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Clinical Lesions and Presumptive Bacterial Associations with Fin and Tail Rot in Ornamental Goldfish

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Abstract

Background: Fin and tail rot is a common clinical syndrome in ornamental goldfish, but distinguishing disease-associated bacteria from background carriage remains challenging. This study characterised clinical and gross pathological abnormalities and compared presumptive Aeromonas and Pseudomonas recovery between affected and apparently healthy goldfish.

Methods: A total of 300 fish from five aquarium shops in Bannu, Pakistan, were examined: 250 clinically affected and 50 apparently healthy. Of the affected fish, 235 underwent postmortem examination and bacterial culture, while 15 were monitored for 24 days under improved husbandry conditions. Isolates were classified presumptively using colony morphology, Gram staining, and microscopy, and group differences were statistically assessed.

Results: Presumptive Aeromonas and Pseudomonas were recovered from 150/235 (63.8%) and 85/235 (36.2%) affected fish, respectively, compared with 15/50 (30.0%) and 4/50 (8.0%) apparently healthy fish. Recovery distributions differed significantly (χ^2 (2) = 164.33, $p < 0.001$; Cramer's V = 0.759), with higher odds of Aeromonas (OR = 4.12) and Pseudomonas (OR = 6.52) recovery in affected fish. Fish length was not associated with bacterial classification. Clinical signs included fin erosion, haemorrhage and ulceration, alongside variable gross internal abnormalities. Twelve of 15 monitored fish (80.0%) showed resolution of visible signs.

Conclusion: These findings indicate an association between clinical disease and increased recovery of presumptive bacterial groups, despite their presence in apparently healthy fish. Species identity, pathogenic causation, and husbandry efficacy remain unconfirmed without molecular identification, controlled infection studies, and untreated intervention controls. Clinical improvement also could not establish bacterial clearance because fish with resolved signs were not retested bacteriologically after observation.

Keywords: Carassius auratus; fin rot; tail rot; ornamental fish; bacterial disease; Aeromonas; Pseudomonas

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Lay summary

Fin and tail rot is an important problem in ornamental goldfish. Aeromonas and Pseudomonas were more common in affected fish, although they were also detected in some apparently healthy fish. Most monitored fish showed visible clinical improvement under improved husbandry conditions.

1. Introduction

Goldfish (Carassius auratus) are among the most widely cultured and traded ornamental fish worldwide. Intensive stocking, poor water quality, nutritional deficiencies, and handling and transport stress can weaken host defences, increase disease susceptibility, and cause mortality and economic losses (Le Verger et al., 2024; Masud et al., 2019). Disease development in ornamental fish involves host-pathogen-environment interactions, with poor water quality, overcrowding, nutritional deficiencies, and physical injury increasing susceptibility to opportunistic infections. Aquarium water, sediments, and biofilms may also serve as reservoirs of potentially pathogenic and antimicrobial-resistant bacteria (Dhanapala et al., 2021). Dietary probiotic supplementation has also been reported to improve growth and immune-related parameters in cultured fish, illustrating how husbandry and nutritional management can influence host condition (Hameed et al., 2025).

Bacterial infections in ornamental goldfish can cause lethargy, reduced feeding, abnormal swimming, fin erosion, ulceration, scale loss, abdominal swelling and haemorrhage, sometimes affecting internal organs. Gram-negative bacteria, particularly Aeromonas and Pseudomonas, have been associated with bacterial diseases in ornamental fish (de Oliveira et al., 2024; Sun et al., 2021). Among these manifestations, progressive fin and tail deterioration is particularly important because it may arise from several infectious and non-infectious processes.

Fin and tail rot are characterised by discolouration, fraying, splitting, progressive erosion and, in advanced cases, necrosis of fin tissue (Estante-Superio et al., 2021). Reliable etiological investigation requires clinical examination, postmortem assessment, aseptic tissue sampling, bacterial isolation and appropriate phenotypic or molecular identification (Cardoso et al., 2021). Recovery of opportunistic bacteria does not establish causation, and phenotypic methods provide only presumptive identification; reliable species-level classification requires biochemical or molecular confirmation (Testerman et al., 2023).

Antimicrobial resistance among fish-associated Aeromonas, Pseudomonas, and other Gram-negative bacteria is an additional concern because ornamental fish and transport water may harbour resistant organisms and resistance determinants (de Oliveira et al., 2024; Dhanapala et al., 2021; Verner-Jeffreys et al., 2009). This highlights the importance of accurate bacterial identification and susceptibility-guided treatment rather than empirical antimicrobial use.

Clinical and bacteriological data on goldfish remain limited in Pakistan, particularly in Khyber Pakhtunkhwa, with scarce comparisons between diseased and apparently healthy cohabiting fish to distinguish disease-associated bacterial enrichment from background carriage. This study addresses this gap through clinical, postmortem, bacteriological, fish-length, and husbandry-observation analyses (Mahmood et al., 2024).

Accordingly, this study aimed to: (i) characterise clinical and gross pathological abnormalities associated with fin and tail rot in ornamental goldfish, (ii) compare presumptive Aeromonas and

Pseudomonas recovery between affected and apparently healthy fish from shared aquarium environments, (iii) assess associations between fish length and bacterial classification, and (iv) document visible clinical changes under improved husbandry conditions in a subset of affected fish. Bacterial identification was limited to the presumptive genus level, and husbandry outcomes were interpreted descriptively rather than as evidence from a controlled therapeutic trial.

2. Methods

2.1 Study design and sample collection

A comparative cross-sectional study examined 300 ornamental goldfish (Carassius auratus), comprising 250 clinically affected and 50 apparently healthy fish from five aquarium shops in District Bannu, Pakistan, between September and December 2024. Affected fish were selected based on visible fin or tail erosion, haemorrhage, ulceration, discolouration, scale loss, lethargy, or abnormal swimming (Jung et al., 2024). Fish measured 2.5-4.0 inches in length. Of the 250 affected fish, 235 underwent postmortem and bacteriological examination, while 15 were assigned to husbandry observation. The 50 apparently healthy fish from the same aquaria served as controls, with subsequently detected internal abnormalities recorded separately (Luo et al., 2024). Clinical examinations were conducted at the Department of Zoology, University of Science and Technology Bannu.

2.2 Acclimatisation, Clinical and Postmortem Examination

Upon arrival, fish were transferred to aerated aquaria for acclimatisation and clinical assessment. Acclimatisation duration, aquarium dimensions, water volume, source, stocking density, and feeding regime were not documented (Khan et al., 2025). During laboratory maintenance, water temperature (25.0-28.5 °C), pH (7.0-7.9), and dissolved oxygen (4.5-4.9 mg L^{-1}) were monitored using a laboratory thermometer, digital pH meter, and dissolved-oxygen meter, respectively, with assistance from the Department of Chemistry, University of Science and Technology Bannu. Instrument manufacturers and models were not recorded.

All fish were examined for behavioural and external abnormalities, including lethargy, abnormal swimming, loss of equilibrium, skin discolouration, scale loss, haemorrhage, ulceration, fin congestion, inter-ray membrane loss, fin-ray erosion and greyish fin-margin lesions (Li et al., 2021; Fudeshima et al., 2025). The 50 apparently healthy fish were assessed using identical criteria and showed no visible abnormalities at examination. Fifteen clinically affected fish were retained for monitoring clinical progression or resolution, while fish assigned to bacteriological investigation underwent postmortem examination and tissue sampling.

The liver, kidney, spleen and intestine were examined for congestion, enlargement, pallor, haemorrhage, inflammation, ascites and necrotic lesions (Magouz et al., 2024). Tissue samples from these organs were subsequently collected aseptically for bacterial isolation.

2.3 Preparation of Culture Media, Tissue Sampling and Bacterial Isolation

Nutrient agar was prepared according to the manufacturer's instructions, autoclaved at 121 °C for 15 min, and aseptically dispensed (approximately 25 mL per plate) into sterile Petri dishes within a laminar-flow cabinet. After solidification at room temperature, plates were labelled by fish identification number and tissue type (Thermo Fisher Scientific, 2024). As a non-selective medium, nutrient

agar was used for primary bacterial isolation, with colony characteristics providing only preliminary differentiation of presumptive Aeromonas and Pseudomonas isolates (Cheok et al., 2020).

Tissue samples were aseptically collected from the liver, kidney, spleen and intestine of 285 goldfish (235 clinically affected and 50 apparently healthy). Internal-organ sampling was prioritised because bacterial recovery from external lesions may include environmental contaminants or secondary colonisers (Walczak et al., 2017).

Collected tissue material was directly streaked onto separately labelled nutrient-agar plates using the quadrant-streak method (Mazumder et al., 2021). Plates were incubated aerobically at 37 °C for 24 h and examined for colony abundance, pigmentation and morphology. Representative colonies were subsequently subcultured onto fresh nutrient agar at 37 °C for 24-48 h, with repeated subculturing until morphologically uniform, contamination-free cultures were obtained (Verner-Jeffreys et al., 2009).

2.4 Gram Staining and Presumptive Phenotypic Identification

Representative purified isolates were Gram-stained using crystal violet, iodine, ethanol, and safranin, followed by microscopic examination under a ×100 oil-immersion objective to assess Gram reaction, cell shape, and arrangement (Tripathi et al., 2025). Gram-negative, rod-shaped isolates were considered broadly compatible with Aeromonas and Pseudomonas spp. (Bureros et al., 2022). Isolates were further characterised by colony colour, pigmentation, shape, size, margin, elevation and surface appearance on nutrient agar (Mazumder et al., 2021). Based on these phenotypic and microscopic characteristics, isolates were provisionally classified as Aeromonas or Pseudomonas (Dubey et al., 2021).

2.5 Interpretation of Bacterial Recovery, Isolate Storage and Husbandry Observation

Bacterial recovery from internal organs indicated bacterial presence but did not confirm systemic infection, as histopathology, tissue-specific data, and molecular identification were unavailable (Sadiq et al., 2024; Shabana et al., 2025). Because opportunistic bacteria may act as primary pathogens, secondary invaders, or components of mixed infections, their association with fin and tail rot did not establish causation without experimental challenge and re-isolation (Behera et al., 2023). Purified presumptive Aeromonas and Pseudomonas isolates were separately labelled and stored in nutrient broth containing 30% glycerol at 4 °C for short-term maintenance rather than long-term cryopreservation at approximately −80 °C (Evans, 2024; Nahm, 2025).

Fifteen affected goldfish were monitored for 24 days under improved husbandry conditions, including aeration, complete water renewal every three days, and NaCl at 4 g L^{-1}, without antimicrobials. Water temperature, pH, and dissolved oxygen were maintained at 25.0-28.5 °C, 7.0-7.9, and 4.5-4.9 mg L^{-1}, respectively. Water renewal aimed to reduce opportunistic bacterial exposure (Smith et al., 2012), while NaCl supplementation remained within reported goldfish tolerance ranges of approximately 6 g L^{-1} and short-term baths at 5 g L^{-1} (Burgdorf-Moisuk et al., 2011). After 24 days, clinical signs were reassessed, and fish with persistent abnormalities underwent bacteriological examination using the previously described procedures.

2.6 Statistical analysis

The association between fish-length category and presumptive bacterial group was assessed using Pearson's chi-square

test of independence, with Cramer's V calculated as a measure of effect size (McHugh, 2013). Bacteriological distributions were compared using Pearson's chi-square test with Cramer's V, while 2 × 2 analyses used odds ratios, 95% CIs, and Fisher's exact test. All analyses were performed in IBM SPSS Statistics 27.0 (McCormick & Salcedo, 2017). Statistical significance was defined as $p < 0.05$.

3. Results

3.1 Characteristics of the examined fish and water quality

A total of 235 clinically affected goldfish (Carassius auratus; 2.5-4.0 inches) were included in the bacterial distribution analysis. The 3.3-inch group was most common (62/235; 26.4%), followed by 3.5-inch (49; 20.9%), 3.7-inch (40; 17.0%), 3.0-inch (36; 15.3%), 2.5-inch (28; 11.9%) and 4.0-inch fish (20; 8.5%) (Figure 1). An additional 50 apparently healthy fish were analysed separately for bacteriological comparison (Figure 2).

Annexure (A)

Among the 15 clinically affected goldfish maintained under improved aquarium hygiene conditions, 12 (80.0%) showed resolution of visible clinical abnormalities by the end of the 24-day observation period, whereas three (20.0%) continued to exhibit visible clinical signs (Table 2).

Table 2. Clinical and bacteriological outcomes of the 15 fish maintained under aquarium-sanitation and water-renewal conditions.

Outcome	Number of fish, n	Percentage (%)
Resolution of visible clinical signs	12	80.0
Persistent signs; presumptive P. fluorescens	1	6.7
Persistent signs; presumptive A. hydropheohila	2	13.3
Total	**15**	**100.0**

Taken together, presumptive Aeromonas was recovered from 150/235 fish (63.8%) and presumptive Pseudomonas from 85/235 (36.2%). Across length categories, Aeromonas recovery ranged from 55.0% to 72.6% and Pseudomonas from 27.4% to 45.0%. The highest Aeromonas recovery occurred in 3.3-inch fish (45/62; 72.6%), whereas Pseudomonas was highest in the 4.0-inch group (9/20; 45.0%). During observation, water temperature was 25.0-28.5 °C, pH 7.0-7.9, and dissolved oxygen 4.5-4.9 mg L^{-1}.

3.2 Association between fish length and bacterial group

Among 235 bacteriologically examined fish, presumptive Aeromonas accounted for 63.8% (150/235) and presumptive Pseudomonas for 36.2% (85/235). Across length categories (2.5-4.0 inches), Aeromonas proportions ranged from 55.0% to 72.6% and Pseudomonas from 27.4% to 45.0%, with the highest Aeromonas proportion in 3.3-inch fish (72.6%) (Figure 2). Bacterial classification did not differ significantly among length categories (χ^2 (5) = 3.76, p = 0.585; Cramer's V = 0.126), and logistic regression likewise showed no association with fish length (OR = 1.00 per inch, 95% CI: 0.52-1.92, p = 0.997). Thus, fish length was not associated with presumptive bacterial classification.

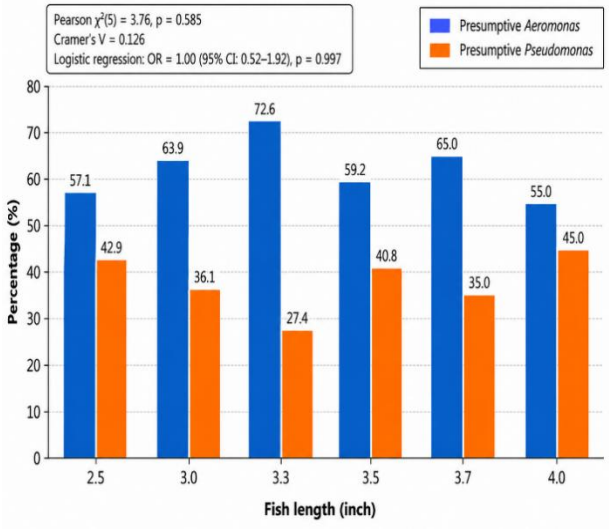


Figure 2. Distribution of presumptive

Aeromonas and presumptive Pseudomonas classifications across fish-length categories with statistical results.

3.3 Bacterial Recovery and Clinical and Postmortem Findings

Among the 50 apparently healthy goldfish, presumptive Aeromonas isolates were recovered from 15 fish (30.0%), presumptive Pseudomonas isolates from 4 fish (8.0%), and neither bacterial group from 31 fish (62.0%). In comparison, among the 235 clinically affected fish, 150 (63.8%) yielded presumptive Aeromonas and 85 (36.2%) yielded presumptive Pseudomonas. The overall distribution differed significantly between clinically affected and apparently healthy fish (χ^2 (2) = 164.33, $p < 0.001$; Cramer's V = 0.759). Clinically affected fish had significantly greater odds of yielding presumptive Aeromonas (OR = 4.12, 95% CI: 2.13-7.97; Fisher's exact $p < 0.001$) and presumptive Pseudomonas (OR = 6.52, 95% CI: 2.27-18.73; Fisher's exact $p < 0.001$).

Table 1. Bacterial recovery from clinically affected and apparently healthy goldfish.

Fish group	N	Tissues sampled	Presumptive Aeromonas	Presumptive Pseudomonas	Neither group
Clinically affected	235	Liver, kidney, spleen, intestine; lesion margins where present	150 (63.8%)	85 (36.2%)	0 (0%)
Apparently healthy	50	Liver, kidney, spleen and intestine	15 (30.0%)	4 (8.0%)	31 (62.0%)

All 250 clinically affected fish exhibited fin or tail abnormalities, including congestion, fraying, membrane loss, ray erosion and greyish discolouration, accompanied by variable lethargy, abnormal swimming, scale loss, ulceration and haemorrhage around the fin bases, abdomen, mouth and anal opening (Figure 3A-C). Postmortem examination revealed variable hepatic congestion, haemorrhage, enlargement, pallor and necrotic-appearing lesions; renal and splenic enlargement or congestion; intestinal inflammation; abdominal distension, dropsy and reddish ascites (Figure 3D).



Figure 3. Clinical and postmortem abnormalities in diseased goldfish (Carassius auratus): (A-C) fin and tail erosion with body discoloration; (D) gross internal abdominal lesions.

3.4 Gram-staining and microscopic characteristics

Repeated subculturing yielded morphologically uniform colonies representing two predominant groups with characteristics provisionally compatible with Aeromonas and Pseudomonas (Figure 4).

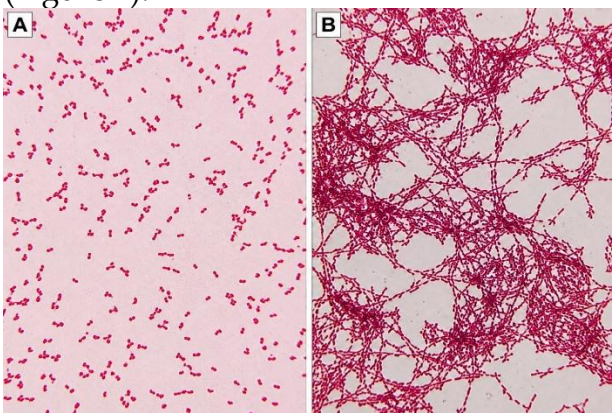


Figure 4. Gram-stained bacterial isolates recovered from diseased ornamental goldfish: (a) Gram-negative rod-shaped cells provisionally assigned to Aeromonas and (b) Gram-negative rod-shaped cells provisionally assigned to Pseudomonas. Magnification: ×1000 under oil immersion.

Gram staining revealed pink-to-red, predominantly rod-shaped cells under the ×100 oil-immersion objective, confirming Gram-negative morphology in both groups (Figure 5). Based on colony pigmentation, morphology, Gram reaction, and microscopic characteristics, isolates recovered from 150 and 85 fish were provisionally classified as Aeromonas and Pseudomonas, respectively. These assignments remained presumptive because species-specific biochemical or molecular confirmation was not performed, and Gram staining alone could not differentiate A. hydrophila from P. fluorescens.

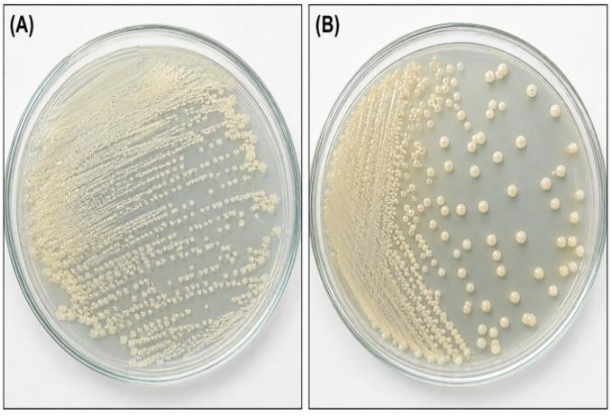


Figure 5. Representative colony morphology of the two predominant bacterial groups on nutrient agar: (A) colonies provisionally assigned to the Pseudomonas group and (B) cream-coloured circular colonies provisionally assigned to the Aeromonas group following streak culture.

4. Discussion

4.1 Clinical, Pathological and Bacteriological Associations with Fin and Tail Rot

The fin fraying, inter-ray membrane loss, fin-ray erosion, haemorrhage and tissue damage observed in this study are consistent with bacterial fin and tail disease reported in cultured fish. Similar lesions have been reproduced experimentally following infection with Aeromonas hydrophila, A. jandaei and Vibrio harveyi-like bacteria (Estante-Superio et al., 2021; Mazumder et al., 2021). Environmental stress and physical injury may damage the protective skin barrier of fish, increasing their susceptibility to opportunistic bacterial infections (Nakatani & Hori, 2021). However, these lesions are not pathogen-specific, as comparable abnormalities have been associated with Aeromonas, Edwardsiella tarda, and Pseudomonas fluorescens infections in goldfish and other freshwater fish (Sun et al., 2021; Mahmood et al., 2024). Thus, gross pathological changes in the liver, kidney, spleen, and intestine could not be attributed to particular bacterial groups or confirmed as systemic infection without histopathological and tissue-specific evidence, supporting a multifactorial interpretation of fin and tail rot (de Oliveira et al., 2024).

Two Gram-negative, rod-shaped bacterial groups were provisionally classified as Aeromonas and Pseudomonas, consistent with bacterial diversity reported in diseased ornamental fish and their environments (Walczak et al., 2017; Cheok et al., 2020). Their identification remained presumptive because closely related species share overlapping phenotypic characteristics influenced by culture conditions and strain variation (Quintieri et al., 2020). Genomic investigations further highlight the taxonomic complexity of pseudomonads (Kumru, 2026). Reliable species identification requires biochemical or molecular confirmation, including housekeeping-gene analysis (gyrB, rpoB and rpoD) or whole-genome sequencing

(Duman et al., 2024). Consequently, the isolates cannot be confirmed as *A. hydrophila* or P. fluorescens, and their recovery does not establish causation because they may represent primary pathogens, secondary colonisers or components of mixed infections. Definitive pathogenicity would require experimental infection followed by re-isolation.

Presumptive Aeromonas and Pseudomonas recovery was significantly higher in affected than apparently healthy goldfish (63.8% vs. 30.0% and 36.2% vs. 8.0%, respectively; χ^2 (2) = 164.33, $p < 0.001$; Cramer's V = 0.759), with approximately fourfold and sixfold greater odds, respectively (Figure 6). Their recovery from healthy fish suggests potential asymptomatic carriage rather than disease-specific occurrence (Dhanapala et al., 2021). Aquarium water and biofilms may harbour diverse Aeromonas species (Cheok et al., 2020), while microbiome studies have reported Aeromonas and Pseudomonas in apparently healthy systems and increased opportunistic bacterial abundance in aquaria containing stressed fish (Shen et al., 2026).

These findings are consistent with a host-pathogen environment framework in which bacterial carriage may coexist with apparent health, while tissue injury, overcrowding, transport stress and deteriorating water quality can increase disease susceptibility (Masud et al., 2019; Vanderzwalmen et al., 2021). Associations between microbial dysbiosis, opportunistic bacterial proliferation and poorer fish-health status have also been reported (Shen et al., 2026). Aquarium water, sediments and biofilms may facilitate persistent bacterial exposure (Cheok et al., 2020; Dhanapala et al., 2021), whereas routine water changes and substrate cleaning have been associated with improved microbial balance (Shen et al., 2026). Accordingly, maintaining water quality, reducing overcrowding, practising sanitation and isolating affected fish may help limit disease occurrence. However, the cross-sectional design cannot establish transmission from asymptomatic carriers or determine whether bacterial recovery preceded disease.

Annexure (B)

4.2 Environmental Factors and Clinical Response to Improved Husbandry Conditions

During laboratory observation, water temperature ranged from 25.0 to 28.5 °C, pH from 7.0 to 7.9, and dissolved oxygen from 4.5 to 4.9 mg L^{-1}. However, these measurements reflect temporary laboratory conditions rather than those in the original market aquaria or during transportation (Vanderzwalmen et al., 2021). Moreover, ammonia, nitrite, and nitrate were not monitored, although these parameters can influence fish physiology and microbial communities (Ortiz et al., 2023). Environmental stressors, including handling, transport, and overcrowding, may compromise skin and mucus barriers, increasing susceptibility to opportunistic infection (Reverter et al., 2018). Aquarium water and biofilms may also serve as bacterial reservoirs, as related Aeromonas strains have been recovered from ornamental fish and surrounding water (Martinez-Murcia et al., 2008). Non-infectious stressors, including copper oxychloride exposure, can produce similar behavioural and external abnormalities (Ullah et al., 2025). Thus, bacterial exposure, physical injury, and environmental stress may have contributed to lesion development (Figure 6), although their specific roles remain uncertain because original aquarium conditions, stocking density, handling history, and disease duration were undocumented.

Among 15 clinically affected goldfish

maintained under improved husbandry conditions, 12 (80%) showed resolution of visible clinical signs. Although improved water quality may support fish health by influencing microbial communities (Ortiz et al., 2023), the contribution of sanitation, water renewal, and salt exposure to clinical recovery remains uncertain. However, salt effects depend on exposure concentration and duration (Burgdorf-Moisuk et al., 2011). Without an untreated control or post-recovery bacteriological testing, clinical improvement cannot be attributed to water renewal or salt exposure, nor can bacterial clearance be confirmed. Persistent signs in three fish may reflect lesion severity, continued colonisation or other causes. Thus, these findings are descriptive rather than evidence of therapeutic efficacy, and persistent cases require bacteriological investigation before antimicrobial treatment.

4.4 Ornamental-fish health and management implications

The recovery of Aeromonas and Pseudomonas bacteria from diseased ornamental goldfish also has practical implications for fish management. Ornamental-fish transport and retail systems can facilitate the movement of microorganisms through fish, transport water, and shared equipment (Amaral-Zettler et al., 2018). Aeromonas species have been isolated from ornamental fish, highlighting their potential presence in aquarium environments (Dias et al., 2012). The recovery of presumptive bacteria from 38.0% of apparently healthy fish suggests potential asymptomatic carriage and bacterial persistence, which may increase disease or transmission risks under stressful conditions. Early detection, isolation, and improved husbandry are therefore essential. Although bacterial identity and antimicrobial susceptibility were not confirmed, multidrug-resistant Aeromonas and resistance determinants have been reported in ornamental fish and associated water (Verner-Jeffreys et al., 2009), including resistant Aeromonas veronii isolated from diseased goldfish (Shameena et al., 2020). Recent evidence from goldfish farms has similarly demonstrated resistance among motile Aeromonas isolates (Fudeshima et al., 2025). These findings reinforce the importance of diagnostic confirmation and antimicrobial-susceptibility testing before treatment.

Management should prioritise quarantine, appropriate water quality and stocking density, prompt isolation of affected fish, tank sanitation and laboratory diagnosis. Antimicrobials should be used only when indicated by confirmed bacterial infection and susceptibility testing. These measures may reduce bacterial transmission, unnecessary antimicrobial use, and treatment failure while improving goldfish health and welfare.

5. Study limitations

Several limitations should be considered: clinically affected fish were purposively selected, and so bacterial recovery frequencies should not be interpreted as population-level prevalence. The apparently healthy comparison group was assessed only cross-sectionally, preventing evaluation of subsequent disease development or transmission. Bacterial identification relied on colony morphology, Gram reaction, and microscopy; therefore, isolates were classified only as presumptive Aeromonas and Pseudomonas. No molecular confirmation, MALDI-TOF, comprehensive biochemical testing, experimental challenge, histopathology, standardised lesion scoring, tissue-specific recovery analysis, co-infection assessment, or antimicrobial-susceptibility testing was

performed. Use of a single incubation temperature (37 °C) may also have biased bacterial recovery.

The husbandry observation lacked an untreated control, and fish showing clinical resolution were not bacteriologically retested; therefore, the 80% resolution cannot be attributed specifically to NaCl or water renewal, nor can bacterial clearance be inferred. Water-quality data were limited to laboratory measurements, with no repeated time-series or original market values, preventing assessment of environmental effects on disease severity.

6. Future directions

Future investigations should validate the observed diseased-versus-asymptomatic differences using prospectively sampled populations from multiple aquarium shops and seasons. Longitudinal monitoring of apparently healthy bacterial carriers, together with tissue-specific bacterial recovery and aquarium-water/biofilm sampling, would clarify transmission dynamics within shared aquarium environments. Molecular characterization and antimicrobial-susceptibility testing of representative isolates would further refine their taxonomic identity and clinical relevance. Controlled intervention studies should also determine the contribution of water renewal, salinity, and other husbandry improvements to recovery from fin- and tail-rot lesions.

7. Conclusion

This study showed that presumptive Aeromonas and Pseudomonas were recovered significantly more often from clinically affected than apparently healthy goldfish from the same aquarium environments. Because identification was based only on phenotypic characteristics, species-level identity and causality could not be confirmed. Most fish in the husbandry observation group showed clinical improvement, but treatment efficacy and bacterial clearance were not established. These findings provide baseline evidence of disease-associated bacterial enrichment and highlight the need for molecular identification, tissue-specific analysis, and controlled follow- up studies.

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Conflicts of Interest

None declared.

Data Availability

The data underlying this study are not publicly available.

Ethics Statement

The study was conducted in accordance with the applicable institutional guidelines and legal requirements for the handling and use of animals in research in Pakistan. The Department of Zoology, University of Science and Technology Bannu, Khyber Pakhtunkhwa, Pakistan, determined that formal ethical approval was not required for this study and granted an exemption.

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Annexure (A)

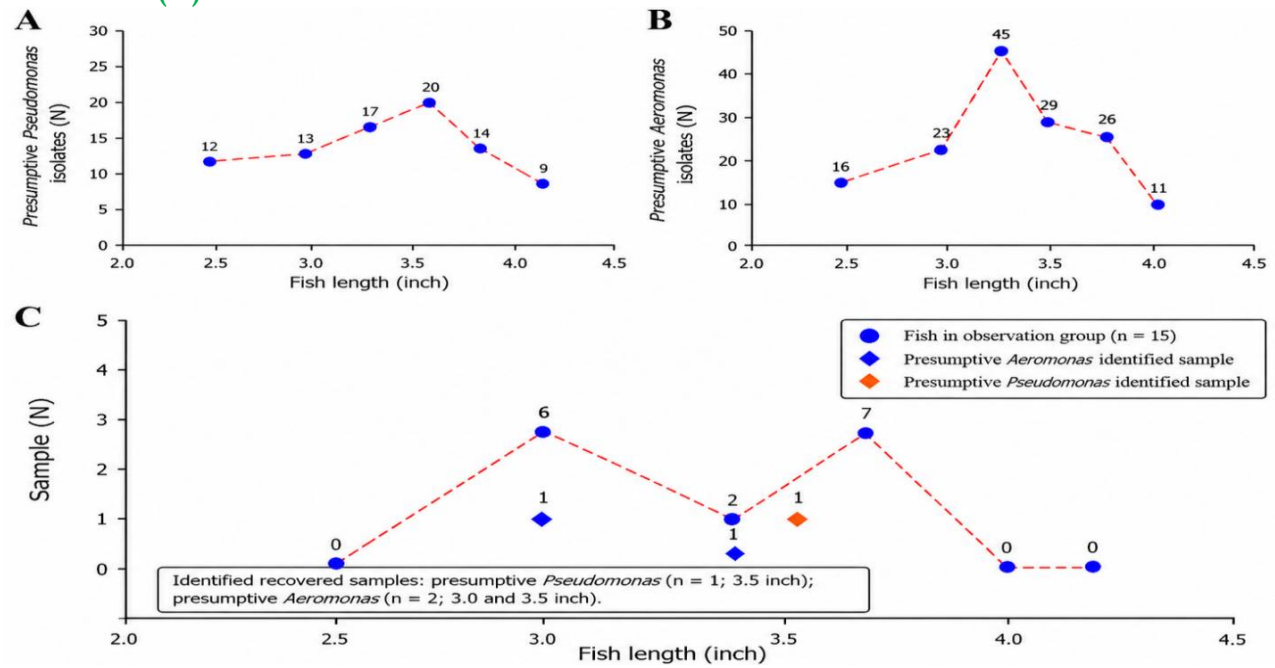


Figure 1. Distribution of (A) presumptive Pseudomonas isolates, (B) presumptive Aeromonas isolates, and (C) fish included in the husbandry-observation group across fish-length categories.

Annexure (B)

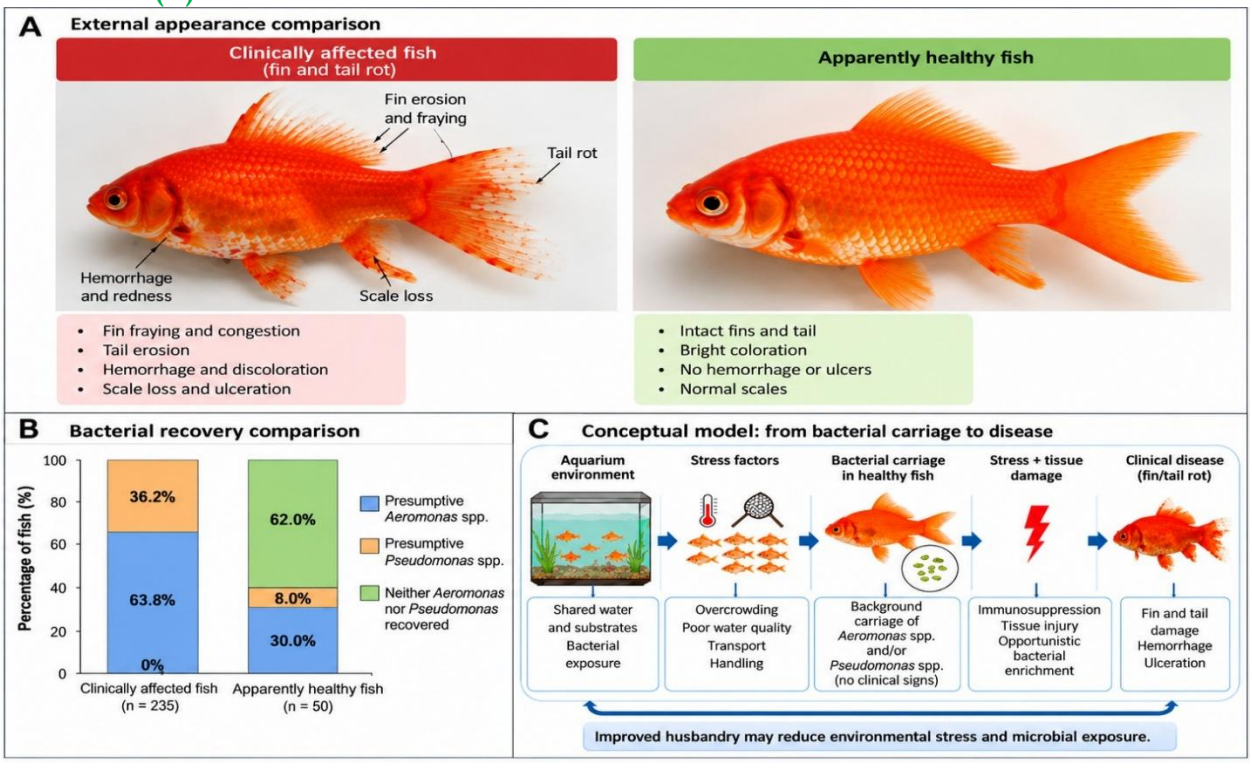


Figure 6. Comparison of clinical characteristics and bacterial recovery patterns between clinically affected and apparently healthy goldfish (Carassius auratus). (A) External features of fin and tail rot compared with apparently healthy fish. (B) Distribution of presumptive Aeromonas and presumptive Pseudomonas in clinically affected and apparently healthy fish. (C) Conceptual model illustrating the potential transition from bacterial carriage to disease under stress conditions.