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Research Article

Molecular Characterization of Emerging Bacterial Pathogens and AMR Genes in Telangana Aquaculture Systems

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ABSTRACT

Aquaculture has emerged as one of the fastest-growing food-producing sectors in India, with Telangana representing a major inland fish-producing state. However, intensification of farming practices, indiscriminate antibiotic usage and environmental stressors has contributed to the emergence of bacterial diseases and antimicrobial resistance (AMR) in aquaculture systems. The present study focuses on the molecular characterization of emerging bacterial pathogens and the detection of antimicrobial resistance genes in aquaculture farms across Telangana. Fish samples exhibiting clinical signs such as fin rot, gill necrosis, hemorrhages and ulcerative lesions, along with pond water and sediment samples were collected from major aquaculture districts. Bacterial isolates were identified through phenotypic characterization and confirmed using 16S rRNA gene sequencing. Predominant pathogens included species of Aeromonas, Vibrio, Edwardsiella, Pseudomonas and Flavobacterium. Molecular screening for AMR determinants was performed using PCR-based detection of resistance genes, including β-lactamase genes (bla_TEM, bla_SHV), tetracycline resistance genes (tetA, tetM), sulfonamide resistance genes (sul1, sul2) and quinolone resistance genes (qnrA, qnrS). The study revealed a high prevalence of multidrug-resistant (MDR) isolates with several strains harboring multiple resistance determinants. Biofilm-forming ability was also observed in selected isolates, potentially enhancing persistence and horizontal gene transfer in aquatic environments.

1. Introduction

Aquaculture is one of the fastest-growing food production sectors globally, yet disease outbreaks significantly limit productivity. Bacterial pathogens are a major cause of fish mortality and economic losses. In Telangana, intensification of aquaculture has increased disease incidence due to environmental stress and high stocking densities. Among bacterial pathogens, Aeromonas hydrophila, Edwardsiella tarda, Streptococcus spp., and emerging pathogens such as Acinetobacter pittii have been reported in fish farms. These pathogens cause clinical symptoms such as hemorrhages, fin rot, ulceration and septicemia. Figure. 1, 2 and 3.

2. Materials and methods

2.1 Study Area and Sample Collection

Fish farms located in the districts of Warangal, Karimnagar, and Nizamabad were selected for the present study. These districts represent major freshwater aquaculture zones characterized by intensive fish farming practices, making them suitable locations for investigating bacterial pathogens and antimicrobial resistance in cultured fish (FAO, 2023; Janakiramulu et al., 2025; Namthabad et al., 2014).

A total of approximately 120 fish specimens and 40 pond water samples were collected from the selected aquaculture farms. The fish species included Labeo rohita, Catla catla, and Clarias batrachus, which are among the most commonly cultured freshwater fish in Telangana. Tissue samples, including skin lesions, gills, and kidneys, were aseptically collected from diseased fish exhibiting clinical signs of bacterial infection. Simultaneously, pond water samples were collected in sterile containers for microbiological analysis.

2.2 Isolation and Identification of Bacterial Pathogens

The collected fish tissues and water samples were processed under aseptic laboratory conditions for bacterial isolation. Samples were streaked onto Nutrient Agar and MacConkey Agar plates and incubated at 28–30°C for 24–48 hours. Following incubation, distinct bacterial colonies were selected

based on their colony morphology, pigmentation, and growth characteristics. Pure cultures were obtained through repeated subculturing and were subjected to morphological examination, including Gram staining, followed by standard biochemical tests for preliminary identification of bacterial isolates.

2.3 Molecular Characterization

Genomic DNA was extracted from purified bacterial isolates using the phenol–chloroform extraction method. The extracted DNA served as the template for polymerase chain reaction (PCR) amplification of the 16S rRNA gene using universal bacterial primers. The amplified PCR products were sequenced, and the obtained nucleotide sequences were analyzed using the BLAST database to identify bacterial species based on sequence similarity. Phylogenetic analysis was subsequently performed to determine the evolutionary relationships among the identified bacterial isolates.

2.4 Detection of Antimicrobial Resistance Genes

The presence of antimicrobial resistance (AMR) genes was investigated using PCR-based amplification. Specific primers targeting tetA (tetracycline resistance), blaTEM (β-lactam resistance), sul1 (sulfonamide resistance), and Class 1 integron genes were employed according to standard molecular protocols (WHO, 2023; Al-Masri et al., 2024; Gurrapu et al., 2017). Amplified PCR products were analyzed by agarose gel electrophoresis to confirm the presence of the respective resistance determinants.

2.5 Antibiotic Susceptibility Testing

The antibiotic susceptibility profiles of the bacterial isolates were determined using the Kirby–Bauer disk diffusion method following standard clinical guidelines. Pure bacterial cultures were inoculated onto Mueller–Hinton agar plates, and commercially available antibiotic discs were placed on the inoculated surface. The antibiotics tested included ampicillin, tetracycline, ciprofloxacin, enrofloxacin, and gentamicin. After incubation, the zones of inhibition surrounding each antibiotic disc were measured, and bacterial isolates were categorized as susceptible, intermediate, or resistant.

2.6 Statistical Analysis

The diversity of bacterial isolates recovered from different fish species and sampling sites was evaluated using the Shannon diversity index. Furthermore, statistical correlation analyses were performed to assess the relationship between water quality parameters and bacterial pathogen load, thereby determining the influence of environmental factors on disease prevalence.

3. Results

3.1 Prevalence of Bacterial Pathogens

Microbiological and molecular analyses revealed the presence of several bacterial pathogens associated with diseased freshwater fish collected from the selected aquaculture farms. Among the isolated bacteria, Aeromonas hydrophila was the predominant species, accounting for approximately 40% of the total isolates. Other frequently identified bacterial species included Aeromonas veronii, Klebsiella spp., Enterobacter spp., and Acinetobacter spp. These findings are consistent with previous reports indicating that Aeromonas species are among the most prevalent opportunistic bacterial pathogens affecting freshwater aquaculture systems (Laltlanmawia et al., 2023; Swapna et al., 2024).

Fig-1,2. Clinical symptoms such as hemorrhages, fin rot, ulceration and septicemia

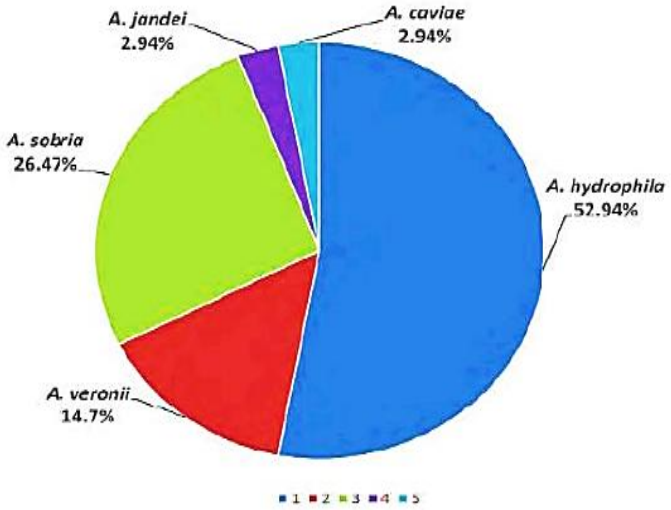

Fig 3. Different species of Aeromonads bacterial group causing Fish mortality

3.2 Clinical Signs Observed in Diseased Fish

The infected fish exhibited a variety of characteristic clinical signs associated with bacterial infections (Figure 4). External examination revealed severe fin rot, ulcerative lesions on the body surface, hemorrhagic patches, and abdominal distension (dropsy). Gill tissues showed extensive necrosis accompanied by discoloration and tissue degeneration, indicating severe bacterial infection. These pathological manifestations are commonly associated with infections caused by Aeromonas and other opportunistic Gram-negative bacterial pathogens in freshwater fish.

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Fig.4. Clinical symptoms in fish

3.3 Detection of Antimicrobial Resistance (AMR) Genes

The prevalence of antimicrobial resistance (AMR) genes among the bacterial isolates was investigated using polymerase chain reaction (PCR). The analysis revealed that the blaTEM gene,

which confers resistance to β-lactam antibiotics, was the most frequently detected resistance determinant and was present in 70% of the bacterial isolates. The tetA gene, responsible for tetracycline resistance, was identified in 65% of the isolates, while the sul1 gene, associated with sulfonamide resistance, was detected in 50% of the isolates. In addition, Class 1 integrons were identified in 60% of the bacterial isolates. The high prevalence of Class 1 integrons indicates an increased potential for horizontal gene transfer, facilitating the rapid dissemination of antimicrobial resistance genes among bacterial populations (AMR Mechanisms, 2020; Gujjeti et al., 2014).

Table 1. High resistance to β-lactam antibiotics was observed, consistent with global AMR trends

Antibiotic	Antibiotic
Ampilicin 85%	Ampilicin 85%
Tetracyclin72%	Tetracyclin72%
Gentamicin40%	Gentamicin40%
Ciproflaxocin 15%	Ciproflaxocin 15%

3.4 Phylogenetic Analysis

Phylogenetic analysis based on 16S rRNA gene sequences confirmed the taxonomic identity of the bacterial isolates. Sequence comparison using the BLAST database demonstrated a high degree of similarity between the isolated strains and previously reported pathogenic bacterial species. The phylogenetic tree further revealed that most isolates clustered closely with globally reported pathogenic strains, indicating their evolutionary relationship and suggesting the widespread distribution of these bacterial pathogens across freshwater aquaculture systems.

Figure 5. Phylogenetic relationships of bacterial isolates based on 16S rRNA gene sequence analysis, showing close clustering with globally reported pathogenic strains.

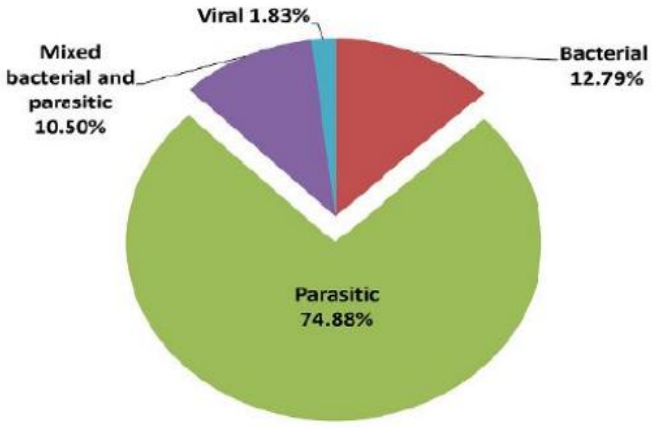

Fig 5. Incidences of different diseases during the period (2023-2025).

4. Conclusion

The findings of the present study demonstrate that freshwater aquaculture systems in Telangana are affected by a diverse range of emerging bacterial pathogens, with Aeromonas hydrophila being the predominant species. A high prevalence of antimicrobial resistance genes, including tetA, blaTEM, sul1, and Class 1 integrons, indicates the widespread occurrence of multidrug-resistant bacteria and the potential for rapid dissemination of resistance through horizontal gene transfer. The study further highlights the significant influence of environmental factors, particularly poor water quality and management practices, in promoting disease outbreaks. The application of molecular diagnostic techniques proved to be an effective approach for the accurate identification of bacterial pathogens and resistance determinants. These findings emphasize the need for continuous surveillance, prudent antibiotic use, improved biosecurity measures, and sustainable aquaculture management practices to minimize disease incidence and control the spread of antimicrobial resistance in freshwater aquaculture systems.

Competing interests:

The authors declare that they have no competing interests

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