

ORIGINAL RESEARCH ARTICLE

Characterization of virulence gene profiles of *Aeromonas hydrophila* and *Lactococcus garvieae* from diseased Nile tilapia in Zambia

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Abstract

Fish production is threatened by frequent disease outbreaks, especially bacterial diseases that cause significant economic losses. This study characterized the key virulence gene profiles of *Aeromonas hydrophila* and *Lactococcus garvieae* isolated from diseased Nile tilapia in southern Zambia and assessed genotype–lesion associations. A total of 163 clinically affected tilapia were examined, from which *A. hydrophila* (43%) and *L. garvieae* (22%) were recovered predominantly from brain and kidney tissues. Virulence gene screening showed that *A. hydrophila* exhibited low-frequency profiles dominated by hemolysin A (*hlyA*) (20.5% in broodstock) and elastase (*ela*) (11.5% in grow-out), while aerolysin (*aerA*) and enterotoxin (*act*) were infrequently detected. *L. garvieae* displayed a hemolysin-skewed profile, with *hly2* being most prevalent (27.3% in broodstock; 21.4% in grow-out), *hly2* detected only in broodstock, and capsule gene cluster (CGC) and fibronectin-binding protein (*fbp*) genes occurring rarely. Significant gene–lesion associations linked *aerA* and *ela* with pale gills ($r = 0.41, p < 0.01$; $r = 0.24, p = 0.05$, respectively), *act* with skin discoloration ($r = 0.27, p = 0.02$), and demonstrated inverse correlations between *hlyA* and fins ($r = -0.4, p < 0.001$) or hepatic hemorrhages ($r = -0.27, p = 0.02$) in *A. hydrophila*. In *L. garvieae*, *hly3* correlated with enlarged liver ($r = 0.23, p < 0.001$), corneal opacity ($r = 0.15, p = 0.05$), and gill necrosis ($r = 0.21, p = 0.01$), while *hly2* and capsule genes were associated with skin discoloration ($r = 0.18, p = 0.02$; $r = 0.24, p < 0.001$, respectively). Overall, virulence determinants occurred at low frequencies and in limited combinations, suggesting the circulation of less virulent strains and underscoring the value of genotype-informed surveillance for improving disease control in tilapia aquaculture.

Keywords: Nile tilapia; *Aeromonas hydrophila*; *Lactococcus garvieae*; Virulence genes; Post-mortem lesions

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1. Introduction

Aquaculture is one of the fastest-growing sectors of food production, projected to surpass 100 million tonnes globally by 2027 and reach 106 million tonnes by 2030.¹ However, this growth is threatened by frequent disease outbreaks, which can cause substantial economic losses if not effectively controlled.² Globally, aquaculture diseases account for an estimated USD 6 billion in annual losses, with bacterial infections among the most significant challenges.³ Pathogenic bacteria such as *Streptococcus*, *Aeromonas*, *Vibrio*, *Lactococcus*, and *Edwardsiella* cause severe infections in farmed fish, leading to high mortality and considerable financial damage.^{4–6}

Understanding the molecular basis of bacterial pathogenicity—particularly the role of virulence factors and their associated genes—is essential for developing targeted disease control strategies and ensuring the sustainability of aquaculture. Virulence genes enable pathogens to invade host tissues, evade immune defenses, and inflict tissue damage.⁷ Research has shown that *Aeromonas hydrophila* carries several key virulence genes—such as aerolysin (*aerA*), cytotoxic heat-stable enterotoxin (*ast*), cytotoxic enterotoxin (*act*), and hemolysin A (*hlyA*)—that facilitate host invasion, immune evasion, and tissue destruction.^{8–10} Similarly, *Lactococcus garvieae* possesses a range of virulence determinants, including the capsule gene cluster (CGC) that confers immune evasion; hemolysin genes (*hly1*, *hly2*, *hly3*) responsible for red blood cell lysis; *sodA* for oxidative stress protection; a plasmid-encoded adenosine diphosphate-ribosyltransferase exotoxin; and multiple adhesion-related genes (*PsaA*, *PavA*, *enolase*, *adhCI*, *adhCII*, *adh*) alongside sortase-anchored proteins, all of which contribute to host colonization and persistence.^{11–13}

In Zambia, bacterial pathogens are also a significant constraint to aquaculture production.^{14,15} *Streptococcus* spp., *L. garvieae*, and *Aeromonas* spp. have been linked to high mortality in both small- and large-scale tilapia farms.^{15,16} Research has demonstrated that disease severity is influenced by multiple factors, including environmental conditions, host immune status, and the intrinsic virulence of the infecting bacteria.¹⁷ Therefore, the detection and characterization of virulence genes provide valuable insight into pathogen behavior and are a critical step in disease epidemiology and control.¹⁸

This study aims to detect and profile key virulence genes in *L. garvieae* and *A. hydrophila* isolates recovered from diseased cage- and pond-cultured Nile tilapia (*Oreochromis niloticus*) in southern Zambia. Although these pathogens have been previously documented to cause outbreaks in farmed tilapia in Zambia, their virulence characteristics remain poorly understood.^{14,15} We hypothesized that

Zambian isolates of *A. hydrophila* and *L. garvieae* would possess distinct virulence gene profiles compared to global reports, and that these profiles would show specific correlations with pathological lesions, thereby reflecting unique pathogenic mechanisms in this region.

2. Materials and methods

2.1. Study area and design

A cross-sectional study was conducted from October 2021 to January 2022—corresponding to the hot season in Zambia, the peak production period, and when reports of fish disease outbreaks are typically highest—in major tilapia-producing zones of the Southern Province. Four high-production districts—Siavonga, Chirundu, Kalomo, and Livingstone—were purposively selected based on their large commercial grow-out farms (>200 t/year) and/or extensive hatchery distribution networks (Figure 1). Sampling targeted ponds and cages that had experienced clinical disease signs or elevated mortality within 14 days prior to the visit. Only moribund tilapias were collected for the study, and the required sample size ($n = 163$) was calculated using the formula by Naing *et al.*¹⁹, assuming an expected prevalence of 13%, 95% confidence, and 5% precision.

2.2. Post-mortem examination and sample collection

At each farm, up to five moribund fish were collected from the affected ponds or cages, euthanized with clove oil (~250 mg/L), and examined for gross lesions, which included gill pallor or necrosis, exophthalmia, corneal opacity, abdominal distension, skin ulcers, hemorrhages, fin erosion, and scale loss. Sterile swabs from the brain, liver, spleen, and kidney were inoculated on-site onto blood agar (Himedia, India) and MacConkey agar (Himedia, India), then transported in cooled, insulated boxes to the bacteriology laboratory at the School of Veterinary Medicine, University of Zambia.

2.3. Identification of bacteria

In the laboratory, the inoculated plates were incubated at 28 to 30 °C and monitored for growth for 24 h; isolates were initially characterized by Gram staining and oxidase and catalase tests. Genus- and species-level identification was performed using conventional biochemical assays, including carbohydrate fermentation (raffinose, lactose, maltose, mannose, D-mannitol, melibiose, sucrose, trehalose, dulcitol, cellobiose, xylose) and other tests (citrate utilization, urease, indole, motility, nitrate reduction, growth in 10 µg/mL ampicillin). Isolates presumptively identified as *A. hydrophila* or *L. garvieae* were stored in

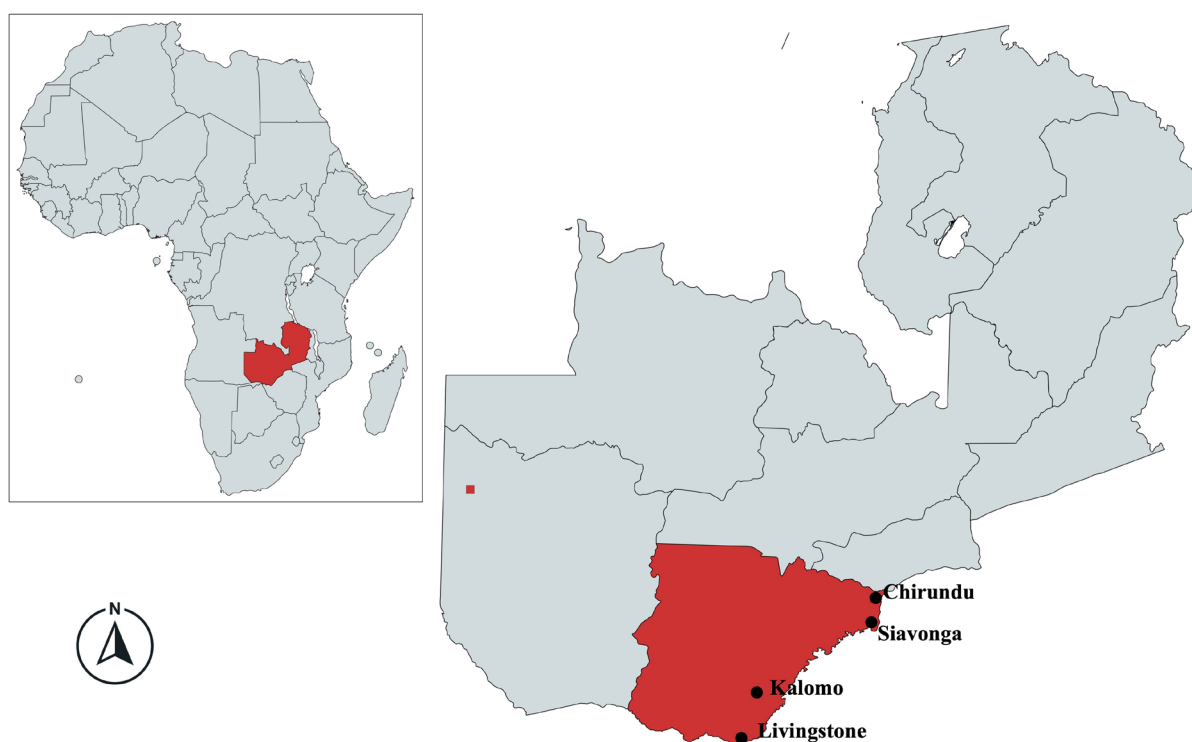


Figure 1. Map of Zambia showing the southern provinces and the nine districts within (Chirundu, Siavonga, Kalomo, and Livingstone)

glycerol at -80°C for subsequent molecular analysis.

2.4. Molecular detection of virulence genes

Biochemically confirmed *A. hydrophila* and *L. garvieae* isolates were screened for virulence genes using polymerase chain reaction (Veriti™ 96-Well Thermal Cycler, Applied Biosystems, USA) following genomic DNA extraction via thermal lysis.²⁰ Target genes for *L. garvieae* included CGC, hemolysin genes (*hly1*, *hly2*, *hly3*), and fibronectin-binding protein (*fbp*), while *A. hydrophila* was screened for *aerA*, *hlyA*, *ela*, and *act* (Table 1). Polymerase chain reactions (25 μL) contained ~100 ng DNA, 2.5 μL 10 \times buffer (Himedia, India), 2.5 mM magnesium chloride, 200 μM dNTPs, 0.5 μM primers (each forward and reverse), and 0.5 U Taq polymerase, with cycling at 94°C for two minutes, followed by 35 cycles of 94°C (30 s), primer-specific annealing (Table 1) (60 s), 72°C (60 s), and a final extension step at 72°C (7 min). Amplicons were resolved on 1.5% agarose gels stained with ethidium bromide, sized against a 100 bp DNA ladder, and visualized under ultraviolet illumination.

2.5. Data analysis

Data were entered into Microsoft Excel 2010 and analyzed using DATAtab (web-based software, Styria, Austria). Categorical variables were summarized as counts and percentages. Associations between virulence gene

detection (present/absent) and post-mortem lesions (seen/not seen) were evaluated using Spearman's rank correlation (ρ) with binary/ordinal coding. Corresponding p -values were calculated, and statistical significance was set at two-sided $\alpha = 0.05$.

3. Results

3.1. Fish sample distribution

A total of 163 diseased tilapia were collected across four districts: Chirundu (66; 40.49%), Siavonga (56; 34.35%), Livingstone (31; 19.02%), and Kalomo (10; 6.13%). Most samples originated from pond systems (107/163; 65.64%), and cage production systems in Siavonga contributed 34.36% (56/163). By growth stage, 100/163 (61.34%) were broodstock and 63/163 were grow-out fish (38.65%) (Table 2).

3.2. Post-mortem lesions

In broodstock fishes, hemorrhages on fins were most frequent (31%), followed by hepatic lesions—hemorrhages on liver (25%) and enlarged liver (22%). Generalized body surface hemorrhages (20%), enlarged spleen (19%), and corneal opacity (19%) were also common. Missing scales (18%), skin discoloration (16%), and eroded fins (11%) were less frequent. Gill lesions were uncommon, with necrotic gills (5%) and pale gills (3%), while pale liver was

Table 1. Primers used for detecting virulence genes in *Lactococcus garvieae* and *Aeromonas hydrophila*

Virulence Gene	Primer	Sequences (5'–3')	Product Size (bp)	Annealing temperature (°C)	Reference
<i>Lactococcus garvieae</i>					
Capsule gene	LGCG	F (5'-TGCTGTCATCATATTGTGTCCA-3') R (5'-GGCTATGGCATTAGTCAGGAAG-3')	744	59	21
Hemolysin 1	hly1	F (5'-TCCTCCGACTAGGAACCAAA 3') R (5'-GCCAGCTTCTCGTGCTTATC3')	522	56	13
Hemolysin 2	hly2	F (5'-GAGCAAAAAGCGAGTGAAGG3') R (5'-GCATCTGGAGCATCAAGTCA3')	796	58	13
Hemolysin 3	hly3	F (5'-CGTGGAGTTATGGCTGGTTT 3') R (5'-CTTGTGGATCTTCGGGTCTT 3')	549	55	13
Fibronectin-binding protein	fbp	F (5'-CGGTTCGTTTCAGGAAGAATCATC3') R (5'-CGGTCATTGCCTACTTGCTCAA3')	181	60	22
<i>Aeromonas hydrophila</i>					
Aerolysin	AHaero	F (5'-CCTATGGCCTGAGCGAGAAG3') R (5'-CCAGTTCCAGTCCCACCACT3')	431	56	21
Hemolysin	AHhemo	F (5'-GCCGAGCGCCCAAGGTGAGTT3') R (5'-GAGCGGCTGGATGCGGTTGT3')	592	59	24
Elastase	AHelast	F (5'-ACACGGTCAAGGAGATCAAC3') R (5'-ATCTTCTCCGACTGGTTCGG3')	513	55	25
Enterotoxin (cytotoxic)	act	F (5'-GAGAAGGTGACCACCAAGAACA3') R (5'-AACTGACATCGGCCTTGAAGTC 3')	232	65	26

Table 2. Distribution of diseased fish samples collected by district, farm, production system, and growth stage in Zambia

S/N	Location	Culture system	Growth stage	Number of samples (%)
1	Chirundu (Farm 1)	Pond	Broodstock	41 (25.15%)
2	Chirundu (Farm 2)	Pond	Broodstock	25 (15.34%)
3	Kalomo	Pond	Broodstock	10 (6.13%)
4	Livingstone	Pond	Broodstock	9 (5.52%)
			Grow-out	22 (13.50%)
5	Siavonga (Farm 1)	Cage	Broodstock	15 (9.20%)
			Grow-out	26 (15.95%)
6	Siavonga (Farm 2)	Cage	Grow-out	15 (9.20%)

rare (2%) (Figures 2 and 3). Overall, hemorrhagic and hepato-splenic changes predominated.

In grow-out tilapia, lesion prevalence was generally higher in pond systems than cage systems, especially for hemorrhagic conditions: hemorrhages on liver (63% pond vs. 30% cage) and body surface (58% vs. 30%), necrotic gills (38% vs. 22%), and hemorrhages on fins (40% vs. 30%). In contrast, cage systems showed higher rates of corneal opacity, enlarged spleen, and missing scales (20–22% in

cages vs. 10% in ponds). Enlarged liver and eroded fins were comparable (20%) (Figures 3 and 4).

3.3. Bacterial isolation and identification

A. hydrophila predominated, with the highest recovery in grow-out ponds—brain and kidney each 31.8%—exceeding broodstocks and grow-out cages (Table 3). Across pathogens, the kidney and brain were the principal isolation sites, liver isolations were rare, and the spleen

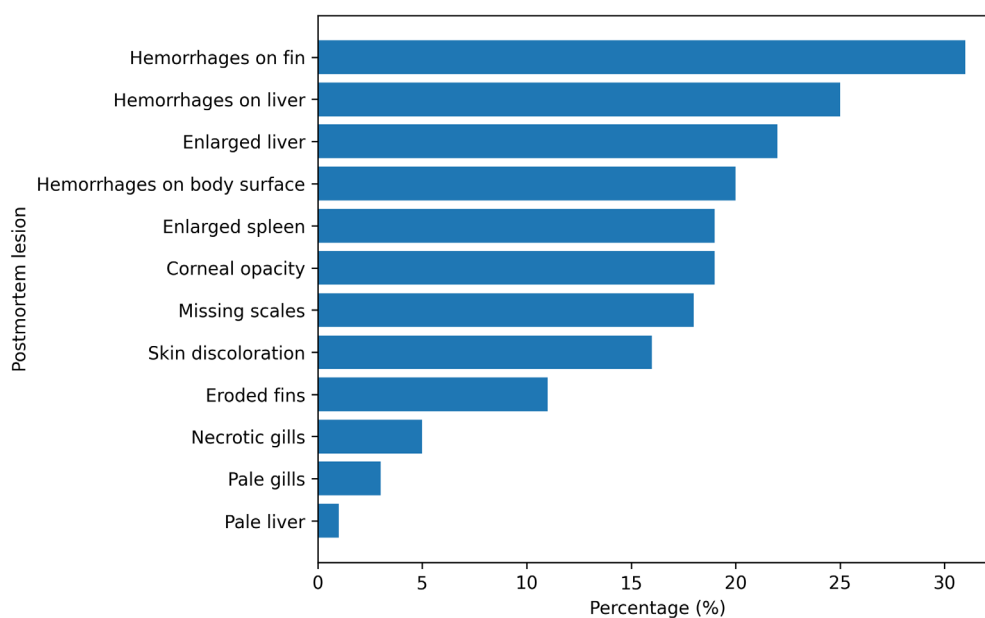


Figure 2. Prevalence of post-mortem lesions in broodstock fishes ($n = 101$)

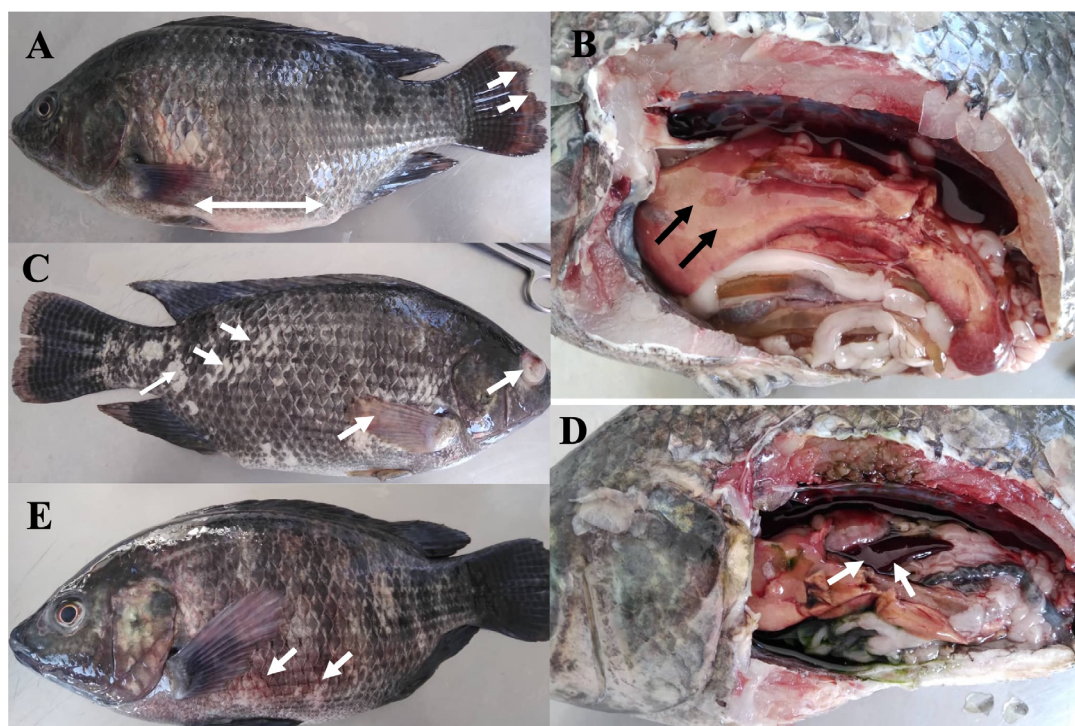


Figure 3. Lesions in fish. (A) Caudal fin erosion, abdominal distension, (B) pale and enlarged liver, (C) skin discoloration, corneal opacity, and hemorrhages on fins, (D) enlarged spleen, and (E) hemorrhages on skin surface.

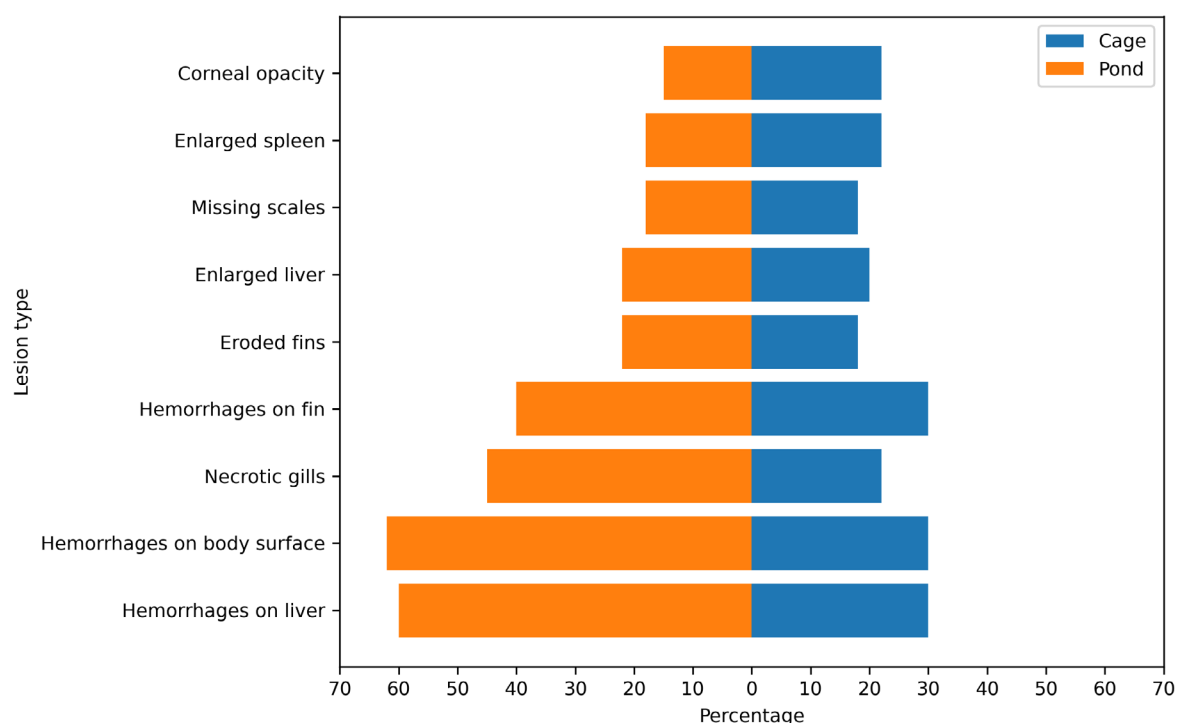


Figure 4. Prevalence of post-mortem lesions in grow-out Nile tilapia by pond ($n = 23$) and cage culture system ($n = 39$)

yielded no isolates for *A. hydrophila*. In contrast, *L. garvieae* occurred less frequently—detected mainly in kidneys—and was absent from liver and spleen in grow-out fish (only 4% liver isolates in broodstock ponds) (Table 3).

3.4. Detection of virulence genes

In *A. hydrophila*, *hlyA* was the predominant virulence gene in broodstock (20.5%) isolates, whereas *ela* was the most frequent in grow-out fish (11.5%); *aerA* was detected at lower levels in both groups (6.8% and 7.7%, respectively), and *act* was rare/absent (Figure 5 and Table 4). Multi-gene profiles such as *aerA* + *ela*, *aerA* + *hlyA*, *ela* + *hlyA*, and *erA* + *ela* + *hlyA* were at very low frequency and only detected in broodstock isolates. In *L. garvieae*, the hemolysin gene *hly3* dominated in both broodstock (27.3%) and grow-out (21.4%) isolates, while *hly2* was detected only in broodstock (13.6%). CGC and *fbp* were infrequent (7.1%). Multiple gene profiles were rare, with *hly2* + *hly3* (4.5%) occurring in broodstock isolates and *hly3* + *fbp* (7.1%) in grow-out isolates (Figure 6 and Table 4).

3.5. Correlation between virulence genes and post-mortem lesions

In *A. hydrophila*, the *act* gene showed a significant positive correlation only with skin discoloration ($r = 0.27$, $p = 0.02$). Both the *aerA* ($r = 0.41$, $p < 0.001$) and *ela* genes ($r = 0.24$,

$p = 0.05$) were positively associated with pale gills. For *hlyA*, significant negative correlations were observed with hemorrhages on fins ($r = -0.40$, $p < 0.001$) and hemorrhages in the liver ($r = -0.27$, $p = 0.02$) (Table 5).

For *L. garvieae*, the CGC was positively associated with corneal opacity ($r = 0.16$, $p = 0.04$), enlarged spleen ($r = 0.15$, $p = 0.04$), and skin discoloration ($r = 0.24$, $p < 0.001$). The *hly3* gene showed positive correlations with corneal opacity ($r = 0.15$, $p = 0.05$), enlarged liver ($r = 0.23$, $p < 0.001$), and necrotic gills ($r = 0.21$, $p = 0.01$). For *hly2*, a single positive association with skin discoloration was observed ($r = 0.18$, $p = 0.02$) (Table 6).

Given the low prevalence of several virulence genes, particularly among *L. garvieae* isolates, the correlation analysis has limited statistical power and should therefore be interpreted with caution.

4. Discussion

Motile *Aeromonas* septicemia caused by *A. hydrophila* and lactococcosis caused by *L. garvieae* represent major challenges to the global freshwater aquaculture sector.^{4–6} This study investigated the occurrence of these pathogens in farmed Nile tilapia and characterized their virulence gene profiles. Additionally, this study examined the associations between these genes and the clinical signs

Table 3. Bacterial isolation rates from different organs in broodstock and grow-out tilapia

Organs	Broodstock (n = 100)	Grow-out Cage (n = 41)	Pond (n = 22)
<i>Aeromonas hydrophila</i>			
Brain	20 (20.0%)	5 (12.2%)	7 (31.8%)
Kidney	18 (18.0%)	6 (14.6%)	7 (31.8%)
Liver	6 (6.0%)	1 (2.4%)	0 (0.0%)
Spleen	0 (0.0%)	0 (0.0%)	0 (0.0%)
<i>Lactococcus garvieae</i>			
Brain	6 (6.0%)	4 (9.8%)	1 (4.5%)
Kidney	12 (12.0%)	5 (12.2%)	4 (18.2%)
Liver	4 (4.0%)	0 (0.0%)	0 (0.0%)
Spleen	0 (0.0%)	0 (0.0%)	0 (0.0%)

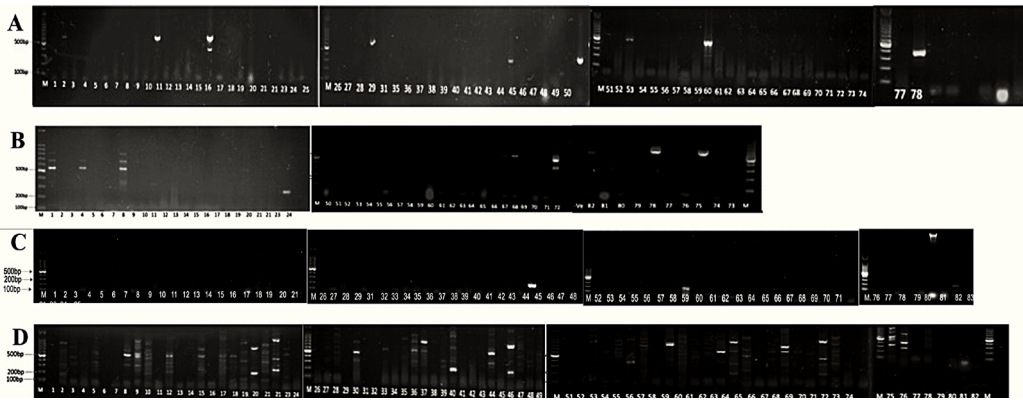


Figure 5. Polymerase chain reaction detection of *Aeromonas hydrophila* virulence-associated genes. (A) Aerolysin gene (*aerA*, 431 bp); (B) elastase gene (*ela*, 513 bp); (C) enterotoxin gene (*act*, 232 bp); and (D) hemolysin gene (*hlyA*, 592 bp). Amplified products were separated by agarose gel electrophoresis and visualized under ultraviolet transillumination. The numbers shown on the gels correspond to individual sample identification numbers.

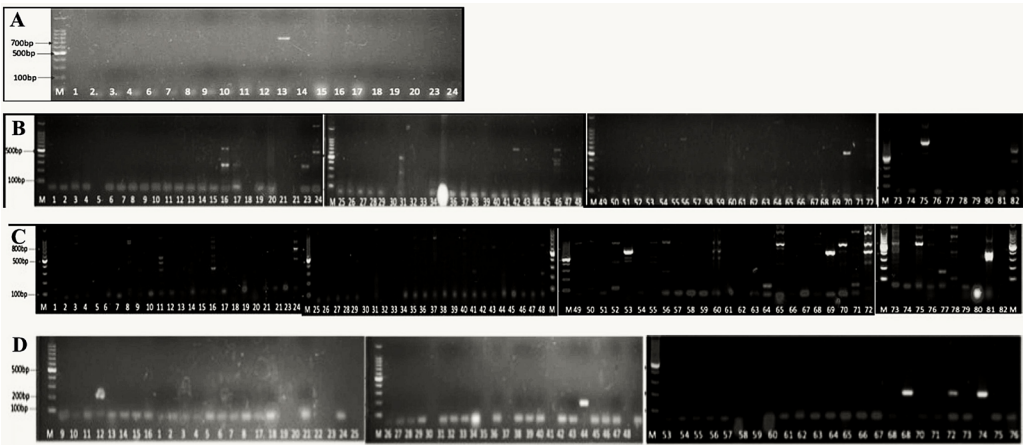


Figure 6. Polymerase chain reaction detection of *Lactococcus garvieae* virulence-associated genes. (A) Capsule gene (*LCGC*, 744 bp); (B) hemolysin 2 gene (*hly2*, 796 bp); (C) hemolysin 3 gene (*hly3*, 549 bp); and (D) fibronectin-binding protein gene (*fbp*, 181 bp). Amplified products were separated by agarose gel electrophoresis and visualized under ultraviolet transillumination. The numbers shown on the gels correspond to individual sample identification numbers.

Table 4. Detection of virulence genes in *Aeromonas hydrophila* and *Lactococcus garvieae* from broodstock and grow-out tilapia

Virulence genes	Tilapia	
<i>Aeromonas hydrophila</i>	Broodstock (n = 44)	Grow out (n = 26)
<i>aerA</i>	3 (6.8%)	2 (7.7%)
<i>ela</i>	4 (9.1%)	3 (11.5%)
<i>act</i>	1 (2.3%)	0 (0.0%)
<i>hlyA</i>	9 (20.5%)	2 (7.7%)
<i>aerA</i> + <i>ela</i>	1 (2.3%)	1 (3.8%)
<i>aerA</i> + <i>hlyA</i>	1 (2.3%)	0 (0.0%)
<i>ela</i> + <i>hlyA</i>	3 (6.8%)	0 (0.0%)
<i>aerA</i> + <i>ela</i> + <i>hlyA</i>	1 (2.3%)	0 (0.0%)
<i>Lactococcus garvieae</i>	Broodstock (n = 22)	Grow out (n = 14)
Capsule gene cluster	1 (4.5%)	0 (0.0%)
<i>fbp</i>	1 (4.5%)	1 (7.1%)
<i>hly2</i>	3 (13.6%)	0 (0.0%)
<i>hly3</i>	6 (27.3%)	3 (21.4%)
<i>hly2</i> + <i>hly3</i>	1 (4.5%)	0 (0.0%)
<i>hly3</i> + <i>fbp</i>	0 (0.0%)	1 (7.1%)

and pathological lesions observed in farmed diseased Nile tilapia in Zambia. Collectively, these findings provide insights into the pathogenicity of the bacterial isolates.

The recovery rates of *A. hydrophila* (43%) and *L. garvieae* (22%) from diseased grow-out and broodstock Nile tilapia, respectively, highlight the likely involvement of these pathogens in the current disease outbreaks. Previous studies in the Zambian aquaculture sector reported markedly lower recovery rates—13% for *Aeromonas* spp. and 7.3% for *Lactococcus* spp.—suggesting a possible shift in pathogen prevalence or disease dynamics.¹⁵ The higher recovery rates observed in the present study may reflect increased pathogen loads associated with active outbreaks, resulting in higher bacterial burdens in infected tissues and, consequently, greater culture success. Furthermore, the targeted sampling approach used—prioritizing clinically diseased fish, collecting samples from multiple diagnostically relevant organs, and ensuring rapid processing—likely enhanced the likelihood of pathogen detection.

The potential of a pathogenic bacterium to cause disease in a susceptible host is largely determined by the virulence genes it carries.⁹ In the present study, *A. hydrophila* isolates exhibited a comparatively narrow virulence profile. Among the detected genes, hemolysin genes predominated in broodstock isolates, whereas *ela* and *aerA* were identified in both broodstock and grow-out fish, whereas *act* was detected exclusively in broodstock.

Multi-gene combinations were uncommon, occurring only in broodstock and at very low prevalence. This contrasts sharply with reports from Asia and other regions where *A. hydrophila* frequently exhibits broader virulence repertoires, with >50% prevalence of *aerA*, *hlyA*, and *act*, and frequent co-carriage of multiple toxin genes.^{8–10,22,26,27} Similarly, the *L. garvieae* isolates demonstrated a hemolysin-skewed profile dominated by *hly3*, with *hly2* detected only in broodstock, while the CGC and *fbp* occurred at low frequencies. This pattern diverges from profiles reported in trout and Mediterranean isolates, where *hly1/2/3* are often ubiquitous and carried in combination^{11,13}, yet aligns with evidence that the CGC is commonly absent outside specific geographic locales.^{11–13} The low prevalence of *fbp* also contrasts with findings from some non-fish hosts, where *fbp* are widespread, highlighting notable strain- and host-associated variability within *L. garvieae*.^{12,21,22} Taken together, the low prevalence of virulence genes—whether individually or in combination—detected in both *A. hydrophila* and *L. garvieae* isolates likely reflects the circulation of less virulent or environmentally adapted strains, with disease expression driven largely by host and environmental stressors rather than by highly toxigenic genotypes. This interpretation is supported by Bwalya *et al.*¹⁶, who reported that *L. garvieae* isolated from farmed Nile tilapia in Lake Kariba exhibited low invasive potential, with infection risk increasing primarily in fish showing skin abrasions.²⁸ Similarly, El-Bahar *et al.*²⁹ demonstrated that isolates possessing two or more virulence genes were

Table 5. Correlation between virulence genes and post-mortem lesions in tilapia infected with *Aeromonas hydrophila*

Lesions	<i>Aeromonas hydrophila</i> virulence genes (n=70)											
	<i>act</i>			<i>aerA</i>			<i>ela</i>			<i>hlyA</i>		
	n (%)	Spearman's r	p-value	n (%)	Spearman's r	p-value	n (%)	Spearman's r	p-value	n (%)	Spearman's r	p-value
Abdominal distention	0 (0%)	-0.01	0.91	0 (0%)	-0.03	0.79	0 (0%)	-0.05	0.68	0 (0%)	-0.06	0.63
Corneal opacity	1 (1.4%)	0.17	0.15	2 (2.7%)	0.04	0.73	4 (5.5%)	0.03	0.79	5 (6.9%)	0.03	0.81
Enlarged liver	1 (1.4%)	0.14	0.25	3 (4.1%)	0.1	0.42	4 (5.5%)	-0.05	0.66	5 (6.9%)	-0.07	0.58
Enlarged spleen	1 (1.4%)	0.15	0.21	2 (2.7%)	0.01	0.94	5 (6.9%)	0.06	0.61	4 (5.5%)	-0.1	0.41
Eroded fins	0 (0%)	-0.08	0.49	1 (1.3%)	-0.07	0.53	3 (4.1%)	-0.05	0.67	2 (2.7%)	-0.19	0.1
Hemorrhages on the body surface	0 (0%)	-0.15	0.19	5 (6.9%)	0.21	0.08	6 (8.2%)	-0.07	0.53	7 (9.6%)	-0.13	0.27
Hemorrhages on the fin	1 (1.4%)	0.07	0.54	3 (4.1%)	-0.08	0.52	7 (9.6%)	-0.08	0.48	5 (6.9%)	-0.4	<0.001
Hemorrhages in the liver	1 (1.4%)	0.08	0.5	3 (4.1%)	-0.05	0.68	6 (8.2%)	-0.13	0.29	6 (8.2%)	-0.27	0.02
Missing scales	1 (1.4%)	0.15	0.22	3 (4.1%)	0.11	0.34	4 (5.5%)	-0.03	0.81	4 (5.5%)	-0.11	0.35
Necrotic gills	0 (0%)	-0.09	0.47	3 (4.1%)	0.15	0.21	3 (4.1%)	-0.06	0.6	2 (2.7%)	-0.2	0.08
Pale gills	0 (0%)	-0.03	0.81	2 (2.7%)	0.41	<0.001	2 (2.7%)	0.24	0.05	2 (2.7%)	0.19	0.11
Pale liver	0 (0%)	-0.02	0.84	0 (0%)	-0.06	0.64	0 (0%)	-0.09	0.46	0 (0%)	-0.1	0.4
Skin discoloration	1 (1.4%)	0.27	0.02	1 (1.4%)	0.03	0.83	2 (2.7%)	0.02	0.87	3 (4.1%)	0.07	0.58

Table 6. Correlation between virulence genes and post-mortem lesions in tilapia infected with *Lactococcus garvieae*

Lesions	<i>Lactococcus garvieae</i> virulence genes (n = 36)											
	CGC			fbp			hly2			hly3		
	n (%)	Spearman's r	p-value	n (%)	Spearman's r	p-value	n (%)	Spearman's r	p-value	n (%)	Spearman's r	p-value
Abdominal distention	0 (0%)	-0.01	0.94	0 (0%)	-0.01	0.86	0 (0%)	-0.02	0.805	0 (0%)	-0.03	0.661
Corneal opacity	1 (2.6%)	0.16	0.04	2 (5.3%)	0.1	0.21	3 (7.9%)	0.07	0.34	9 (23.7%)	0.15	0.05
Enlarged liver	1 (2.6%)	0.15	0.06	1 (2.6%)	-0.01	0.93	3 (7.9%)	0.11	0.14	10 (26.3%)	0.23	0
Enlarged spleen	1 (2.6%)	0.15	0.04	0 (0%)	-0.09	0.26	2 (5.3%)	0	0.99	6 (15.8%)	0.06	0.45
Eroded fins	0 (0%)	-0.03	0.66	1 (2.6%)	0.02	0.82	0 (0%)	-0.11	0.15	7 (18.4%)	0.1	0.17
Hemorrhages on the body surface	0 (0%)	-0.05	0.53	0 (0%)	-0.03	0.67	1 (2.6%)	-0.05	0.54	5 (13.2%)	0.14	0.07
Hemorrhages on the fin	0 (0%)	-0.05	0.5	0 (0%)	-0.04	0.56	1 (2.6%)	-0.06	0.41	5 (13.2%)	0.1	0.18
Hemorrhages in the liver	0 (0%)	-0.05	0.5	0 (0%)	-0.04	0.58	1 (2.6%)	-0.01	0.92	5 (13.2%)	0.14	0.06
Missing scales	0 (0%)	-0.04	0.63	0 (0%)	-0.08	0.28	0 (0%)	-0.12	0.12	5 (13.2%)	0.07	0.35
Necrotic gills	0 (0%)	-0.03	0.67	0 (0%)	0.02	0.76	2 (5.3%)	0.1	0.18	4 (10.5%)	0.21	0.01
Pale gills	0 (0%)	-0.01	0.88	0 (0%)	-0.03	0.73	1 (2.6%)	0.13	0.1	1 (2.6%)	0.04	0.64
Pale liver	0 (0%)	-0.01	0.89	0 (0%)	-0.02	0.76	0 (0%)	-0.03	0.67	1 (2.6%)	0.06	0.42
Skin discoloration	1 (2.6%)	0.24	0.00	1 (2.6%)	0.06	0.41	3 (7.9%)	0.18	0.02	4 (10.5%)	0.08	0.324

significantly more pathogenic than those carrying only a single gene. Therefore, the predominance of single-gene profiles in the Zambian *A. hydrophila* and *L. garvieae* isolates suggests comparatively lower pathogenicity, consistent with the observations reported by Bwalya *et al.*¹⁶

By linking genotype to phenotype, our correlation analyses identified biologically plausible gene–lesion associations. In *A. hydrophila*, *aerA* was positively associated with pale gills, and *act* was positively associated with skin discoloration, whereas *hlyA* showed inverse correlations with hemorrhages on fins and liver. Systematically, both *hlyA* and *aerA* are pore-forming cytolysins capable of erythrocyte lysis (inducing anemia) and tissue injury, and *ela* is implicated in proteolysis and necrosis.^{8,10,23–25} The modest effect sizes and few significant signals are consistent with multifactorial lesion pathogenesis in tilapia, in which environmental stressors, such as temperature, water quality, crowding, and coinfections, modulate clinical expression.^{30–35} For *L. garvieae*, *hly3* correlated positively with enlarged liver, corneal opacity, and necrotic gills, and *hly2* with necrotic gills. Hemolysins are central to *L. garvieae* virulence and can drive hemorrhagic septicemia and organ damage.^{11,12,36} The weak positive association of CGC with organomegaly in our data, despite its low prevalence, is consistent with its proposed role in immune evasion, as reported in previous studies.^{11,21} The limited occurrence of *fbp* and the absence of strong associations in our isolates may indicate a lesser contribution of fibronectin-mediated adhesion in these tilapia outbreaks; however, the small number of detected genes cautions against overinterpretation of the results.²²

Several limitations of the study should be acknowledged. First, although molecular identification is considered the gold standard, biochemical characterization was selected as the primary identification approach due to its cost-effectiveness and feasibility for processing a large number of field isolates. Second, the final number of isolates included in the correlation analysis was relatively modest, particularly for less frequently detected virulence genes. This constraint may reduce statistical power and increase the likelihood of type II errors, potentially leading to the failure to detect existing associations. Despite these limitations, the dataset offers valuable preliminary insights into virulence–gene interactions in important aquaculture pathogens and establishes a foundation for future studies. Expanding the isolate collection in subsequent work will further strengthen the robustness of correlation outcomes and provide a more comprehensive understanding of virulence dynamics in *A. hydrophila* and *L. garvieae* affecting tilapia production systems.

The findings of the present study suggest that Zambian

isolates carry the same core virulence determinants reported globally, but at lower frequencies and in different combinations, particularly for *A. hydrophila* (*hlyA*-dominant, low *act/aerA/ela*) and *L. garvieae* (*hly3*-dominant, sparse CGC/*fbp*). The lesion correlations reinforce the pathogenic roles of virulence genes while highlighting epidemiological complexity driven by environment, host stage, and production system.^{31,37} These regional differences have practical implications: surveillance that includes genotype–phenotype linkage can refine risk assessment and support targeted control, such as management of temperature stress for *A. hydrophila* outbreaks and vaccine antigen selection for *L. garvieae* focused on prevalent hemolysins.^{8,10,12} Future work should increase sample size and incorporate quantitative expression data to better understand the temporal dynamics of virulence gene activity in tilapia aquaculture systems.

5. Conclusion

The study profiled virulence genes in *A. hydrophila* and *L. garvieae* from diseased Nile tilapia in southern Zambia and evaluated their associations with lesions. *A. hydrophila* showed higher frequencies of *hlyA* and *ela* genes, while *aerA* and *act* were less common; *L. garvieae* occurred at lower prevalence and was dominated by *hly3*. Gene–lesion correlations included *aerA* with pale gills, *act* with skin discoloration, and *hlyA*, which showed inverse associations with fin and liver hemorrhages in *A. hydrophila*. In *L. garvieae*, *hly3* correlated with hepatomegaly, corneal opacity, and gill necrosis, while *hly2* was linked to skin discoloration. These findings underscore the value of genotype-informed surveillance for farm-level decision-making, risk assessment, and targeted disease control strategies in tilapia aquaculture.

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Conflict of interest

The authors declare that they have no conflicts of interest relevant to this publication.

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Ethics approval and consent to participate

The study was approved by the Institutional Review Board (or Ethics Committee) of ERES Converge IRB (reference number: 2019-AUG-024).

Consent for publication

Not applicable.

Availability of data

All data analyzed have been presented in the paper.

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