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Multidrug-Resistant Strain of *Listeria monocytogenes* Infection in the African Catfish (*Clarias gariepinus* L.): Biological Infection, Clinical Lesions, and Tissue Architecture Alteration

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ABSTRACT

One of the main challenges in aquaculture is bacterial infections, which cause substantial fish mortality worldwide. In this study, a multidrug-resistant strain of *Listeria monocytogenes* (PQ524490) was isolated from *Clarias gariepinus* collected from various fish markets in Egypt. For the *in vivo* experiment, *C. gariepinus* specimens were randomly divided into three equal groups, each with three replicates of 10 fish. The first group was intraperitoneally (I/P) injected with 0.1mL of *L. monocytogenes* PQ524490 at a concentration of 2.0 × 10^8 CFU/mL. The second group served as a negative control and was injected with 0.1mL of sterile saline. The third group served as an untreated control, receiving only an artificial diet without any injection. Infection with *L. monocytogenes* PQ524490 significantly impaired ($P< 0.05$) hepatorenal function, nonspecific immunity, and antioxidant activity compared with the control groups. Histological examination further confirmed these findings, revealing pronounced tissue damage in the liver, kidney, and spleen of the infected fish.

INTRODUCTION

Globally, aquaculture suffers significant financial losses each year due to outbreaks of bacterial, fungal, parasitic, and viral diseases that can occur at any stage of the production cycle **(Dy *et al*., 2018)**. *Listeria monocytogenes* (*L. monocytogenes*) is a Gram-positive rod belonging to the class Bacilli, family Listeriaceae, and genus *Listeria* **(McLauchlin *et al*., 2014)**. It is a facultative anaerobe, motile, non-spore-forming, catalase-positive, and oxidase-negative bacterium. Importantly, it exhibits beta-hemolytic activity, which leads to the breakdown of red blood cells and the onset of listeriosis **(Yehia *et al*., 2016)**.

Clarias gariepinus is among the most important aquaculture species worldwide, second only to tilapia in terms of production volume, and represents the most inexpensive

fish in Egypt **(Heikal *et al*., 2024)**. Previous studies have reported that *L. monocytogenes* was detected in *C. gariepinus* at a prevalence rate of 8%, compared with only 5% in fresh tilapia **(Saleh *et al*., 2024)**. This higher occurrence is likely linked to the bottom-feeding habits of *C. gariepinus*, which increase exposure to sediment-associated contaminants. The species is also well known for its scavenging behavior and strong environmental adaptability, particularly in polluted waters with low oxygen availability. As a result, *C. gariepinus* can survive in sewage-rich habitats, making it more vulnerable to infection by harmful bacteria such as *L. monocytogenes* **(Saleh *et al*., 2024)**.

The World Health Organization (WHO) has classified multidrug-resistant (MDR) bacteria, including *L. monocytogenes*, as priority pathogens requiring urgent attention and the development of alternative treatment strategies **(WHO, 2017)**. With antibiotics increasingly losing their effectiveness, coupled with stricter regulations and rising social concerns, the cost of maintaining healthy aquaculture systems has already grown significantly and is expected to increase further if effective alternatives are not implemented. Finfish diseases are estimated to cause annual losses between US$1 and 9.6 billion, representing 1–10% of the total global value of finfish aquaculture production **(FAO, 2016)**.

The present study therefore aimed to evaluate the prevalence and potential risks of *L. monocytogenes* infection in commercially marketed *C. gariepinus* in Egypt. In addition, it sought to assess the immunological, pathological, and behavioral responses of infected fish, thereby contributing to the limited body of research on this emerging aquaculture pathogen.

MATERIALS AND METHODS

Listeria monocytogenes isolation and identification

A total of 100 live, naturally infected African catfish (*Clarias gariepinus*) were collected from a private fish farm in Abbassa and various fish markets in Alsharqia Governorate, Egypt. The fish were immediately transported to the Laboratory of Aquatic Animal Medicine, Zagazig University, for bacteriological examination. From each fish, 25g of internal organs (liver, kidney, and spleen) were homogenized in 225 mL of Listeria-specific broth base (HiMedia, M889, India) for two minutes. Samples were enriched for *Listeria* spp. by incubation in selective broth (HiMedia, M889) supplemented with Listeria selective supplement II (HiMedia) at 37 °C for 48 h **(Anthony *et al*., 2022)**.

A loopful from the enrichment culture was streaked onto Oxford medium base (HiMedia, M1145) supplemented with Oxford Listeria supplement (Modified FD172) and incubated for 48h at 37°C. One presumptive colony from each plate was transferred to tryptone soy agar (HiMedia, M291) with 0.6% yeast extract (TM Media) and incubated

overnight at 37°C. Isolates were identified using Gram staining, catalase test, β-hemolytic activity, motility test, and molecular characterization via 16S rRNA sequencing **(Aygun & Pehlivanlar, 2006)**.

Phenotypic detection of antibiotic resistance

Antibiotic selection was based on their common use in human and veterinary medicine in the study region. Antimicrobial susceptibility was tested using the disc diffusion method on Mueller–Hinton agar supplemented with 5–10% sheep red blood cells, following CLSI guidelines **(CLSI, 2018)**. Ten antibiotics (Oxoid®) were tested at the following concentrations (µg/mL): chloramphenicol (30), ampicillin (10), tetracycline (30), sulfamethoxazole (25), ciprofloxacin (5), gentamicin (10), nalidixic acid (30), amoxicillin/clavulanate (20/10), erythromycin (10), and cefotaxime (30). The multiple antibiotic resistance (MAR) index was calculated for each isolate **(Singh *et al.*, 2010)**. The isolate with the highest MAR index, *L. monocytogenes* PQ524490, was selected for further analysis.

Phylogenetic analysis of *L. monocytogenes* PQ524490

The evolutionary history was inferred using the UPGMA method **(Sneath & Sokal, 1973)**. Evolutionary distances, expressed as the number of base substitutions per site, were calculated using the Maximum Composite Likelihood approach **(Tamura *et al.*, 2004)**. The percentage of replicate trees in which associated taxa clustered together was shown next to the nodes. The analysis included 10 nucleotide sequences, with codon positions set at 1st + 2nd + 3rd + noncoding. Ambiguous positions were removed by pairwise deletion, leaving a total of 11,884 positions in the final dataset. Phylogenetic analyses were performed using MEGA11 software **(Tamura *et al.*, 2021)**.

Fish and culture conditions

A total of 200 healthy *C. gariepinus* (average body weight 200 ± 0.5g) were acclimatized under the standards of the Canadian Council for Animal Care **(CCAC, 2005)**. Fish were maintained in 100-L glass aquaria containing dechlorinated tap water, with continuous aeration provided by electric compressors and air stones. They were fed a basal diet and acclimatized for two weeks prior to experimentation. Water was completely replaced every two days. Environmental parameters were maintained as follows: temperature 28.3 ± 1.1 °C, dissolved oxygen 6.18 ± 0.4 mg/L, pH 6.9 ± 0.1, and total ammonia 0.035 ± 0.01mg/ L, under a controlled 12h light:12h dark photoperiod **(APHA, 1998)**.

Biological infection and LD50 determination

The median lethal dose (LD50) of *L. monocytogenes* was determined following **Mahboub *et al.* (2024)**. A total of 110 fish were divided into five groups (20 fish each; 10 per duplicate) plus a control group. After 24h fasting, fish were injected

intraperitoneally with 0.2 mL of *L. monocytogenes* suspensions at concentrations ranging from 10^5 to 10^9 CFU/mL. The control group received 0.2mL of sterile phosphate-buffered saline. Mortality was recorded at 24, 48, 72, and 96h post-infection. LD50 was calculated using Probit analysis (SPSS v21), yielding a value of 1.0 × 10^8 CFU/mL. A sub-lethal dose of 2.0 × 10^7 CFU/mL was used for subsequent trials.

For the treatment study, after 14 days, 0.1mL of bacterial suspension (2.0 × 10^8 CFU/mL) was injected intraperitoneally into five fish per replicate (n = 15 per group). Fish were monitored for 14 days, and clinical signs and mortality were recorded.

Experimental design

A total of 90 *C. gariepinus* were randomly assigned to three equal groups, each with three replicates (10 fish per replicate). Group 1 (G1) was injected intraperitoneally with 0.1mL of *L. monocytogenes* PQ524490 (2.0 × 10^8 CFU/mL). Group 2 (G2, negative control) was injected with 0.1mL of sterile saline. Group 3 (G3, control) received only the basal diet without injection. Fish were daily monitored for 14 days for clinical signs and mortality. All procedures were approved by the Institutional Animal Care and Use Committee of Zagazig University (ZU-IACUC/1/F/206/2024) and conducted in compliance with NIH guidelines.

Sampling

Five fish from each aquarium were anesthetized with clove oil (80mg/ L; Oleum, Egypt) within three minutes **(Woody *et al.*, 2002)**. Blood was collected from the caudal vessels using sterile syringes, centrifuged at 1075 × g for 20min, and serum was stored at –20 °C. Tissue samples (liver, kidney, spleen) were collected for pathological evaluation.

Hepatorenal indicators

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined following **Sahoo *et al.* (2015)**. Serum urea and creatinine levels were analyzed according to **Coulombe & Favreau (1963)** and **Larsen (1972)**, respectively.

Antioxidant and innate immune parameters

Total antioxidant capacity (TAC) and catalase (CAT) activity were measured according to **Aebi (1984)**. Nitric oxide (NO) was determined using the method of **Montgomery and Dymock (1961)**, reduced glutathione (GSH) concentration assessed by **Beutler *et al.* (1963)**, superoxide dismutase (SOD) activity by **Nishikimi *et al.* (1972)**, and lysozyme (LYZ) activity by **Ellis (1990)**.

Histopathological evaluation

Tissues (liver, kidney, spleen) were fixed in 10% neutral-buffered formalin for 48h, dehydrated in ascending ethanol series, cleared in xylene, and embedded in paraffin.

Sections (5µm) were cut with a microtome (Leica RM 2155, UK), stained with hematoxylin and eosin, and examined under a light microscope. Photomicrographs were taken using an AmScope® digital camera attached to a Leica® microscope **(Bancroft *et al.*, 2013)**.

Statistical analysis

Data were analyzed using one-way ANOVA in SPSS v18. Post hoc comparisons were performed with Duncan's Multiple Range Test at a significance level of $P< 0.05$.

RESULTS

Isolation of *L. monocytogenes* and testing the antibiotic resistance profile

The findings revealed that *Clarias gariepinus* specimens were infested with *Listeria monocytogenes*, from which four strains were successfully isolated. The overall prevalence of *L. monocytogenes* in *C. gariepinus* was 5.71%. All four isolates exhibited complete resistance (100%) to gentamicin, whereas the highest susceptibility (100%) was recorded for ampicillin. Among these isolates, *L. monocytogenes* PQ524490 demonstrated the highest multiple antibiotic resistance (MAR) index (0.7), while isolates 2, 3, and 4 showed lower MAR values of 0.4, 0.2, and 0.4, respectively. A single colony of the *L. monocytogenes* PQ524490 isolate was subsequently streaked on Oxford Listeria selective agar medium (Fig. 1), identified, and subjected to phylogenetic analysis.

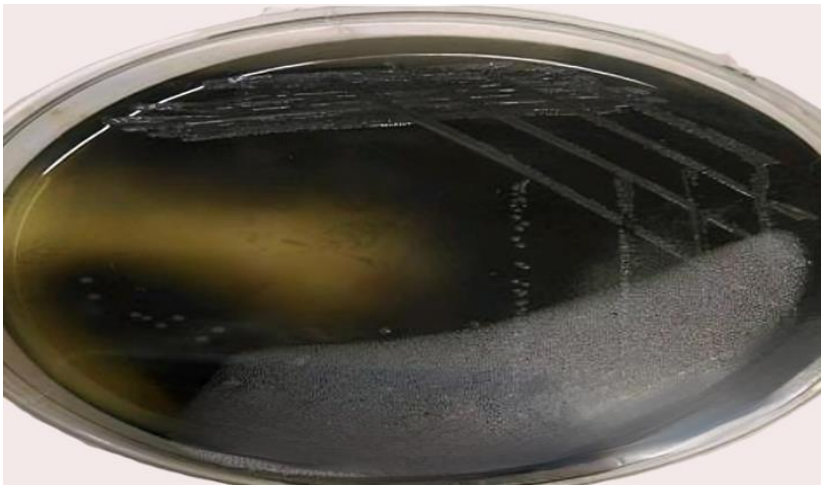

Fig. 1. *L. monocytogenes* PQ524490 colonies on Oxford agar measure being black, with a sunken center and a black halo on Oxford *Listeria* selective agar medium

Phylogenetic analysis for multi-drug-resistant *L. monocytogenes* PQ524490

L. monocytogenes PQ524490 was subjected to phylogenetic analysis. The results demonstrated that the tested isolate shared high genetic similarity with other *L. monocytogenes*

strains from diverse sources available in the GenBank database. The sequence was deposited in GenBank under the accession number PQ524490. The resulting phylogenetic tree is presented in Fig. (2).

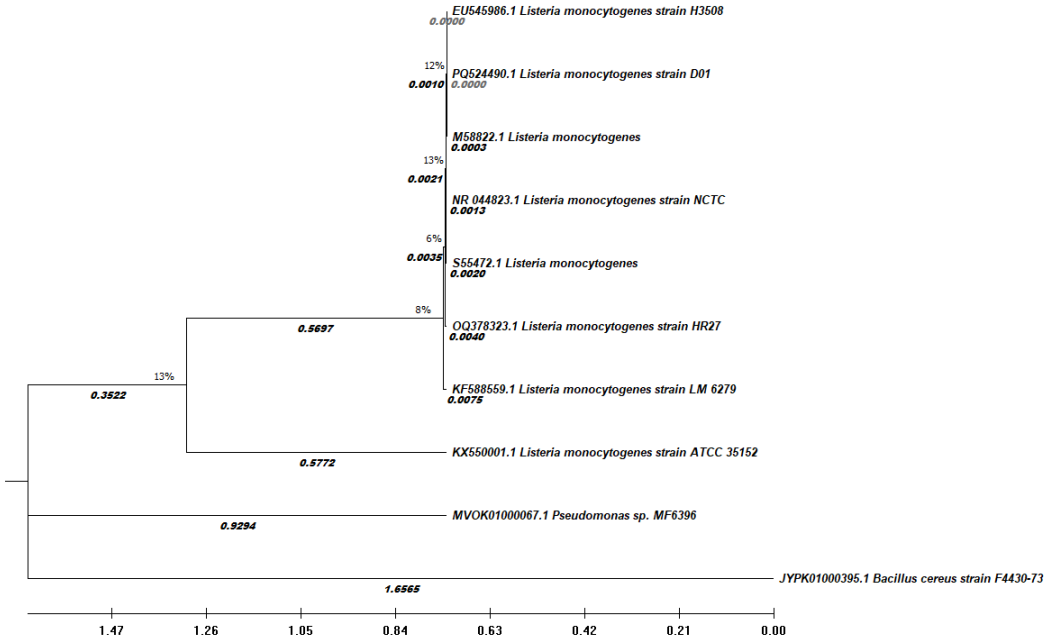

Fig. 2. Phylogenetic tree of the tested *L. monocytogenes* isolate based on the *16S rRNA* gene sequences generated via the neighbor-joining technique. Our examination indicated *L. monocytogenes* PQ524490.

Hepatorenal function indicators

Serum ALT, AST, urea, and creatinine mean values were considerably higher ($P< 0.05$) in *C. gariepinus* infected with *L. monocytogenes* PQ524490 in G1 after 14 days of the experiment compared to the control groups (Table 1).

Table 1. The effect of *L. monocytogenes* PQ524490 experimental infection on hepatorenal functions of *C. gariepinus* at the end of the 14-day experiment

Group	ALT (U/L)	AST (U/L)	CREATININE (mg-dL)	UREA (mg-dL)
G1	20.32± 1.80^{a}	274.82± 7.84^{a}	0.185± 0.003^{a}	2.03± 0.10^{a}
G2	2.31± 0.26^{b}	57.47±4.74^{b}	0.08± 0.003^{b}	0.92± 0.10^{b}
G3	2.50± 0.43^{b}	62.34± 4.24^{b}	0.09± 0.006^{b}	0.95± 0.037^{b}

Note: G1: Injected I\P with *L. monocytogenes*. **G2**: Injected I\P with sterile saline. **G3:** Control negative (neither *L. monocytogenes* nor sterile saline). **ALT:** Alanine aminotransferase enzyme. **AST:** Aspartate transferase enzyme. **$^{a, b, c, d}$:** Means with different superscripts differed significantly ($P< 0.05$) (n =90).

Antioxidant and immune indicators

Table (2) reveals a substantial decrease in G1 serum antioxidant and immunological markers after 14 days of the experiment when compared with G2 and G3 ($P< 0.05$).

Table 2. The effect of the experimental infection of *L. monocytogenes* PQ524490 on *C. gariepinus* serum antioxidant and immune parameters at the end of the 14-day experiment

Group	TAC (ng/ml)	CAT (ng/ml)	SOD (U/ml)	GSH (ng/ml)	NO (µmol/L)	LYZ (ng/ml)
G1	0.81± 0.22^{b}	0.91± 0.064^{b}	15.39± 1.38^{b}	11.59± 1.49^{b}	6.80±0.84^{b}	0.43± 0.074^{b}
G2	5.38±0.80^{a}	5.91±0.48^{a}	126.39±5.77^{a}	94.49±4.71^{a}	23.95±3.08^{a}	7.82±0.91^{a}
G3	4.89± 0.20^{a}	6.10± 0.15^{a}	131.38± 2.39^{a}	95.94± 5.35^{a}	26.25± 1.50^{a}	8.06± 0.30^{a}

Note: G1: Injected I\P with *L. monocytogenes*. **G2:** Injected I\P with sterile saline. **G3:** Control negative (neither *L. monocytogenes* nor sterile saline). **TAC:** Total antioxidant capacity. **CAT:** Catalase enzyme. **LYZ:** Lysozyme enzyme. **SOD:** Superoxide Dismutase enzyme. **GSH:** Glutathione enzyme. **$^{a, b, c, d}$:** Means with different superscripts differed significantly ($P< 0.05$) (n =90).

Clinical signs and postmortem lesions

A typical slow swimming, which caused the fish to swim to the water's surface with sluggishness, and the fish isolate themselves in the corner of the aquarium, was one of the clinical signs of *L. monocytogenes* PQ524490 infection of fish in G1. Fish stop feeding and lose their escape response. In addition to varied degrees of internal organ enlargement, congestion, and hemorrhages, fish dissection revealed severe fin rot, erythema, and hemorrhages with deep ulcers.

Histopathological investigation

The liver's G3 "control groups" (Fig. 3A) showed normal histology of hepatic acini, sinusoids, and central veins. However, G1" infected group by *L. monocytogenes* PQ524490 (Fig. 3B) revealed perivascular necrotic hepatocytes invaded by inflammatory cell infiltrates beside endotheliosis of a hepatic artery with fibrinoid necrosis within the arterial wall. The control kidney groups (Fig. 4A) exhibited normal histological configurations of renal corpuscles, renal tubules, and interstitial hematopoietic series with melanomacrophages. Congestion of renal vasculatures, hemorrhages within interstitial tissue, shrunk glomerular tufts, and destruction of some renal tubular epitheliums were demonstrated in G1 (Fig. 4B). The control groups of the spleen (Fig. 5A) showed normal histological structures of white pulp that alternated with red pulp around ellipsoidal arterioles beside randomly distributed melanomacrophages. However, G1 (Fig. 5B)

showed marked depletion of lymphoid populations that may be due to necrotic or apoptotic lymphoid elements around ellipsoidal arterioles.

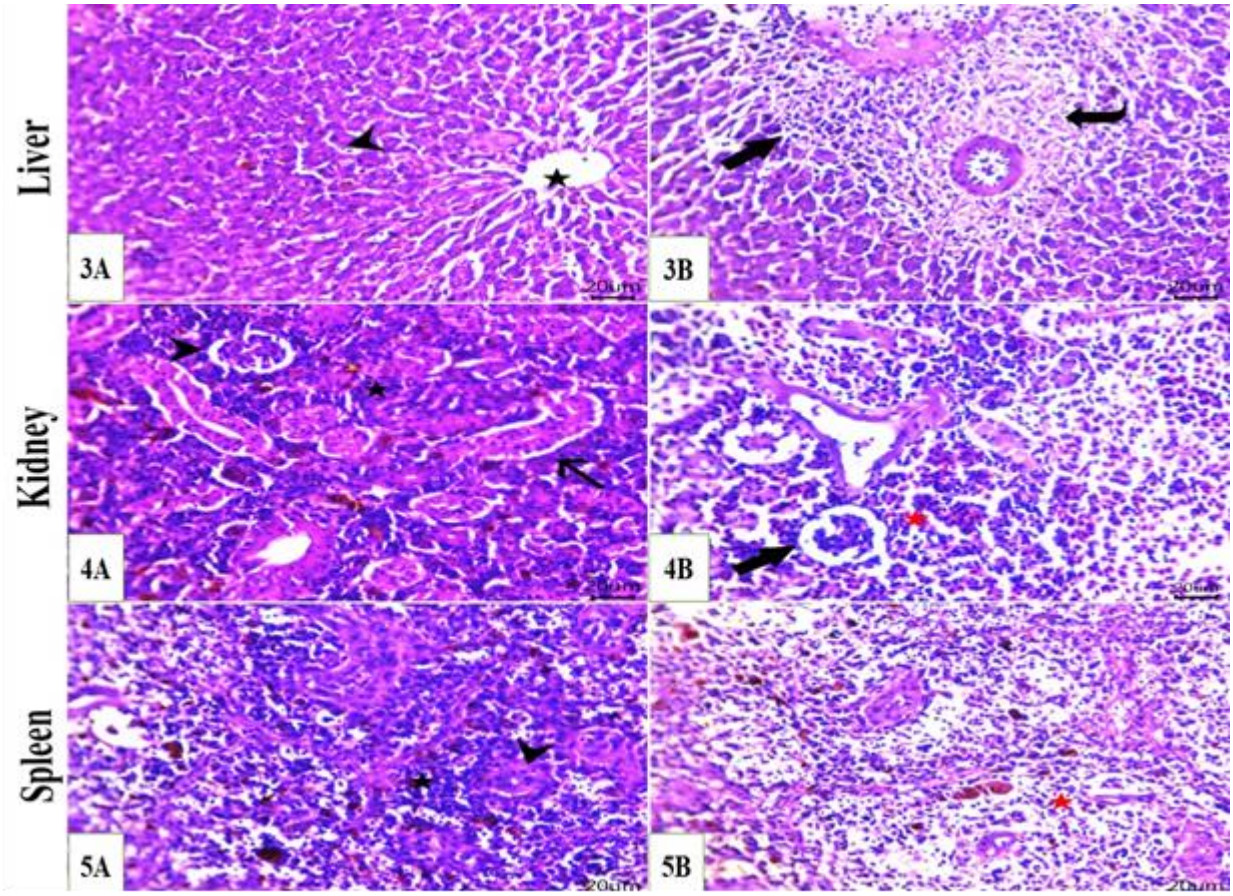

Fig. 3. Photomicrographs of H&E-stained sections of the liver (Figure 3A-3B) from *C. gariepinus* showing: **(3A)** Normal histology of hepatic acini (arrowhead), sinusoids, and central vein (star) in G3 "Control groups". **(3B)** Perivascular necrotic hepatocytes invaded by inflammatory cells infiltrates (thick arrow) beside endotheliosis of a hepatic artery with fibrinoid necrosis within an arterial wall (curved arrow) in G1 "infected groups" (Scale bar 20 µm).

Fig. 4. Photomicrographs of H&E-stained sections of the kidney from African catfish showing: **(4A)** Normal histological configurations of renal corpuscle (arrowhead), renal tubule (arrow), and interstitial hematopoietic series (star) with melanomacrophages in G3 "Control groups". **(4B)** Congestion of renal vasculatures, hemorrhages (red star) within interstitial tissue, shrunk glomerular tufts (thick arrow), and destruction of some renal tubular epithelium in G1 "infected groups" (Scale bar 20 µm).

Fig. 5. Photomicrographs of H&E-stained sections of the spleen from catfish showing: **(5A)** Normal histological structures of white pulp (star) that alternated with red pulp around ellipsoidal arteriole (arrowhead) beside randomly distributed melanomacrophages in G3 "Control groups". **(5B)** Marked depletion of lymphoid populations (red star) around ellipsoidal arterioles in G1"infected groups" (Scale bar 20µm).

DISCUSSION

One of the major challenges facing aquaculture is fish disease, particularly bacterial and infectious diseases, which cause multi-billion-dollar losses annually **(Assefa & Abunna, 2017)**. Fish provide the main source of animal protein for nearly one billion people worldwide, making them an essential component of global diets **(Aboagye *et al.*, 2020)**. However, fish can also act as reservoirs of *Listeria* infection. *Listeria* species are widely distributed in the environment and are considered ubiquitous organisms, having been isolated from soil and aquatic ecosystems **(Derevyanchenko & Kraeva, 2024)**. *L. monocytogenes* has been recovered from surface waters, including streams, rivers, and inland canals **(Raschle *et al.*, 2021)**, making its association with aquatic animals ecologically plausible. Environmental conditions, including weather, have been shown to significantly affect the likelihood of *Listeria* contamination in fish farms **(Miettinen & Wirtanen, 2006)**.

Our study investigated the prevalence of *L. monocytogenes* in *Clarias gariepinus*, a widely consumed traditional fish in Egypt previously identified as a possible source of this pathogen **(Chen *et al.*, 2010)**. The prevalence observed (5.71%; 4/70) is comparable to findings by **Abdallah-Ruiz *et al.*, 2022**, who reported a 2% prevalence in live catfish.

The emergence of antibiotic resistance represents an additional hazard in *L. monocytogenes* infections. The isolate PQ524490 exhibited the highest MAR index (0.7), demonstrating resistance to ciprofloxacin, gentamicin, sulfamethoxazole, tetracycline, amoxicillin/clavulanate, nalidixic acid, and cefotaxime, while remaining susceptible to chloramphenicol, cefotaxime, and ampicillin. These findings are consistent with those of **Kuan *et al.* (2017)**, who reported no resistance to ampicillin. Conversely, **Gana *et al.* (2024)** reported a lower prevalence of resistance to gentamicin and doxycycline but higher resistance to nalidixic acid and cefotaxime. The extensive use of antibiotics in aquaculture and livestock production likely drives resistance development in environmental bacteria, which may subsequently transfer to humans through the food chain **(Ed-Dra *et al.*, 2019)**. Antibiotic use in aquaculture can also enrich resistant bacterial communities in sediments, water columns, and fish-associated microbiota. Horizontal gene transfer further facilitates the dissemination of resistance determinants, enabling rapid spread across aquatic microbial populations and eventually to human pathogens **(Pepi & Focardi, 2021)**.

In vivo, experimentally infected fish displayed higher mortality rates compared to negative controls, which showed no signs of infection. Infected fish exhibited slow swimming, poor feeding, and lethargy, consistent with immune suppression **(Maule *et al.*, 1989)**. Gross pathology revealed fin rot, erythema, ulceration, enlargement of internal organs, congestion, and hemorrhage. Histopathology confirmed hepatic, splenic, and renal necrosis, consistent with earlier studies showing that *L. monocytogenes* toxins damage these organs **(Yin *et al.*, 2011; Hoelzer *et al.*, 2012)**.

The liver, the largest internal organ receiving blood supply, is the primary filter against bacterial infections, helping maintain blood sterility **(Wollina, 2017)**. Elevated AST and ALT levels in infected fish can be attributed to hepatocellular damage caused by PQ524490, which compromises membrane integrity and increases leakage of hepatic enzymes into the circulation. This agrees with the predicted notion of **López-Prieto *et al.*, 2000**, who suggested that liver infection by *L. monocytogenes* may occur via primary bacteremia or through the portal system. Histological findings in our study—endotheliosis of the hepatic artery, fibrinoid necrosis in arterial walls, perivascular necrotic hepatocytes, inflammatory infiltrates, and vascular changes—further confirm hepatic damage, consistent with reports by **Ikeh *et al*. (2010)**.

Renal impairment was also evident. *L. monocytogenes* may damage the endothelium and release toxins that cause tissue degeneration, reflected in elevated serum urea and creatinine. These findings agree with **Adeshina *et al*. (2020)**. Histopathological analysis revealed degeneration and inflammation of renal tissue, while the spleen exhibited pathological changes consistent with impaired immune function, as previously reported by **Olufemi (2020)**.

Regarding antioxidant defense, *L. monocytogenes* infection induced oxidative stress, disrupting the balance between reactive oxygen species (ROS) and antioxidant systems. Infected *C. gariepinus* showed elevated ROS levels due to immune responses against the pathogen. Excess ROS triggered lipid peroxidation, reflected by increased malondialdehyde (MDA) levels, and caused DNA, protein, and membrane damage **(Aebi, 1984; Livingstone, 2001)**. Antioxidant enzymes were impaired: CAT activity declined, leading to hydrogen peroxide accumulation **(Farombi *et al*., 2007)**; GPx activity was suppressed, likely due to glutathione depletion **(Adeyemi *et al*., 2016)**; GSH levels dropped significantly **(Anyanwu *et al*., 2017)** and SOD activity decreased, reducing superoxide radical scavenging **(Olajide *et al*., 2020)**.

Immune parameters were also affected, with lysozyme activity and immunoglobulin levels significantly reduced. This suppression may be linked to bacterial-mediated downregulation of immune genes **(Akinbowale *et al*., 2007)** and reduced lymphocyte and macrophage counts **(Harikrishnan *et al*., 2011)**.

Taken together, the biochemical, oxidative stress, and histopathological findings confirm that *L. monocytogenes* PQ524490 infection severely compromises immune competence and organ function in *C. gariepinus*.

CONCLUSION

The detection of *Listeria monocytogenes* in African catfish underscores the urgent need for continuous surveillance and preventive measures to safeguard both aquaculture productivity and public health. Infection in *Clarias gariepinus* was associated with profound behavioral changes, impaired hepatorenal function, dysregulated immune

responses, and reduced antioxidant defenses, collectively leading to immune suppression, chronic inflammation, and oxidative stress. These pathological alterations compromised the fish's ability to neutralize reactive oxygen species (ROS), resulting in tissue damage, heightened vulnerability to secondary infections, and diminished survival and performance within aquaculture systems. Given the escalating threat of antibiotic resistance among aquaculture pathogens, future research should focus on developing sustainable and eco-friendly therapeutic strategies to support disease management in *C. gariepinus* farming.

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الملخص العربي

سلالة مقاومة للأدوية المتعددة من عدوى بكتيريا *Listeria monocytogenes* في سمك السلور الأفريقي (*Clarias gariepinus*): العدوى البيولوجية، الأعراض الأكلينيكية، وتغيير البنية النسجية

تُعد العدوى البكتيرية إحدى المشكلات الرئيسية في تربية الأحياء المائية، والتي قد تؤدي إلى معدلات نفوق هائلة للأسماك. تم عزل بكتيريا *Listeria monocytogenes* PQ524490 المقاومة للأدوية المتعددة من سمك السلور الأفريقي. قُسِّمت بكتيريا *Listeria monocytogenes* إلى ثلاث مجموعات متساوية؛ كل مجموعة تتكون من ثلاث مكررات (10 أسماك/مكرر). حُقنت المجموعة الأولى داخل التجويف البروتوني (I/P) بجرعة 0.1 مل من البكتريا (2.0×10^8 وحدة تشكيل مستعمرة/مل). أما المجموعة الثانية، فكانت بمثابة مجموعة ضابطة سلبية، وحُقنت داخل التجويف البروتوني بجرعة 0.1 مل من محلول ملحي معقم. أما المجموعة الثالثة، فكانت بمثابة مجموعة ضابطة (لا تحتوي على البكتريا ولا على محلول ملحي معقم)، وتغذت على نظام غذائي صناعي فقط. تؤدي بكتيريا *Listeria monocytogenes* PQ524490 إلى تأثيرات سلبية شديدة على مؤشرات الكبد والكُلى، وانخفاض ملحوظ في المناعة غير النوعية، ونشاط مضادات الأكسدة ($P< 0.05$) مقارنةً بمجموعات الضبط. أظهرت المجموعة المصابة تلفًا في الكبد والكُلى والطحال في المقاطع النسيجية.