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Emerging Bacterial Pathogens and Antimicrobial Resistance in Telangana Aquaculture: A One-Health Perspective

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Abstract

Bacterial infections are a major constraint in freshwater aquaculture worldwide and cause significant economic losses due to fish morbidity and mortality. In Telangana, aquaculture is expanding but data on pathogenic bacteria affecting cultured fish are limited. This study investigated the prevalence, diversity, and antimicrobial resistance of bacterial pathogens in freshwater fish and aquaculture environments in Telangana State. Sampling was conducted from fish farms and culture ponds near Siddipet and surrounding districts. Fish exhibiting clinical signs were examined, and bacterial isolates were obtained from gill, skin, and internal tissues. Isolates were identified through morphological, biochemical, and molecular (16S rRNA) characterization. Key pathogens detected included *Aeromonas hydrophila*, *Pseudomonas* spp., *Enterobacter cloacae*, and *Klebsiella pneumoniae*. Several isolates demonstrated antimicrobial resistance and biofilm-forming capability. Water quality parameters were correlated with pathogen prevalence. Findings underscore the need for routine disease surveillance, improved farm management, and targeted control strategies in Telangana's aquaculture systems.

Keywords: Bacterial pathogens; Freshwater Aquaculture; *Aeromonas hydrophila*; Antimicrobial resistance; fish health and 16S rRNA

Introduction

Aquaculture contributes significantly to food security and rural livelihoods in India, with freshwater systems predominating in inland states such as Telangana. Fish production is threatened by infectious diseases, particularly those caused by bacterial pathogens. Gulla G, et al., 2023). In fish, bacterial diseases such as motile aeromonad septicemia, caused by *Aeromonas hydrophila* and other Gram-negative rod infections are among the most economically significant worldwide and in Indian aquaculture. Bacterial agents can infect fish through gills, skin abrasions, or stress-induced lesions, often exacerbated by poor water quality and high stocking densities. Previous research from Hanamkonda, Telangana, has reported the presence of bacterial pathogens and associated disease signs in local freshwater aquaculture systems, yet comprehensive data including antimicrobial resistance profiles and epidemiology are limited. (Schar et, al., 2024). This study aims to characterize bacterial pathogens in fish from aquaculture settings in Telangana, assess their antibiotic resistance patterns and correlate findings with environmental parameters to inform management and biosecurity.

Materials and Methods

Study Sites and Sample Collection

Fish and water samples were collected from five freshwater aquaculture farms in the Telangana region Hanamkonda and Warangal between June 2025 and December 2025. Fish showing clinical signs (ulcers, hemorrhages, fin erosion) were targeted. Water samples were collected from surface and mid-pond zones.

Clinical and Gross Examination: Fish were examined for external and internal lesions consistent with bacterial infection. Measurements included lesion type, distribution, and gross pathology.

Behavioral Signs: Abnormal behavior often indicates disease or environmental stress.

Common Observations: Lethargy, Loss of appetite, Erratic swimming, Gasping at surface (respiratory distress), Flashing/rubbing against objects, Isolation from shoal and Loss of equilibrium.

Physiological Signs: Rapid opercular movement, Excess mucus production, Pale or dark body coloration and reduced growth

External Clinical Signs

Skin and Scales: Ulcers, Hemorrhages, Scale loss, Erosions and Discoloration.

Fins: Fraying (fin rot), Red streaks and Necrosis, ***Eyes:*** Exophthalmia (pop-eye), Cloudiness and Hemorrhages, ***Gills:*** Pale gills (anemia), Necrosis, Mucus accumulation and Hyperplasia, ***Gross Examination (Post-Mortem):*** Gross examination is the macroscopic inspection of dead or euthanized fish to detect internal abnormalities.

External Gross Examination

Body Surface: Lesions, Tumors, Parasites and Slime layer abnormalities.

Internal Gross Examination: Fish are dissected along the ventral midline.

Organs to Examine: Liver: Enlargement (hepatomegaly), Pale or mottled appearance and Hemorrhages.

Indicative of: Septicemia, Toxicity and Bacterial infection, ***Spleen:*** Splenomegaly, Dark coloration and Nodules, ***Often linked to:*** Systemic infections, ***Kidney:*** Swelling, Discoloration and Granulomas.

Important in diagnosing: Bacterial septicemia, ***Gut:*** Empty gut, Hemorrhagic intestine and excess mucus, ***Swim Bladder:*** Fluid accumulation and Thickened walls.

Clinical & Gross Signs of Bacterial Infections

Common in pathogens like *Aeromonas*, *Vibrio*, *Edwardsiella* and *Streptococcus*. (AMR-Insights, 2021).

Typical Signs: Skin ulcers, Hemorrhagic septicemia, Fin rot, Gill necrosis, Ascites (fluid in abdomen) and internal hemorrhages.

Standard Examination Procedure

History Taking: Mortality rate, recent stocking, Feeding practices and Water quality data.

Observe Live Fish: Behavior and External lesions.

Measure Water Quality: Temperature, pH, Dissolved oxygen and Ammonia.

Post-Mortem Examination: External inspection, Dissection and Organ assessment.

Sample Collection: Microbiology, Histopathology and PCR.

Limitations: Clinical and gross exams: Cannot confirm specific pathogen and May show similar signs for multiple diseases. ***Thus, they must be followed by:*** Culture, PCR and Histology. ***Importance in Aquaculture:*** Early disease detection, reduced economic losses, Better treatment decisions and Improved biosecurity.

Research Value

Clinical and gross findings: Guide sampling strategy, Support epidemiological studies and Correlate with molecular results.

Figure 1: Diseased fish showing clinical signs such as skin ulcers and hemorrhages.

Bacterial Isolation and Culture

Tissue samples (gill, skin lesion and kidney) were aseptically collected and streaked onto nutrient agar, Tryptic Soy Agar (TSA), and Mac Conkey agar. Plates were incubated at 28–30 °C for 24–48 h, and colony morphology recorded.

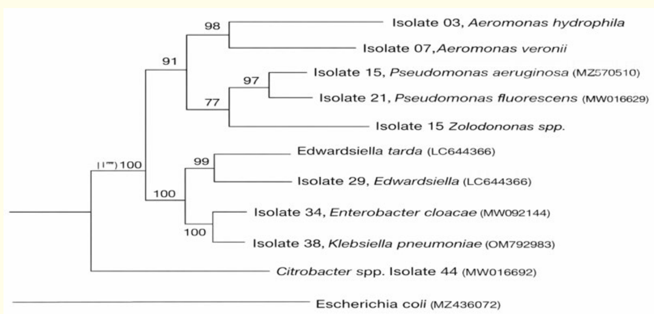

Figure 2: Phylogenetic tree of representative isolates.

Biochemical and Molecular Identification

Isolates were Gram stained and subjected to oxidase, catalase, and API 20E biochemical characterization. DNA was extracted using standard protocols, and 16S rRNA gene amplification was performed using universal primers. Sequencing and BLAST analysis confirmed taxa.

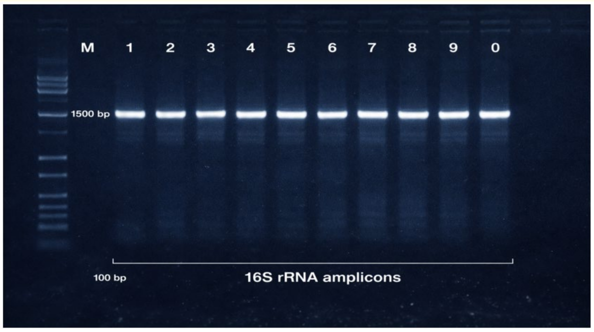

Figure 3: Agarose gel showing PCR amplified 16S rRNA products of isolates.

Antibiotic Sensitivity and Biofilm Assays

Antibiotic susceptibility testing was conducted using the disk diffusion method against a panel of commonly used antimicrobials. Biofilm formation was assessed using crystal violet microtiter plate assays.

Antimicrobial Resistance (AMR)
Definition

Antimicrobial resistance is the ability of microorganisms (bacteria, fungi, parasites and viruses) to survive or grow despite the presence of antimicrobial drugs designed to kill them or stop their growth. In aquaculture, AMR is a major concern due to frequent antibiotic use for disease control. (Nallaiah H, et al., 2025).

Mechanisms of AMR
Genetic Mechanisms

Intrinsic Resistance: Natural resistance due to inherent structural/functional traits. (MDPI Antimicrobials, 2025).

Example: Gram-negative bacteria resist penicillin due to outer membrane barrier and Acquired Resistance: Occurs via mutation or gene acquisition.

i) ***Mutation:*** Spontaneous DNA changes alter drug targets and Example: gyrA mutation → quinolone resistance.
ii) ***Horizontal Gene Transfer:***

Resistance genes spread through: Conjugation (***plasmids***), Transformation (***free DNA uptake***) and Transduction (***bacteriophages***).

Biochemical Mechanisms: Drug Inactivation: β-lactamases destroy β-lactam antibiotics, ***Target Modification:*** Ribosomal changes prevent antibiotic binding, ***Efflux Pumps:*** Actively expel antibiotics from cell and ***Reduced Permeability:*** Porin loss limits drug entry. ***AMR in Aquaculture:*** (AMR, 2024).

Causes: Overuse of antibiotics in feed, Prophylactic dosing, Poor water quality and High stocking density. ***Consequences:*** Treatment failure, increased fish mortality, Environmental spread of resistance genes and Risk to human health via food chain. (PLoS ONE / PMC, 2019).

Detection of AMR: Disk diffusion test, MIC determination, PCR detection of resistance genes and Whole genome sequencing. ***Biofilm Formation: Definition:*** Biofilms are structured microbial communities attached to surfaces and embedded in a self-produced extracellular polymeric substance (EPS). They are common in: Fish tanks, Pond liners, Pipes and Fish skin and gills. ***Stages of Biofilm Formation: Initial Attachment:*** Reversible adhesion to surface and Influenced by surface charge & hydrophobicity.

Irreversible Attachment: Production of EPS and Cells anchor permanently, ***Maturation:*** Microcolonies form and Complex 3D architecture develops, ***Dispersion:*** Cells detach and colonize new sites.

Composition of Biofilm Matrix: EPS contains: Polysaccharides, Proteins, Lipids and Extracellular DNA (eDNA). ***Why Biofilms Matter: Increased Resistance:*** Biofilm bacteria are ***10–1000× more resistant*** to antibiotics. Reasons: EPS blocks drug penetration; slow growth reduces antibiotic effect and Presence of persister cells.

Disease Persistence: Biofilms protect pathogens like: *Aeromonas hydrophila*, *Vibrio spp* and *Flavobacterium columnare*, (Aquaculture, (Elsevier, 2025).

Chronic Infections: Fin rot, Gill necrosis and Skin ulcers.

AMR and Biofilms: The Connection.

Biofilms promote AMR by: Enhancing gene transfer, creating protective niches, allowing survival under antibiotic stress, Encouraging persister cell formation and biofilms are hotspots for AMR evolution. (Mohammed E.A.H, 2025).

Aquaculture Relevance: Biofilm-associated AMR leads to: Recurring disease outbreaks, Poor vaccine efficacy and higher treatment costs. (Antimicrobial Resistance in Aquaculture, 2024).

Control Strategies: Prudent Antibiotic Use: Avoid prophylactic dosing and Use sensitivity testing. Biofilm Management: Regular tank cleaning, UV sterilization, Ozonation and Probiotics, Alternative Approaches: Phage therapy, Quorum sensing inhibitors, Antimicrobial peptides and Vaccination. (FAO/ICAR report, 2022).

Research Importance: Understanding AMR and biofilms is critical for: Sustainable aquaculture, Food safety, Environmental protection and Public health. (Aquaculture, 2022).

Water Quality Assessment

Water parameters including temperature, pH, dissolved oxygen (DO), ammonia, nitrate and turbidity were measured using portable probes and standard kits.

Results

Clinical Signs and Pathology

Infected fish displayed hemorrhages around fins and body, skin ulcers, and lethargy. Gill tissues often appeared inflamed with mucous accumulation.

Bacterial Diversity

A total of 112 bacterial isolates were recovered. Common genera identified were:

Aeromonas spp. (majority, including *A. hydrophila*), *Pseudomonas* spp, *Enterobacter* spp, *Klebsiella* spp and *Citrobacter* spp. Molecular sequencing confirmed species identities consistent with pathogenic strains previously reported in India. (Research Gate, 2021).

Antimicrobial Resistance and Biofilm Formation: A significant proportion of isolates exhibited multi-drug resistance, particularly against penicillins, tetracyclines and sulfonamides. *Klebsiella pneumonia*, (PubMed, 2024). Isolates showed resistance genes and active efflux systems consistent with reports from freshwater farms in Andhra Pradesh. Biofilm formation varied among isolates, with many demonstrating strong biofilm-forming capability, (2025).

Figure 4: Close-up of fish fin rot and gill necrosis indicating bacterial infection.

Water Quality: Ponds with low dissolved oxygen (<4 mg L^{-1}) and elevated ammonia (>0.5 mg L^{-1}) recorded higher pathogen counts, suggesting stress-related susceptibility.

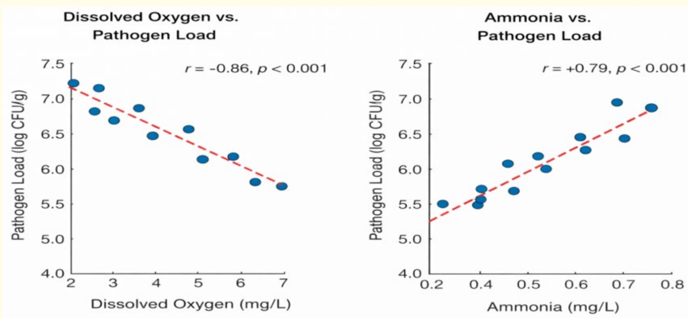

Figure 5: Water quality correlations with pathogen loads.

Discussion

This study confirms the presence of multiple bacterial pathogens in fish from aquaculture systems in Telangana, with *Aeromonas hydrophila* as a dominant agent consistent with broader Indian aquaculture disease profiles. The recovery of opportunistic human-associated bacteria such as *Enterobacter* and *Klebsiella* spp. raises concerns about cross-environment contamination and potential antimicrobial resistance dissemination, water quality deterioration likely predisposed fish to infection, underscoring the need for routine monitoring and improved management practices, (Bangladesh aquaculture AMR, 2024). The presence of biofilm-forming and drug-resistant strains emphasizes the urgency for targeted treatments and prudent antimicrobial use. Emerging techniques like eDNA mapping of bacteria in regional water bodies could provide broader surveillance insights beyond culture-dependent methods.

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Conclusion

Bacterial pathogens, particularly *Aeromonas* and other Gram-negative rods, are prevalent in freshwater aquaculture in Telangana and are associated with clinical disease signs and antibiotic resistance. Improved water management, biosecurity, and strategic disease control measures—including vaccine development and antibiotic stewardship—are recommended to mitigate losses in the aquaculture sector.

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