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Article

Isolation, molecular identification and antibiogram profiling of *Aeromonas hydrophila* (Chester, 1901) from farmed mrigal (*Cirrhinus mrigala*, Hamilton 1822) in Mymensingh region, Bangladesh

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Abstract: Motile Aeromonas Septicemia (MAS) is the most significant bacterial disease in freshwater aquaculture, causing substantial economic losses. This study aimed to isolate, identify, and evaluate the antibiogram profile of *Aeromonas hydrophila* from diseased mrigal. A total of 85 infected fish samples displaying clinical signs of MAS, including hemorrhages, fin erosion, scale loss, and dropsy, were collected from ten different farms. Bacterial loads were determined from the skin, liver, spleen, and kidney, and isolates were characterized using phenotypic, biochemical, physiological, and molecular methods. Molecular confirmation was performed by PCR, targeting a 760 bp amplicon specific to the isolated bacterium. Quantitative analysis revealed varying bacterial loads across organs, with high concentrations in visceral organs such as the kidney, liver, and spleen, indicative of systemic infection. The kidney showed the highest bacterial load, at 5.21 × 10^9 CFU/g, in fish samples from Bhaluka upazila. Antibiotic susceptibility testing against ten commonly used antibiotics demonstrated complete resistance to ampicillin (100%) and penicillin (90%), high resistance to novobiocin (80%) and oxytetracyclines (60%), moderate sensitivity to gentamicin (60%), and high sensitivity to fluoroquinolones (ciprofloxacin 90% and levofloxacin 80%). The calculated Multiple Antibiotic Resistance (MAR) index was 0.41, indicating low but significant multidrug resistance by *A. hydrophila*. These findings confirm the role of *A. hydrophila* in MAS infection in farmed mrigal and underscore the need for accurate disease diagnostics, rational antibiotic use, and continuous antimicrobial resistance surveillance in Bangladeshi aquaculture systems. The results provide a foundation for enhancing disease diagnosis, promoting prudent antibiotic use, and strengthening antimicrobial resistance surveillance in freshwater aquaculture.

Keywords: bacterial disease; motile aeromonas septicemia; aquaculture; multiple antibiotic resistance

1. Introduction

Aquaculture is a growing global sector that significantly contributes to food security, nutrition, and poverty alleviation. Indian major carps are the most widely cultured species in freshwater aquaculture and play an important role in food production of Bangladesh due to consumer preference, high market demand, and suitability for pond-based polyculture systems (Hossain *et al*., 2020). Mrigal is one of the preferred cultured carps, valued for its palatability, soft flesh, and high nutritional value. Its lower degree of natural reproduction also makes it a suitable candidate for freshwater farming systems (Ahmed *et al*., 2015; Hughes *et al*., 2025).

However, the intensification of aquaculture has increased the risk of disease outbreaks, particularly bacterial infections, leading to mortality or significantly diminished farm productivity (Ahmed *et al*., 2024).

Bacterial diseases are a major limiting factor for the sustainable farming of healthy carp, often occurring in stressful environments characterized by poor water quality, high stocking density, handling stress, and inadequate health management (Al-Jubouri *et al*., 2024; Diao *et al*., 2024). *Aeromonas hydrophila* is one of the most common bacterial pathogens, frequently reported as the cause of Motile Aeromonas Septicemia (MAS) in cultured carps and other freshwater fish (Ahmed *et al*., 2024). *A. hydrophila* is a Gram-negative, rod-shaped, and facultatively anaerobic opportunistic bacterium capable of causing systemic infection in stressed or immunocompromised fish (Hossain *et al*., 2020; Abdella *et al*., 2024; Jebesa *et al*., 2025).

Accurate and rapid diagnosis is essential for properly responding to outbreaks of MAS. Existing phenotypic and biochemical assays for *A. hydrophila* are laborious and prone to false positives due to phenotypic similarities among *Aeromonas* species (Ayoub *et al*., 2024). Consequently, molecular identification methods, particularly polymerase chain reaction (PCR) assays targeting conserved genetic regions like 16S rRNA or species-specific virulence genes, are now employed for definitive pathogen confirmation (Abdella *et al*., 2023; Ayoub *et al*., 2024).

Aquaculture practitioners often use chemotherapeutic interventions to control bacterial outbreaks. However, the untargeted and unrestricted use of antibiotics in aquatic environments has led to the emergence of multiple antibiotic-resistant *A. hydrophila* strains. The dissemination of aquaculture-associatedmultidrug-resistant (MDR) bacteria not only threatens effective treatment of infections but also poses a serious public health concern. This is due to the well-documented zoonotic exchange of resistance determinants through the food chain and aquatic environments (Janda and Abbott, 2010; Fernández-Bravo and Figueras, 2020).

The Mymensingh region plays a critical role in national carp production. However, there is a lack of contemporary studies integrating clinical diagnosis, molecular confirmation, and antimicrobial susceptibility profiling of *A. hydrophila* in cultured mrigal. This limits evidence-based disease management and antimicrobial stewardship. This study addressed the following research question: Can *A. hydrophila* isolated from diseased farmed mrigal in the Mymensingh region be reliably identified using conventional and molecular methods, and what is its current antibiotic susceptibility profile? We hypothesized that *A. hydrophila* is the primary bacterial pathogen associated with MAS in farmed mrigal and that circulating isolates exhibit multidrug resistance to commonly used antibiotics. Therefore, this study aimed to isolate and molecularly confirm *A. hydrophila* from clinically infected mrigal and determine its antibiogram profile. This will generate baseline evidence for improved disease diagnosis, antimicrobial stewardship, and sustainable freshwater aquaculture management. The novelty of this study lies in its integrated assessment of clinical findings, organ-wise bacterial load, conventional bacteriology, molecular confirmation, and antibiogram profiling of *A. hydrophila* from cultured mrigal in Bangladesh. The findings will provide crucial baseline evidence to support effective disease diagnosis, antimicrobial stewardship, and sustainable freshwater aquaculture management in the region.

2. Materials and Methods

2.1. Ethical approval

No ethical approval was required to conduct the study.

2.2. Study area and period

Diseased mrigal sampling was conducted on ten different mrigal farms in the Mymensingh region (24°38'3"N 90°16'4"E), encompassing six upazilas: Mymensingh Sadar, Trishal, Muktagacha, Fulbaria, Bhaluka, and Gauripur (Figure 1). The study area map was generated using ArcGIS 10.7 software and shows the sampling locations along with the corresponding samples collected from each upazila. This study was conducted for ten months, from May 2025 to February 2026.

2.3. Fish sample collection, transportation and biosafety

A total of 85 samples were collected from ten mrigal farms in the Mymensingh area where fish exhibited clinical signs of MAS i.e., including hemorrhagic lesions, fin rot, scale loss, abdominal dropsy, and high mortality. These guidelines were adapted from earlier literature on MAS pathology in koi and catfish in Bangladesh (Sarkar and Rashid, 2012; Borty *et al*., 2016). To ensure minimal contamination, diseased fish were aseptically collected from infected farms and transported in sterile boxes with ice packs (4-8 °C) to the Fish Disease and Health Management laboratory, Bangladesh Fisheries Research Institute (BFRI), within 24 hours of collection. This rapid transport aimed to facilitate accurate diagnosis and prevent microbial overgrowth (Nahar *et al*., 2016). Exterior lesions were swabbed with clean cotton swabs for bacteriological culture. Before

collecting bacterial samples from the skin, the fish surface was sterilized with 70% ethanol to prevent contamination. Visceral organs (i.e., liver, kidney, and spleen) were also aseptically collected. To prevent cross-contamination, tissue specimens were handled separately. All laboratory work adhered to hygiene and biosafety procedures, maintaining Biosafety Level 2 (BSL-2).

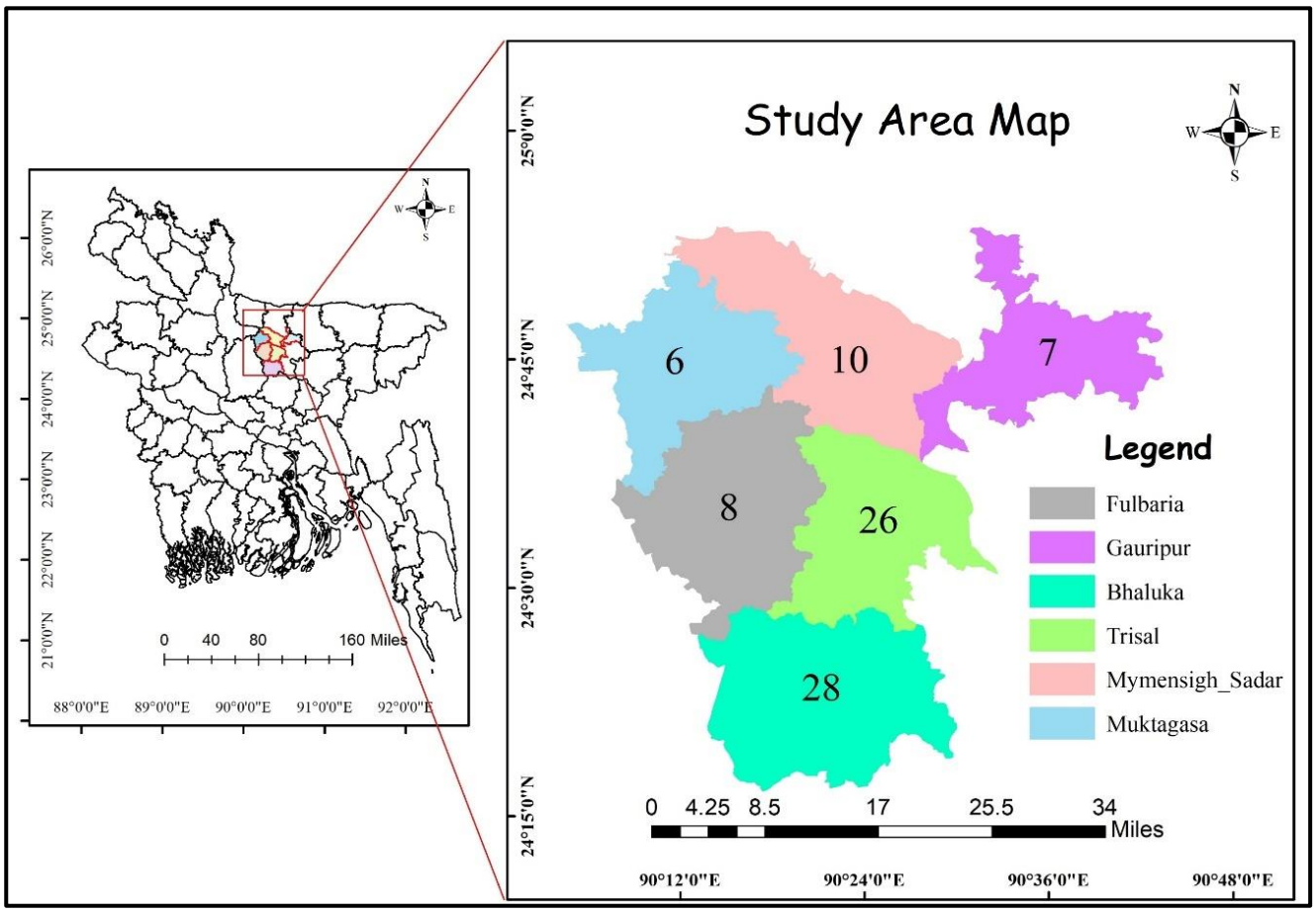

Figure 1. Sampling locations of diseased mrigal in the Mymensingh region, Bangladesh.

2.4. Clinical and postmortem examination

Infected mrigal from the study areas were examined to define the pathological alterations of MAS through detailed clinical and postmortem study. Gross clinical signs, including hemorrhages, ulcers, fin rot, and gill congestion, were recorded. Postmortem examination revealed marked changes in the visceral organs, including enlargement of the liver, kidneys, and spleen. The hepatic and renal parenchyma showed heavy congestion and hemorrhages, while the liver exhibited discoloration and patches of necrosis. Kidneys were swollen and pale, with occasional focal necrosis, and spleens were dark and enlarged. In a few cases, ascitic fluid accumulation (dropsy) in the abdominal cavity was also observed, indicating systemic bacterial infection. These clinical and postmortem findings were consistent with previous fish disease assessment methods in Bangladesh (Sarkar and Rashid, 2012).

2.5. Bacterial isolation and enumeration

2.5.1. Primary and selective culture

Skin, spleen, liver, and kidney tissues from infected mrigal were used as specimens for primary bacterial culture. After aseptic collection, the specimens were streaked onto tryptic soy agar plates and incubated for 24 hours at 30 °C. Following incubation, isolated colonies exhibiting unique *Aeromonas* spp. morphology were selected based on their biochemical properties, according to Narejo *et al.* (2005). For purity and successful isolation, these colonies were re-streaked and sub-cultured on Aeromonas Isolation Medium (AIM), a selective medium for *Aeromonas* spp. proliferation (Borty *et al.*, 2016). The resulting pure cultures were preserved for subsequent biochemical and molecular characterization.

2.5.2. Total viable count

Six fish, one from each upazila, were used for the quantitative investigations of *A. hydrophila*. First, a drop of blood was spread evenly on a Tryptic Soy Agar (TSA) plate and incubated for 24 hours for bacterial enumeration. The fish were then dissected, and samples of skin, gill, kidney, liver, and intestine were collected. A weighed amount of each tissue was placed in a sterile mortar, crushed with Phosphate-buffered saline (PBS) (0.1 g tissue: 0.9 ml PBS), and used to prepare a stock solution. Serial dilutions (10-1 to 10-8) were performed to enumerate *Aeromonas* colonies. From these dilutions, 0.1 ml of the 10-2 and 10-3 suspensions for skin, liver, and spleen, and the 10-3 and 10-4 suspensions for kidney, were spread onto AIM for the enumeration of *A. hydrophila* colonies. The total bacterial load for each organ was expressed using the formula described by Rashid *et al.* (2014).

$$\text{Total Bacterial Load} = \frac{\text{Average number of colonies on plates}}{\text{Dilution factor} \times \text{Volume plated}} \text{ CFU/g (CFU=Colony-forming unit)}$$

2.6. Bacterial characterization

Conventional identification of *A. hydrophila* was conducted based on phenotypic and biochemical characteristics. Presumed colonies from infected mrigal were sub-cultured on TSA plates, incubated for 24 hours, and then streaked onto AIM for confirmation of selective growth. Colonies exhibiting typical growth on AIM were sub-cultured onto TSA plates for biochemical tests. The conventional identification of *A. hydrophila* is depicted in Figure 2.

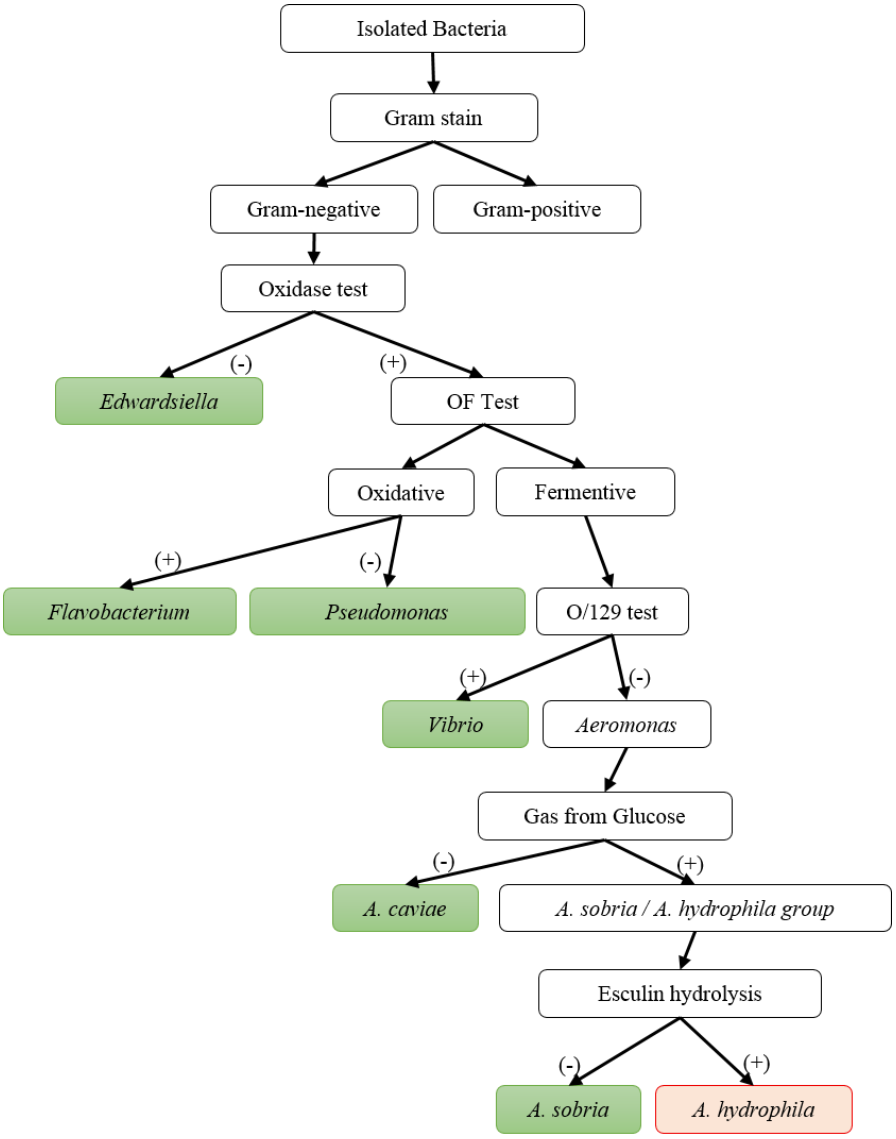

Figure 2. Schematic diagram for traditional detection of *A. hydrophila* [adopted from Ahmed *et al.* (2024)].

2.6.1. Phenotypic characterization

First, pure isolates from AIM were sub-cultured on TSA plates and incubated for 24 hours. Freshly grown colonies were then Gram-stained and observed under a 100× oil immersion microscope (LEICA MC170 HD, Leica Microsystems GmbH, Wetzlar, Germany) to determine their shape and Gram reaction. Motility of the isolated bacteria was assessed using the hanging drop method, and the results were observed on a PC screen.

2.6.2. Biochemical characterization

Young colonies were used for each biochemical test. Biochemical tests, including oxidase, catalase, OF, O/129 sensitivity, esculin hydrolysis, methyl red, Voges-Proskauer, indole, hydrogen sulfide (H_2S) production, citrate utilization, and TSI tests, were performed to detect the suspected bacterium from infected mrigal using a previously described method (Iqbal *et al*., 1996).

2.6.3. Physiological characterization

Physiological characteristics of the *A. hydrophila* isolates were analyzed in relation to temperature (4 °C, 37 °C, and 40 °C) and salinity (0%, 1%, 2%, 3.5%, and 4% NaCl).

2.7. Molecular detection

2.7.1. DNA extraction

Genomic DNA was extracted from overnight cultures of suspected bacteria in nutrient broth (NB) using lysozyme, SDS, and proteinase-K lysis, followed by ethanol precipitation, as per the protocols described by Rathore *et al.* (2005) and Sjogren and Goller (2023). A pure colony was inoculated into 10 ml of NB and incubated at 29 °C overnight. After centrifugation at 5000 rpm for 10 minutes, the cell pellet was resuspended in Solution I (Tris-HCl, EDTA, sucrose, and lysozyme) and incubated at 37 °C for 15 minutes to lyse the bacterial cells. Solution II (Tris-HCl, EDTA, SDS, and Proteinase K) was then added, and the mixture was incubated at 55 °C for approximately 3 hours to digest bacterial proteins. The samples were centrifuged again at 6000 rpm for 10 minutes, and the supernatant was transferred to a fresh tube. DNA was precipitated using chilled ethanol, and the pellet was washed with 70% ethanol, air-dried, and dissolved in 100 µl of TE buffer (pH 7.6).

2.7.2. PCR amplification

PCR amplification of the 760 bp *A. hydrophila*-specific fragment was performed described by Rathore *et al*. (2005). The primer sequences used for amplification are presented in Table 1. PCR was carried out in a DNA thermal cycler with slight modifications to the protocol of Borty *et al.* (2016). Amplification conditions included an initial denaturation at 94 °C for 4 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 65 °C for 1.5 min, and extension at 72 °C for 5 min, with a final extension at 72 °C for 5 min. The amplified PCR products were separated by electrophoresis on a 1.5% agarose gel and visualized under ultraviolet (UV) light.

Table 1. Primer sequences and expected amplicon size for PCR amplification of the *Aeromonas hydrophila* 16S rRNA gene.

Primers	Sequence (5′ → 3′)	Amplicon size (bp)	Reference
Forward	AACCTGGTTCCGCTCAAGCCGTTG	760	Nerjeo *et al.*
Reverse	TTGCCTCGCCTCGGCCCAGCAGCT		(2005)

2.8. Antibiogram profiling

Antibiotic sensitivity profiling was conducted on ten isolates against ten reference antibiotics using the disc diffusion method (Kirby-Bauer method) (Bauer *et al*., 1966). The antibiotics used were, including, Ampicillin (10 µg), Penicillin (10 µg), Novobiocin (5 µg), Chlortetracycline (25 µg), Oxytetracycline (10 µg), Tetracycline (30 µg), Azithromycin (15 µg), Gentamicin (10 µg), Levofloxacin (5 µg), and Ciprofloxacin (5 µg). Bacterial isolates were categorized as sensitive, intermediate, or resistant based on inhibition zone diameters, as per CLSI (2024) guidelines highlighted in Table 2. All laboratory analyses were performed at the Fish Disease and Health Management Laboratory, BFRI.

Table 2. Interpretation criterion for *Aeromonas hydrophila* (CLSI , 2024).

Name of antimicrobial agent	Disc concentration (µg)	Interpretation of results (zone in diameter in mm)		
		R	I	S
Ciprofloxacin	5	≥ 16	12 – 15	≤ 17
Levofloxacin	5	≥ 22	17 – 21	≤ 23
Gentamicin	10	≥ 14	10 – 13	≤ 15
Azithromycin	15	≥ 16	12 – 15	≤ 17
Tetracycline	30	≥ 14	09 – 13	≤ 15
Oxytetracycline	10	≥ 15	11 – 14	≤ 16
Chlortetracycline	25	≥ 16	13 – 15	≤ 17
Novobiocin	5	≥ 17	14 – 17	≤ 18
Ampicillin	10	≥ 22	16 – 21	≤ 23
Penicillin	10	≥ 14	10 – 13	≤ 15

R = resistant, I = intermediately resistant, S = susceptible; µg = microgram, mm = millimeter, ≥ = greater than or equal to, ≤ = less than or equal to.

2.9. Multiple Antibiotic Resistance (MAR)

MAR index of *A. hydrophila* was determined using the formula by Krumperman (1983). The results for the MAR index are presented in Table 3.

$$\text{MAR index} = \frac{\text{Number of antibiotics resisted}}{\text{Total number of antibiotics tested}}$$

Table 3. Interpretation of MAR index for *Aeromonas hydrophila* isolates.

MAR index range	Interpretation	References
≥ 0.5	High multidrug resistance	Krumperman (1983); Sandhu *et al.* (2016)
< 0.5	Low multidrug resistance	

3. Results

3.1. Clinical and post-mortem findings

Clinical signs observed in infected mrigal included erratic movement or loss of equilibrium, a few lesions on the body (Figure 3d), erosion of the caudal fin (Figure 3c), hemorrhage at the base of the fins and margin of the head (Figure 3a), and loss of scales (Figure 3b). High mortality was observed in the field. Postmortem findings revealed congestion and enlargement of internal organs, specifically the kidneys, liver, and spleen (Figure 3e).

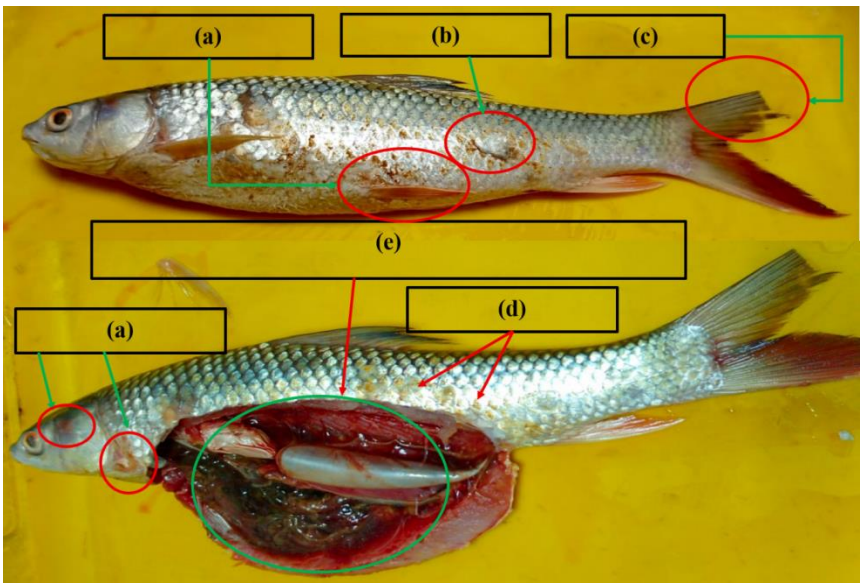

Figure 3. Clinical and post-mortem findings of infected mrigal, (a) highlights hemorrhage; (b) depicts scale loss; (c) erosion of caudal fin; (d) highlights lesions; (e) reflects congestion and enlargement of internal organs especially liver kidney.

3.2. Bacterial load in diseased mrigal

The visceral organs, predominantly the liver and spleen, were the major niches for heavy bacterial colonization. Hepatic loads peaked strikingly in cohorts from Bhaluka (5.21×10^9 CFU/g) and Trisal (3.90×10^9 CFU/g), while splenic tissues generally preserved elevated concentrations (1×10^7 CFU/g) across all sampled sites. In contrast, peripheral cutaneous lesions consistently remained at a steady-state, resting in the range of 1×10^5 CFU/g, with the exception of Trishal, which was skewed by a high cutaneous load (1×10^6 CFU/g). Renal colonization appeared to show higher geographical heterogeneity, with the greatest pathogen recovery seen in Sadar (1×10^7 CFU/g) and an unusual failure to colonize Gauripur (1×10^7 CFU/g) (Table 4).

Table 4. Bacterial load (CFU/g) in skin lesions, liver, spleen and kidney of diseased mrigal from Mymensingh district.

Location	Skin lesions (CFU/g)	Liver (CFU/g)	Spleen (CFU/g)	Kidney (CFU/g)
Sadar	1.88×10^5	2.52×10^7	2.54×10^8	5.28×10^7
Trishal	1.20×10^6	3.90×10^9	2.41×10^8	1.85×10^7
Bhaluka	3.21×10^5	5.21×10^9	3.45×10^8	3.78×10^6
Muktagacha	2.14×10^5	5.41×10^8	1.09×10^8	3.51×10^7
Fulbaria	3.41×10^5	3.54×10^5	1.90×10^7	4.89×10^7
Gauripur	2.70×10^5	2.54×10^8	2.32×10^7	3.63×10^5

CFU= colony-forming units

3.3. Bacterial characterization (traditional detection)

All presumptive isolates were rod-shaped, motile, oxidase-positive, catalase-positive, and fermentative in O/F tests, according to biochemical characterizations. Isolates fermented glucose, lactose, sucrose, maltose, and mannitol, but not inositol, sorbitol, or rhamnose. Esculin hydrolysis, methyl-red, Voges-Proskauer, indole, H2S production, arginine decomposition, and citrate utilization were all positive; however, lysine and ornithine decarboxylation reactions were negative. The isolates were K/A on TSI agar and grew at 5 °C and 37 °C, but not at 4 °C or 40 °C (Table 5).

Table 5. Comparative phenotypic, biochemical, and physiological characterization of presumptive *Aeromonas hydrophila* isolates.

Traits	Characterization by Mostafa and Ahamed (2008)	Characterization by Borty *et al.* (2016)	Present result
"Gram's stain"	–	–	–
"Shape"	"Rod"	"Rod"	"Rod"
"Motility"	+	+	+
"O/129"	"NP"	–	–
"Oxidase"	+	+	+
"Catalase"	+	+	+
"OF test"	"F"	"F"	"F"
"Glucose"	+	+	+
"Lactose"	+	+	+
"Sucrose"	+	+	+
"Maltose"	+	+	+
"Manitol"	+	+	+
"Inositol"	–	–	–
"Sorbitol"	–	–	–
"Rhamnose"	–	–	–
"Esculin hydrolysis"	+	+	+
"Methyl-red test"	+	+	+
"Voges-Proskauer"	+	+	+
"Indole"	+	+	+
"H_2S production"	+	+	+
"Arginine decomposition"	+	+	+
"Lysine decarboxylation"	–	–	–
"Ornithine decarboxylation"	–	–	NP

Table 5. Contd.

Traits	Characterization by Mostafa and Ahamed (2008)	Characterization by Borty *et al.* (2016)	Present result
"Citrate utilization"	+	+	+
"TSI"	"NP"	"K/A"	"K/A"
"Growth at 4 °C"	–	–	–
"Growth at 5 °C"	+	+	+
"Growth at 37 °C"	+	+	+
"Growth at 40 °C"	–	–	–

"+" = positive; "–" = negative; "NP" = not performed; "F" = fermentative; "K/A" = 'K' (Alkaline) in slants but 'A' (acidic) in butt; TSI = triple sugar iron

3.4. Molecular detection

Molecular identification of *A. hydrophila* was performed using PCR amplification of the species-specific 16S rRNA gene (760 bp). Figure 4 confirms the suspected isolates as *A. hydrophila*.

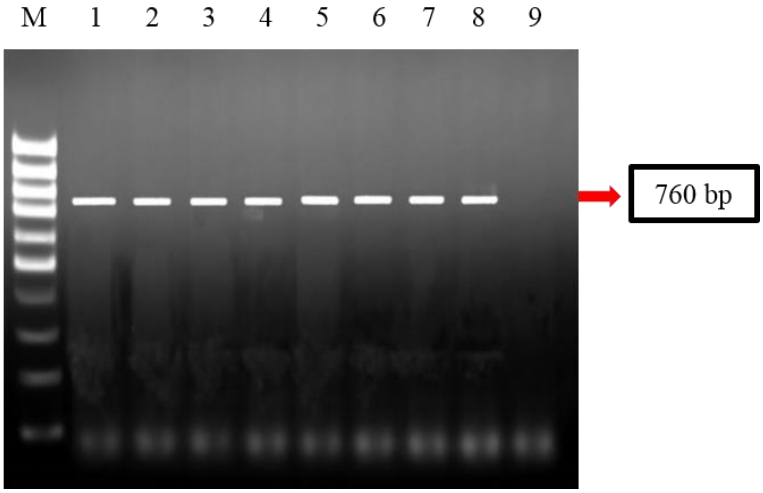

Figure 4. Agarose gel electrophoresis of PCR amplification generated by *Aeromonas hydrophila.* Lanes: (M) 100 bp DNA marker; 1-8 positive samples from field; 9 is negative control.

3.5. Antibiogram profiling

Aeromonas hydrophila isolates were examined for susceptibility to ten commercially available antibiotics, and the results are presented in Table 6. *Aeromonas hydrophila* showed complete resistance to novobiocin (80%), ampicillin (100%), and penicillin (90%). Moderate resistance was exhibited to chlortetracycline (40%) and oxytetracycline (60%). On the other hand, gentamicin (60%) and azithromycin (60%) exhibited moderate sensitivity, while ciprofloxacin (90%) and levofloxacin (80%) were found to be highly effective against *A. hydrophila* isolates.

Table 6. Antibiogram profile of isolated *Aeromonas hydrophila* (n=10).

Antibiotics	No. (%)		
	Sensitive (count)	Intermediate (count)	Resistant (count)
Ciprofloxacin (5 µg)	9 (90)	1 (10)	0 (0)
Levofloxacin (5 µg)	8 (80)	2 (20)	0 (0)
Gentamicin (10 µg)	6 (60)	4 (40)	0 (0)
Azithromycin (15 µg)	6 (60)	3 (30)	1 (10)
Tetracycline (30 µg)	2 (20)	5 (50)	3 (30)
Oxytetracycline (10 µg)	0 (0)	4 (40)	6 (60)
Chlortetracycline (25 µg)	1 (10)	5 (50)	4 (40)
Novobiocin (5 µg)	0 (0)	2 (20)	8 (80)
Ampicillin (10 µg)	0 (0)	0 (0)	10 (100)
Penicillin (10 µg)	0 (0)	1 (10)	9 (90)

3.6. MAR index

The MAR index was calculated to confirm the extent of multidrug resistance in the *A. hydrophila* isolates. A total of 100 antibiotic samples were used, and 41 were found to be resistant, resulting in a MAR index value of 0.41.

4. Discussion

The present study provides valuable evidence regarding the isolation, identification, and antibiotic sensitivity profiling of *A. hydrophila* from diseased *C. mrigala* in the Mymensingh region of Bangladesh. The clinical and postmortem findings, including hemorrhages, fin erosion, scale loss, dropsy, and congestion of internal organs, are consistent with the characteristic features of MAS. The presence of both external lesions and internal organ congestion suggests that the infection was not restricted to the skin or gills but had progressed to septicemia, affecting multiple organs. These results validate previous studies in carp and catfish, where *A. hydrophila* has been identified as a prominent causative organism of septicemia and mortality (Sarkar and Rashid, 2012; Borty *et al*., 2016; Anyanwu *et al*., 2020; Hasan *et al*., 2021; Das *et al*., 2022).Therefore, the clinical and post-mortem observations of the present study support the role of *A. hydrophila* as an important bacterial pathogen in mrigal culture systems in the Mymensingh region.

The isolation of bacteria from vital organs such as the liver, spleen, and kidney is particularly noteworthy. These organs are frequently targeted during systemic bacterial infections due to their central roles in blood filtration, immune sensitization, and detoxification. The isolation of *A. hydrophila* from these organs suggests that the pathogen entered through the fish's circulatory system, leading to systemic disease. This finding aligns with necropsy and histopathology observations typically seen in MAS, where *Aeromonas* species infect internal tissues, causing hemorrhage, congestion, necrosis, and organ dysfunction. Ahmed *et al*. (2024) also reported that *A. hydrophila* causes systemic infection. Thus, the organ-wise isolation pattern in this study further indicates that systemic aeromonad infection, rather than localized bacterial illness, could be attributed to mrigal.

Gram staining, motility testing, and biochemical properties of the bacterial isolates were initially assessed for the presumptive identification of *A. hydrophila*. These isolates exhibited a Gram-negative reaction and motility, characteristics consistent with motile *Aeromonas* species. These classical diagnostic techniques remain valuable due to their cost-effectiveness, simplicity, and practicality for routine use in fish disease laboratories. However, phenotypic and biochemical properties alone are insufficient for definitive species-level identification, as closely related *Aeromonas* species often share indistinguishable morphological and biochemical traits. Therefore, molecular confirmation was necessary to enhance identification reliability (Ahammed *et al*., 2016; Ayoub *et al*., 2024).

In this study, PCR amplification generated the predicted 760 bp amplicon for *A. hydrophila*. This specific PCR product enabled more secure identification of the isolates compared to phenotypic testing alone. Furthermore, integrating conventional bacteriological methods with molecular PCR detection enhanced the significance of the results. While previous studies in Bangladesh identified presumptive *A. hydrophila* isolates from diseased fish using cultural, staining, and biochemical procedures (Borty *et al*., 2016; Monir *et al*., 2017; Ferdousi *et al*., 2025), PCR-based amplification of the 760 bp DNA fragment was performed here to provide further confirmatory evidence. Molecular confirmation is crucial because the *Aeromonas* genus comprises several closely related species with similar biochemical characteristics. Most importantly, the presence of *A. hydrophila* was reaffirmed, providing double confirmation of its involvement in the disease affecting mrigal.

One of this most startling outcomes of this study is the antibiogram profile. All isolates exhibited complete resistance to Ampicillin and Penicillin, with prevalent resistance also observed for Novobiocin and Tetracyclines. This resistance pattern aligns with previous observations that *A. hydrophila* inherently possesses resistance to multiple β-lactam antibiotics (Ahammed *et al*., 2016; Nhinh *et al*., 2021; Khan *et al*., 2023) and is acquiring resistance to widely used agents due to the overutilization of antibiotics in aquaculture (Janda and Abbott, 2010). Conversely, Ciprofloxacin and Levofloxacin demonstrated exceptional efficacy, with sensitivity rates of 90% and 80%, respectively, while gentamicin showed moderate efficacy. These findings mirror previous studies in Bangladesh and other Asian countries, where fluoroquinolones continue to emerge as the most efficacious class of antibiotics against *A. hydrophila* (Ahammed *et al*., 2016; Monir *et al*., 2017; Rahman *et al*., 2021; Ferdousi *et al*., 2025; Siddiquee *et al*., 2026). However, these latest findings should not be interpreted as a blanket recommendation for widespread fluoroquinolone use in fish farms. In vitro susceptibility, as measured by disc diffusion testing, only partially reflects their effectiveness against infections. Treatment decisions in aquaculture must adhere to national regulations and withdrawal periods, consider the fish species or genotypes present in culture facilities and disease severity, and uphold the principles of antimicrobial stewardship.

The MAR index obtained for the different samples is a true indicator of the level of antimicrobial resistance in the aquaculture environment. The isolates in the current study achieved a mean MAR index of 0.41, with some individual isolates reaching values of 0.5. A MAR index higher than 0.2 could therefore classify a pathogen as originating from a "high-risk" environment, as it would be exposed to uninterrupted and unregulated antimicrobial pressures, both biologically and epidemiologically (Krumperman, 1983). A mean of 0.41 indicates that the pathogen has evolved functional immunity to approximately 40% of the commercial therapeutics surveyed, while an index of 0.5 indicates resistance to half of the available medical arsenal. This disturbing quantification is in exact harmony with recent findings where observed comparably higher MAR indices across farmed areas of domestic catfish (Sughra *et al*., 2022; Ferdousi *et al*., 2025). Apart from these, commercial mrigal culture systems have been directly revealed as potential high-density reservoirs for MDR bacteria. Consequently, the very high MAR indices reported here not only impact aquaculture economics but also present an urgent and compounding risk of temperature-mediated zoonotic transmission of *A. hydrophila* to humans, which can cause both gastroenteritis and septicemia. This elevates One Health and public safety concerns at the regional scale, particularly since these threats are occurring in areas where they have previously been unrecognized globally.

5. Conclusions

This study confirms the baseline emergence of MAS in farmed mrigal from Mymensingh. Phenotypic and molecular validations established the specific pathogenic threat. Alarming multi-drug resistance patterns emphasize a critical public health risk, requiring strict antibiotic surveillance and enhanced biosecurity. Future research should investigate plasmid-mediated resistance transmission pathways to address current epidemiological knowledge gaps.

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Data availability

Upon reasonable request, the corresponding author will provide the raw data supporting the research article’s conclusions.

Conflict of interest

None to declare.

Authors’ contribution

Farjana Jannat Akhi: designed the study, conducted research work, and interpreted the data, wrote the first draft of the manuscript; Kutub Uddin: data collection, analyzed and interpreted the data, wrote the first draft of the manuscript; Mushfika Jannat: writing-reviewing and editing; Md. Shirajum Monir: facilitated to conduct research work, provide finding support, supervision and critically reviewed the manuscript. All authors have read and approved the final manuscript.

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