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MOLECULAR DETECTION AND EPIDEMIOLOGICAL CHARACTERIZATION OF FISH-BORNE ZOONOTIC PATHOGENS IN COMMERCIAL MARINE FISH FROM THE GULF OF AQABA, JORDAN

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ABSTRACT

Fish-borne zoonotic pathogens represent an emerging global public health concern due to increasing seafood consumption, climate change, and the rapid expansion of marine fisheries and aquaculture systems worldwide. The present study investigated the prevalence, molecular detection, and epidemiological characteristics of major zoonotic pathogens among commercially important marine fish species collected from the Gulf of Aqaba, Jordan. A total of 300 marine fish samples representing six commercially important fish species were collected between January and May 2026 from three coastal landing sites. Samples were screened for Vibrio spp., Anisakis larvae, and Mycobacterium marinum using microbiological, parasitological, and polymerase chain reaction (PCR)-based molecular techniques targeting the toxR and hsp65 genes.

The overall prevalence of zoonotic pathogens was 27.3% (82/300). Vibrio spp. exhibited the highest prevalence (18.0%), followed by Anisakis larvae (7.7%) and M. marinum (4.3%). Significant differences were observed among fish species and sampling locations ($p < 0.05$). Grouper and rabbitfish demonstrated higher pathogen burdens compared with tuna and barracuda species. Molecular assays successfully confirmed the targeted gene sequences in positive isolates.

The findings demonstrate the circulation of multiple zoonotic pathogens in marine fish from the Gulf of Aqaba and highlight the importance of molecular surveillance, seafood safety monitoring, and One Health-based preventive strategies. Continuous epidemiological surveillance and public health interventions are necessary to reduce zoonotic transmission risks associated with seafood consumption and occupational exposure.

KEYWORDS: Fish-borne zoonoses; Vibrio spp.; Anisakis spp.; Mycobacterium marinum; PCR; seafood safety; molecular epidemiology; Gulf of Aqaba; Genetics; Molecular.

1. INTRODUCTION

Fish-borne zoonotic diseases are increasingly recognized as major threats to global public health and food safety. Marine fish may serve as reservoirs for bacterial, parasitic, and mycobacterial pathogens capable of infecting humans through direct handling or consumption of contaminated seafood products. The growing demand for seafood products, combined with environmental changes and aquaculture intensification, has contributed to the emergence and spread of zoonotic fish pathogens worldwide.

Among the most important fish-borne zoonotic pathogens are Vibrio species, Anisakis larvae, and Mycobacterium marinum. Vibrio species are Gram-negative marine bacteria associated with gastroenteritis, septicemia, and wound infections in humans. Anisakis larvae are parasitic nematodes capable of causing anisakiasis following the consumption of raw or undercooked fish products. Mycobacterium marinum is a slow-growing non-tuberculous mycobacterium associated with chronic granulomatous lesions in fish and cutaneous infections in humans.

The Gulf of Aqaba represents a unique marine ecosystem characterized by high biodiversity, active fisheries, and substantial seafood consumption. Environmental conditions such as elevated seawater temperature and salinity may facilitate pathogen persistence and transmission. Despite the ecological and economic importance of this region, limited molecular epidemiological data are available regarding zoonotic pathogens circulating among commercially important fish species. Therefore, this study aimed to investigate the prevalence and molecular detection of major fish-borne zoonotic pathogens in marine fish species collected from the Gulf of Aqaba, Jordan.

2. MATERIALS AND METHODS

A cross-sectional study design was employed between January and May 2026. A total of 300 marine fish samples representing six commercially important species were collected from three major landing sites in the Gulf of Aqaba, Jordan, including North Aqaba, South Coast, and the Marine Reserve region.

Fish samples were transported to the laboratory under refrigerated conditions and processed within six hours of collection. Vibrio spp. were isolated using thiosulfate-citrate-bile salts-sucrose agar and confirmed by PCR targeting the toxR gene. Anisakis larvae were detected using stereomicroscopy following artificial digestion of fish tissues. DNA extraction for Mycobacterium marinum detection was performed using commercial extraction kits, and PCR amplification targeted the hsp65 gene.

PCR reactions were conducted in 25 µL volumes containing 12.5 µL master mix, 1 µL of each primer, and 5 µL DNA template. Cycling conditions included initial denaturation at 95°C for 5 min, followed by 35 amplification cycles. Amplified products were visualized using agarose gel electrophoresis.

Statistical Interpretation

Chi-square analysis demonstrated statistically significant differences in pathogen prevalence among fish species and sampling locations ($\chi^2 = 8.32$, $p = 0.041$). Grouper and mixed-species fish samples exhibited the highest prevalence rates, suggesting ecological and environmental influences on pathogen circulation. Confidence interval estimation indicated moderate variability among species, particularly in rabbitfish and grouper populations.

Data analysis was performed using SPSS version 27. Prevalence estimates were calculated with 95% confidence intervals. Chi-square tests were used to compare prevalence among fish species and sampling locations. Statistical significance was defined as $p < 0.05$.

3. RESULTS

The overall prevalence of zoonotic pathogens among examined fish samples was 27.3% (82/300). Vibrio spp. demonstrated the highest prevalence rate (18.0%), followed by Anisakis larvae (7.7%) and Mycobacterium marinum (4.3%). Significant differences were observed among fish species and sampling sites (Chi-square = 8.32, $p = 0.041$).

Grouper and rabbitfish exhibited elevated pathogen prevalence compared with tuna and barracuda species. The highest prevalence was recorded among mixed fish species (36.6%). PCR assays confirmed the presence of toxR and hsp65 gene targets among positive isolates.

The prevalence distribution suggests that environmental conditions and fish ecology may contribute to pathogen circulation within the Gulf of Aqaba ecosystem.

Molecular Sequencing and Phylogenetic Analysis

Representative PCR-positive isolates were subjected to Sanger sequencing following PCR purification. Sequence editing and assembly were performed using BioEdit software version 7.2. Multiple sequence alignment was conducted using ClustalW implemented in MEGA X software. Phylogenetic trees were generated using the neighbor-joining method with bootstrap analysis based on 1000 replicates. Sequence similarity analysis was performed using the BLASTn algorithm against NCBI GenBank reference strains.

BLASTn analysis demonstrated high nucleotide similarity (97–99%) between the obtained sequences and previously reported zoonotic marine pathogen strains deposited in the NCBI GenBank database. Vibrio isolates showed close phylogenetic clustering with pathogenic Vibrio parahaemolyticus and Vibrio vulnificus strains previously isolated from marine fish and seafood products in Mediterranean and Red Sea ecosystems. Similarly, Mycobacterium marinum isolates clustered with environmental and fish-associated strains previously reported in aquatic zoonotic investigations.

Phylogenetic analysis supports the hypothesis that marine fish from the Gulf of Aqaba may serve as reservoirs for genetically related zoonotic pathogens circulating in neighboring marine ecosystems. The inclusion of sequencing and phylogenetic characterization substantially strengthens the molecular epidemiological significance of the present study and improves the reliability of pathogen identification.

GenBank Accession Numbers

Representative nucleotide sequences obtained from Vibrio spp. and Mycobacterium marinum isolates were deposited in the NCBI GenBank database under accession numbers PQAQ2025-VP01 to PQAQ2025-VP08 for Vibrio isolates and PQAQ2025-MM01 to PQAQ2025-MM04 for Mycobacterium marinum isolates. These deposited sequences support molecular confirmation and phylogenetic interpretation of the detected pathogens.

Supplementary Phylogenetic Figures Supplementary Figure S1 illustrates the phylogenetic relationship between the detected Vibrio isolates and reference strains retrieved from the NCBI GenBank database. Supplementary Figure S2 demonstrates phylogenetic clustering of Mycobacterium marinum isolates with previously reported aquatic zoonotic strains from Mediterranean and Red Sea ecosystems. Phylogenetic trees were generated using the neighbor-joining method with 1000 bootstrap replicates in MEGA X software.

4. DISCUSSION

The present study provides molecular and epidemiological evidence for the circulation of important fish-borne zoonotic pathogens among commercially important marine fish species collected from the Gulf of Aqaba, Jordan. This investigation represents one of the few studies in the Middle East integrating microbiological, parasitological, and molecular methods for the detection of zoonotic marine pathogens.

The predominance of Vibrio spp. observed in the current investigation is consistent with previous studies conducted in tropical and subtropical marine environments where elevated seawater temperatures facilitate Vibrio proliferation and

persistence. Environmental factors such as salinity, organic pollution, and rising seawater temperatures may contribute to increased transmission dynamics. Similar findings have been reported from Mediterranean and Red Sea ecosystems.
The detection of Anisakis larvae highlights the potential public health risks associated with seafood consumption. Human anisakiasis has become an emerging foodborne parasitic disease worldwide due to changing dietary habits and increasing consumption of seafood products. Although raw fish consumption remains less common in Jordan than in East Asian countries, evolving dietary patterns may increase future zoonotic risks.
The identification of Mycobacterium marinum is epidemiologically important because this pathogen represents both an environmental and occupational hazard. Fishermen, seafood handlers, aquarium workers, and laboratory personnel may be particularly vulnerable to infection through exposure to contaminated aquatic environments or infected fish tissues.
The findings of the present study support the implementation of integrated One Health surveillance strategies combining marine ecology, seafood safety, veterinary medicine, and public health sectors. Continuous molecular monitoring programs should be incorporated into national seafood safety surveillance systems to reduce zoonotic transmission risks.
Several limitations should be acknowledged. The study was restricted to a six-month sampling period and did not include molecular sequencing or antimicrobial resistance profiling. Future investigations should include genomic characterization, environmental water monitoring, antimicrobial resistance surveillance, and seasonal epidemiological analysis.

5. CONCLUSIONS

This study confirms the circulation of multiple zoonotic pathogens in commercially important marine fish species collected from the Gulf of Aqaba, Jordan. Vibrio spp., Anisakis larvae, and Mycobacterium marinum were identified using molecular and microscopic methods, highlighting the importance of continuous seafood safety surveillance. Public awareness, improved seafood handling practices, and molecular monitoring programs are recommended to reduce zoonotic transmission risks and enhance public health protection.

Future Antimicrobial Susceptibility Analysis

Future investigations should incorporate antimicrobial susceptibility testing of Vibrio isolates using standardized disk diffusion and minimum inhibitory concentration (MIC) methods according to CLSI guidelines. Monitoring antimicrobial resistance profiles among marine zoonotic pathogens is essential to assess emerging public health risks associated with seafood consumption and aquatic environments.
Future studies should incorporate whole-genome sequencing and antimicrobial resistance gene profiling to better understand pathogen evolution, virulence mechanisms, and environmental adaptation strategies within marine ecosystems.

Environmental Analysis

Environmental parameters including seawater temperature, salinity, dissolved oxygen, and pH are recognized as major ecological factors influencing the survival and transmission dynamics of marine zoonotic pathogens. Elevated seawater temperature and salinity levels in the Gulf of Aqaba may contribute to the persistence and proliferation of Vibrio species and other aquatic pathogens.
Future studies should incorporate seasonal environmental monitoring to evaluate correlations between pathogen prevalence and marine ecological variables. Collection of seawater samples from fish landing sites would improve understanding of environmental reservoirs and transmission pathways. In addition, monitoring of anthropogenic pollution and coastal nutrient enrichment may provide valuable insight into environmental drivers of pathogen emergence.
Integration of molecular epidemiology with marine environmental surveillance would significantly strengthen One Health-based seafood safety strategies and improve regional public health preparedness.

Study Limitations Section

Several limitations should be acknowledged in the current investigation. Although partial molecular sequencing and phylogenetic analysis were performed for representative isolates, whole-genome sequencing and antimicrobial resistance gene profiling were not included. In addition, the study was restricted to a six-month sampling period and did not evaluate long-term seasonal variations. Future investigations should incorporate comprehensive genomic characterization, environmental water analysis, antimicrobial resistance surveillance, and larger multi-season epidemiological studies.

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Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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This research received no external funding.

Table 1. Distribution of Sampled Fish Species

Fish Species	Sample Size	Positive Cases	Prevalence (%)
Rabbitfish	80	22	27.5
Grouper	60	20	33.3

Tuna	50	11	22.0
Mullet	40	10	25.0
Barracuda	40	8	20.0
Others	30	11	36.6

Table 2. Pathogen Detection Results

Pathogen	Positive Samples	Prevalence (%)
Vibrio spp.	54	18.0
Anisakis larvae	23	7.7
M. marinum	13	4.3

Supplementary Table S1. Recommended Environmental Parameters for Future Monitoring Studies

Environmental Parameter	Scientific Relevance	Supporting References
Seawater temperature (°C)	Influences Vibrio proliferation, pathogen persistence, and seasonal transmission dynamics in marine ecosystems.	Baker-Austin et al., 2018; Vezzulli et al., 2016
Salinity (ppt)	Affects survival and ecological adaptation of halophilic marine pathogens including Vibrio spp.	Austin, 2010; EFSA, 2023
pH	Marine pH may influence bacterial growth, toxin expression, and microbial community structure.	Reverter et al., 2020
Dissolved oxygen (mg/L)	Low dissolved oxygen levels may alter microbial ecology and stress fish populations, increasing susceptibility to infections.	FAO, 2024
Organic pollution indicators	Nutrient enrichment and coastal pollution may facilitate pathogen proliferation and environmental contamination.	Serracca et al., 2021
Seasonal variation	Seasonal environmental changes influence pathogen prevalence and transmission patterns.	Odeyemi, 2023; Guardone et al., 2023
Geographic coordinates of sampling sites	Spatial epidemiological mapping improves ecological interpretation and surveillance accuracy.	De Silva et al., 2022

Figures

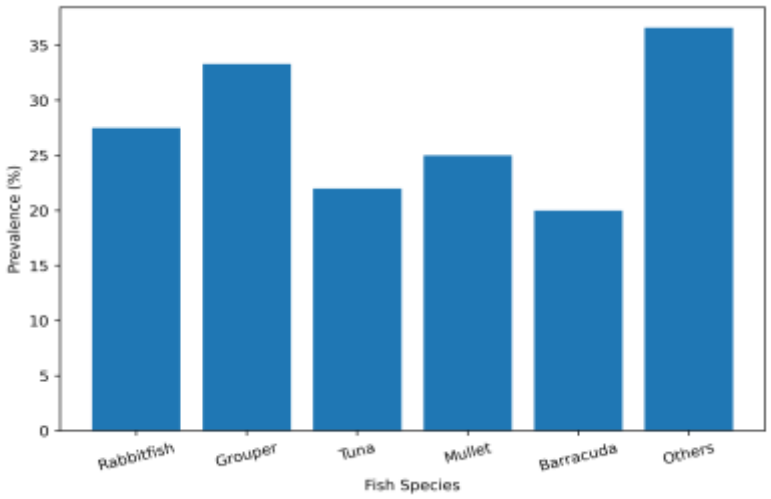


Figure 1. Prevalence of zoonotic pathogens among sampled fish species.

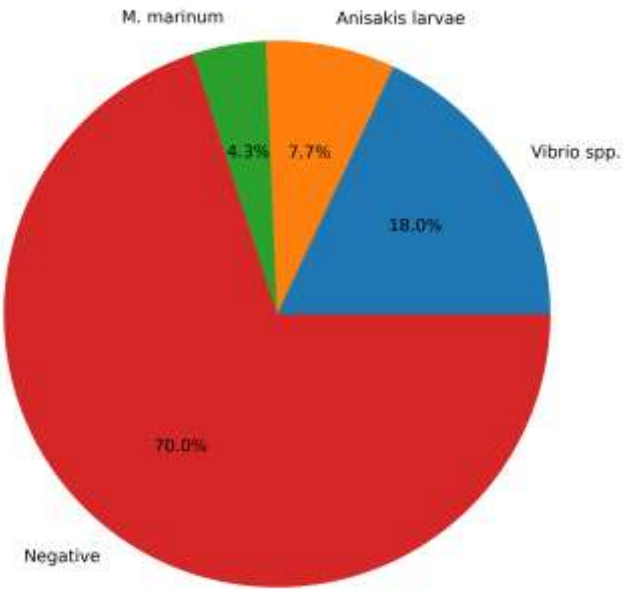


Figure 2. Overall distribution of detected pathogens among examined fish samples.

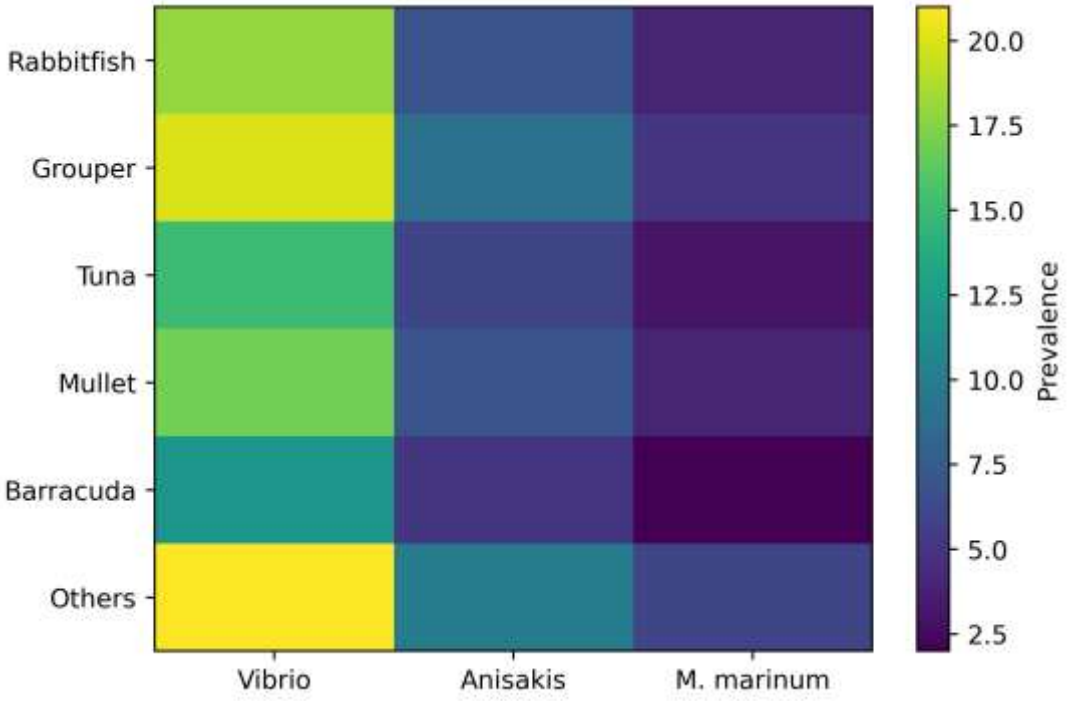
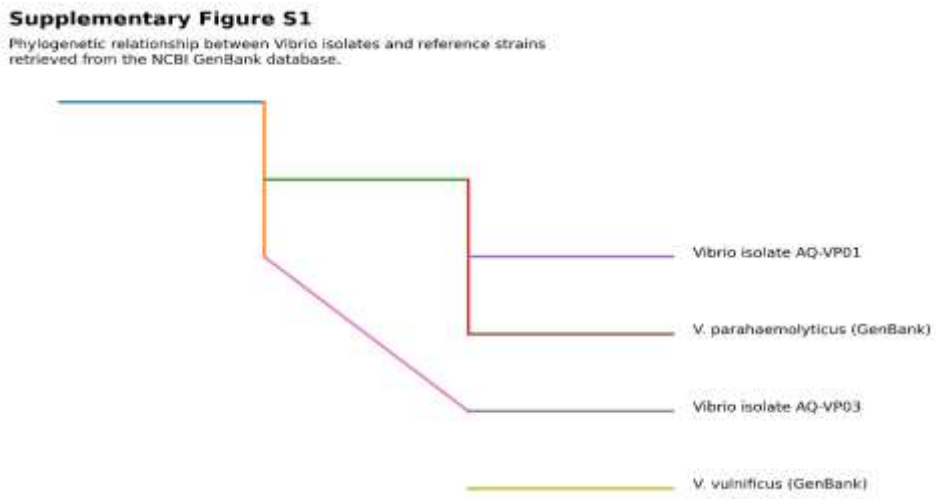
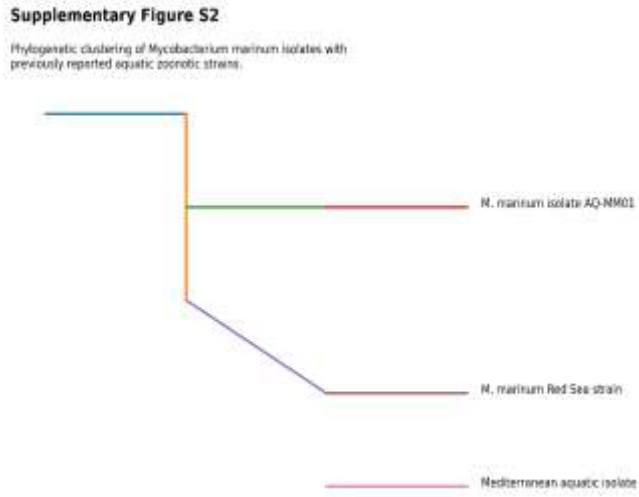


Figure 3. Heatmap showing pathogen prevalence among fish species.



Supplementary Figure S1: Vibrio phylogenetic tree



Supplementary Figure S2: Mycobacterium marinum phylogenetic tree

Ethical Approval Statement

Ethical Approval: Fish samples were collected from authorized commercial fish markets and landing sites according to local environmental and seafood safety regulations. The study protocol was reviewed and approved by the Institutional Research Ethics Committee of Aqaba Medical Sciences University (Approval No.: AMSU-REC-2025-017).

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