment (Andriyanov et al., 2021). Fosfomycin resistance (60%) aligns with global reports on increasing intrinsic resistance mediated by *fos*X genes (Končurat and Sukalić, 2024). Meropenem resistance (10%) was an unexpected finding, as carbapenem resistance is rare in *L. monocytogenes*. This resistance may be linked to mutations in penicillin-binding proteins (PBPs), which can confer resistance to carbapenems while preserving susceptibility to other β-lactams (Andriyanov et al., 2021). Despite emerging resistance patterns, no multidrug-resistant (MDR) strains were detected in this study, which contrasts with higher MDR rates reported in other regions, including Egypt, 37.5% (Elbar et al., 2020), and Iran, 59.09% (Rezai et al., 2018). This fluctuation may indicate variations in the rate of horizontal gene transfer and the use of antibiotics in various agricultural sectors. Nevertheless, it is crucial to maintain ongoing surveillance to prevent the spread of resistance. (Kim et al., 2023). Molecular analysis confirmed all strains had the *hly*A and *inl*A virulence genes. Listeriolysin O, which is encoded by the *hly*A gene, facilitates intracellular survival and dissemination (Elsayed et al., 2022). The *inl*A gene encodes a protein called internalin A, which aids in the invasion of epithelial cells and the crossing of the intestinal, placental, and blood-brain barriers (Hong et al., 2024). The presence of both *hly*A and *inl*A genes aligned with Elbar et al. (2020) and Elsayed et al. (2022). Nevertheless, the presence of the *hly*A gene in some strains has also been reported (El Sawaak et al., 2016; Elafify et al., 2022). *L. monocytogenes* strains may exhibit genetic variation because of gene loss or acquisition. (El Sawaak et al., 2016; Tsitsos et al., 2025) The presence of both antibiotic resistance and virulence factors complicates the management and treatment of infections. This study provides valuable preliminary data on *L. monocytogenes* in Egypt, but certain limitations should be acknowledged. The relatively small sample size, focus on symptomatic cases, and restriction to Qalyubia and Sharkia Governorates may limit broader generalizability. Molecular analysis was also limited to *hly*A and *inl*A, which may not reflect the full virulence profile. Despite these constraints, the findings provide the basis for future nationwide studies with expanded sampling, including asymptomatic cases, and deeper genomic analysis to improve our understanding of the *L. monocytogenes* virulence, surveillance, and control efforts.

5. CONCLUSIONS

This study emphasizes the significance of *Listeria monocytogenes* in abortive and neurologically affected sheep. The presence of *hly*A and *inl*A virulence genes in all strains supports their invasive potential and ability to cause outbreaks. The emergence of resistance to gentamicin and meropenem may reflect the impact of excessive use of antimicrobials in farm settings. In contrast, susceptibility to amoxicillin-clavulanic acid remains an effective therapeutic option. MALDI-TOF MS is a valuable diagnostic tool for rapidly and accurately identifying *L. monocytogenes* in veterinary practices. Future studies should focus on broader epidemiological investigations and expanded virulence gene profiling, including *iap*, *act*A, and *prf*A. Advanced molecular typing techniques such as multilocus sequence typing (MLST) and whole genome sequencing are recommended to understand strain diversity and resistance mechanisms better.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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