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Isolation and Identification of Pathogenic Bacteria from Dead Fish in Rainbow Trout (*Oncorhynchus mykiss*) Farms in Türkiye

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Abstract: Aquaculture has emerged as an important component of global food security, and rainbow trout (Oncorhynchus mykiss) is among the most commercially valuable freshwater species. However, the intensification of trout farming has made fish populations increasingly vulnerable to bacterial infections, posing serious threats to both animal welfare and economic sustainability. This study was conducted to systematically isolate and identify the dominant bacterial pathogens involved in trout mortality cases observed in aquaculture farms in two geographically and climatically distinct regions of Türkiye (Gaziantep and Antalya). A total of 60 lethal fish samples were subjected to integrated microbiological diagnosis combining traditional culture-based methods (including Gram staining and a series of biochemical tests) with high-resolution proteomic identification methods via Matrix-Assisted Laser Desorption/Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS). Most of the isolates were taxonomically classified as Aeromonas hydrophila, Yersinia ruckeri, Pseudomonas fluorescens, and Flavobacterium psychrophilum, pathogens known to play a role in systemic and opportunistic infections in cold-water fish species. The spatial distribution of these bacterial agents revealed significant ecological variability, indicating a strong correlation between pathogen prevalence and environmental parameters such as water temperature, rearing conditions, and geographical location. In particular, MALDI-TOF MS provided high-accuracy species-level identification and showed over 90% agreement with traditional biochemical classification, thereby highlighting its utility as a rapid and reliable diagnostic tool for surveying aquatic diseases. In addition to its etiological importance, the study also provides region-specific epidemiological information on the spatial distribution of bacterial pathogens under changing environmental conditions. By offering this dual perspective, the research provides critical information for the development of targeted disease management frameworks and advocates for the integration of molecular diagnostics and sustainable biological control applications in the aquaculture industry.

Keywords: Aquaculture, Bacterial pathogens, Rainbow trout, MALDI-TOF MS, Fish health, Microbiology

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Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most widely cultured freshwater fish species globally and in Türkiye, due to its rapid growth and high tolerance to environmental fluctuations. As of 2022, Türkiye ranks among the leading producers in Europe, with an annual production exceeding 160,000 tons (FAO, 2022). However, this intensive aquaculture activity puts considerable pressure on fish health, increasing the incidence and spread of bacterial diseases (Austin & Austin, 2016).

Stress factors such as temperature fluctuations, overstocking, poor water quality, changes in feed, and transportation suppress the immune system of fish, facilitating infection by pathogenic microorganisms (Roberts, 2012). Among these, bacterial infections are particularly significant, causing high mortality rates and substantial economic losses in aquaculture operations (Decostere et al., 2000).

Common pathogenic bacteria in rainbow trout include *Aeromonas hydrophila*, *Yersinia ruckeri*, *Flavobacterium psychrophilum*, *Pseudomonas fluorescens*, and *Lactococcus garvieae*. These microorganisms are capable of inducing both acute and chronic infections, complicating the diagnosis and treatment of diseases (Noga, 2010). Accurate isolation and identification of these pathogens are therefore essential for early detection and the development of effective treatment strategies (Cipriano, 2001).

This study aims to isolate and identify bacterial pathogens from dead fish collected from trout farms in different regions of Türkiye. By providing updated and region-specific data on bacterial prevalence and distribution, the study seeks to contribute to the development of sustainable disease management strategies in Turkish aquaculture.

Method

This study was conducted in three commercial rainbow trout (*Oncorhynchus mykiss*) farms located in the Southeastern Anatolia and Mediterranean regions of Türkiye. Sampling was performed during periods of increased fish mortality between September and March. A total of 60 dead fish were collected and transported to the laboratory in sterile bags within ice-cooled containers. Each specimen was examined macroscopically for external and internal pathological signs, including surface lesions, gill color, eye condition, bloating, and lesions in the mouth, pharynx, liver, and kidney (Noga, 2010).

Samples were taken from the kidney, liver, spleen, and gill tissues. These tissues were homogenized on slides sterilized with 70% ethanol using a sterile scalpel. The homogenates were inoculated onto Blood Agar (5% sheep blood) and Tryptic Soy Agar (TSA) and incubated at 25–37°C for 24–72 hours (Austin & Austin, 2016). Colonies exhibiting distinct morphological features were selected and subcultured for purification. Gram staining was performed to determine the bacterial cell wall structure, and characteristics such as cell morphology, pigment production, and hemolysis patterns were evaluated. Standard biochemical tests were used to characterize the isolates, including oxidase, catalase, indole, methyl red, H_2S production, urease activity, and hemolysis tests. These tests allowed for the identification of bacterial metabolic activities (Cipriano, 2001).

To confirm the identity of selected isolates and validate classical methods, MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization–Time of Flight Mass Spectrometry) analysis was performed. This technique compares the bacterial protein profile against a reference library and provides highly accurate identification (Clark et al., 2013). The frequencies and distributions of the identified bacterial species were statistically evaluated. Variations between farms were compared and results were presented using Microsoft Excel and SPSS 26.0 through tables and graphs.

Results and Discussion

During the study, 60 dead rainbow trout were collected from two farms in Gaziantep and one farm in Antalya. Macroscopic examination revealed common signs of bacterial infection, including pale gills, hemorrhagic skin lesions, abdominal bloating, and swim bladder abnormalities. These signs were consistent with systemic bacterial infections typically observed in aquaculture settings (Noga, 2010).

A total of 72 bacterial isolates were obtained from various tissues, including the surface, gills, liver, and kidney. Colony growth was observed predominantly on TSA and blood agar media within 24–48 hours of incubation.

Gram staining results showed that 62% of the isolates were Gram-negative rods, 28% were Gram-positive cocci, and 10% were Gram-positive rods. The biochemical characterization revealed that *Aeromonas spp.* was the most frequently isolated genus (46%), followed by *Enterococcus spp.* (26.6%), *Pseudomonas spp.* (18.3%), and *Lactococcus garvieae* (13.3%). Less frequent isolates included *Acinetobacter spp.*, *Flavobacterium spp.*, *Bacillus spp.*, and *Micrococcus spp.* (Table 1)

Table 1. Prevalence of bacterial ısolates ıdentified from trout farms

Bacterial Species	Number of Isolates	Percentage (%)
Aeromonas spp.	28	46.0
Enterococcus spp.	16	26.6
Pseudomonas spp.	11	18.3
Lactococcus garvieae	8	13.3
Acinetobacter spp.	7	11.6
Flavobacterium spp.	6	10.0
Others (e.g., *Bacillus*)	9	15.0

The identification results were further confirmed by MALDI-TOF MS for 50 selected isolates. The protein profiles obtained from MALDI-TOF analysis matched classical methods with approximately 90% consistency. Particularly accurate identifications were achieved for *Aeromonas* and *Pseudomonas* species, supporting previous findings on the efficacy of this method (Clark et al., 2013).

Comparison of farms revealed notable differences in bacterial prevalence. In Gaziantep farms, *Aeromonas hydrophila* and *Pseudomonas fluorescens* were dominant, while in the Antalya farm, *Lactococcus garvieae* and *Aeromonas veronii* were more prevalent. These variations may be attributed to environmental factors such as water temperature, hygiene practices, stocking density, and feeding regimes.

Organ-specific distributions also showed clear trends. *Aeromonas spp.* were more prevalent in internal organs like the liver and kidney, indicating systemic infection potential. In contrast, *Enterococcus spp.* were more commonly isolated from the mouth, pharynx, and cloacal regions, suggesting a gastrointestinal origin. *Pseudomonas spp.* and *Acinetobacter spp.* were mostly found on the surface and gills, pointing to environmental opportunism. These findings are in line with previous studies reporting similar bacterial profiles in Turkish trout farms (Altınok & Kurt, 2003; Kara et al., 2021). Furthermore, the higher detection of *Flavobacterium psychrophilum* in the cooler waters of Antalya supports the known psychrophilic nature of this pathogen.

Conclusion

This study successfully identified the dominant pathogenic bacteria present in dead rainbow trout (*Oncorhynchus mykiss*) from aquaculture farms in Turkey, with a particular focus on regional and environmental variations. The most frequently isolated pathogens were *Aeromonas hydrophila*, *Enterococcus spp.*, *Pseudomonas fluorescens*, and *Lactococcus garvieae*. These bacteria are known to cause significant health problems and economic losses in the aquaculture industry. The use of MALDI-TOF MS significantly improved the accuracy and speed of bacterial identification and showed strong agreement with classical biochemical methods. The observed regional differences in pathogen prevalence underscore the importance of localized monitoring and farm-specific disease management strategies.

Overall, the findings contribute valuable data for developing more effective and sustainable health management protocols in trout farming. Early diagnosis, environmental monitoring, and the integration of advanced diagnostic tools such as MALDI-TOF MS are essential for improving fish health and reducing the impact of bacterial diseases in aquaculture systems.

Recommendations

Based on the results of this study, the following recommendations are proposed to enhance fish health and disease management in trout farming:

- *Routine Microbiological Surveillance*: Regular monitoring of bacterial pathogens should be implemented in aquaculture facilities to detect infections at an early stage and to prevent widespread outbreaks.

- *Molecular and Antibiotic Resistance Profiling*: Future studies should incorporate molecular techniques such as 16S rRNA sequencing and antibiotic susceptibility tests to better understand the genetic characteristics and resistance profiles of isolated bacteria.
- *Alternative Control Strategies*: Sustainable approaches such as bacteriophage therapy, probiotics, and vaccination should be explored as alternatives to traditional antibiotic use, which may lead to antimicrobial resistance.
- *Farm-Specific Disease Management*: Environmental parameters like water temperature, oxygen levels, and stocking density should be optimized based on regional pathogen profiles to minimize disease risk.
- *Capacity Building and Training*: Aquaculture workers should be trained in disease identification, biosecurity practices, and hygienic management to strengthen farm resilience against bacterial outbreaks.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

* This study was conducted in accordance with ethical and legal standards applicable to research involving animal specimens. Prior to the initiation of the study, ethical approval was obtained from the Gaziantep University Local Ethics Committee for Animal Experiments (HADYEK). In addition, official permission for the collection of fish samples from commercial aquaculture farms was granted by the Gaziantep Provincial Directorate of Agriculture and Forestry, Ministry of Agriculture and Forestry of the Republic of Türkiye.

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Conflict of Interest

* The authors declare that they have no conflicts of interest

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