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Original Article

Pathological Alterations Associated with Antibiotic-Resistant Bacteria in the Liver of Mugil cephalus (Linnaeus, 1758) from Anthropogenically Impacted Sites in Tripoli Coast, Libya

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Abstract:

Background: Coastal waters around Tripoli, Libya, are severely polluted by urban expansion, industrial discharges, and untreated sewage, creating conditions that threaten marine organisms and human consumers. **Objectives:** This preliminary study aimed to isolate and identify aquatic bacterial species from the liver of *Mugil cephalus*, determine the antibiotic susceptibility profiles of the isolated bacteria, and investigate histopathological lesions associated with bacterial stress in *Mugil cephalus.* **Results:** Gross examination revealed mild lordosis, gill marbling, severe hepatic congestion, and hemorrhages involving the liver, swim bladder, and kidneys. Histological analysis demonstrated pronounced vascular congestion, widespread vacuolar degeneration of hepatocytes, nuclear pyknosis, leukocyte infiltration, and activation of melano-macrophage centers in 13 out of 15 specimens (87%); unidentified parasitic structures were observed in 12 out of 15 specimens (80%). Bacteriological examination of liver tissue yielded Gram-negative isolates from all 15 samples, predominantly *Aeromonas* and *Vibrio* species, of which 12 isolates (80%) exhibited multidrug resistance. These findings suggest an association between environmental contamination, bacterial colonization, and progressive liver damage, underscoring the species' potential role as a sentinel for antimicrobial resistance and highlighting possible zoonotic risks. **Conclusion:** The observed hepatic lesions may serve as valuable bioindicators for ongoing ecosystem monitoring and underscore the need for improved wastewater management and antimicrobial stewardship within a One Health framework.

Keywords: *Mugil cephalus*, Histopathological Changes, Multidrug-resistant Bacteria, Liver Pathology, Antimicrobial Resistance, One Health.

Introduction

The *flathead grey mullet* (*Mugil cephalus*) is a vital species for Libya's commercial fisheries [1]. With its ability to tolerate salinity changes, feed on bottom detritus, and thrive in various environments, it accumulates significant amounts of pollutants and microbes in its tissues [2]. Consequently, *M. cephalus* often carries high levels of drug-resistant bacteria, making it a useful indicator for monitoring these microbes and related tissue diseases [3].

The Tripoli coastline experiences considerable pollution due to rapid urbanization and expanding industrial activity, which discharge untreated wastewater and agricultural runoff into the marine ecosystem [4]. Such contamination facilitates the proliferation of antibiotic-resistant bacteria within seawater and marine organisms, posing risks to biodiversity and presenting significant public health concerns [5]. Fish may serve as vectors for the transmission of multidrug-resistant (MDR) pathogens to humans via the food chain or occupational exposure [6]. Furthermore, the elevated prevalence of resistant strains in Libyan waters raises concerns regarding zoonotic transmission and wider health implications [7].

Histopathological analysis of the liver is an effective way to assess bacterial infections in teleost fish, as the liver quickly responds to environmental stress [8]. Typical lesions, such as vascular congestion, hepatocyte degeneration, nuclear pyknosis, and increased melano-macrophage activity, often appear before clinical signs [9] and are usually linked to Gram-negative bacteria such as *Vibrio* [10].

Although *Mugil cephalus* is a vital component of Libya's marine economy, regional research has remained largely fragmented, focusing primarily on isolated aspects such as Vibrio pathogenesis in the Benghazi region [11]. The study lacked histopathological evidence and antimicrobial resistance (AMR) profiling necessary for a comprehensive health assessment, or it examined heavy-metal bioaccumulation in Eastern Libya without evaluating the corresponding tissue damage [12].

Notably, within the Tripoli region, there is a distinct lack of integrated studies bridging the gap between histopathological biomarkers and the molecular characterization of antimicrobial-resistant bacteria (AMR) [13]. Addressing this research deficit is critical, as the direct associations between the presence of multidrug-resistant (MDR) pathogens and the severity of resulting tissue damage in aquatic hosts remain poorly documented globally [14].

Addressing these limitations, this study characterizes hepatic histopathological changes in Mugil fish as bioindicators of environmental pollution along the Tripoli coast. Additionally, it isolates, identifies, and

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determines the antibiotic susceptibility profiles of bacteria from the liver. Finally, this investigation correlates tissue lesions with the AMR profiles of these isolates, providing essential data for ecosystem surveillance and the assessment of zoonotic public health risks.

Materials and Methods

Sample processing

Fifteen *Mugil cephalus* specimens were collected at random from fishermen returning to the western Tripoli coast during the winter season. Fish were placed on crushed ice inside insulated containers and transported to the diagnostic laboratory at the Faculty of Veterinary Medicine, University of Tripoli, Libya. Total length and weight were recorded. A complete macroscopic examination was conducted to detect external and internal gross abnormalities, which were documented photographically. All data were analysed descriptively.

Histopathological Examination

Liver tissue samples were carefully removed and cut into small pieces, each about 0.5 cm^3. The samples were placed in 10% neutral buffered formalin for at least 48 hours at room temperature. Once fixed, the tissues underwent dehydration using increasing concentrations of ethanol (70%, 80%, 90%, 100%), followed by two rounds of xylene clearing, and then were embedded in paraffin wax heated to 58°C. Thin sections (4–5 µm thick) were prepared using a Leica RM 2125 RTS rotary microtome from Germany. These slices were mounted on glass slides and stained with Harris haematoxylin and eosin (H&E) for routine examination. Finally, the stained slides were viewed and photographed under a light microscope (Leica, Germany) at various magnifications.

Bacteriological Identification and Antimicrobial Susceptibility Testing

The external surface of each fish was disinfected with 70% ethanol to eliminate potential contaminants before bacteriological examination. Under aseptic conditions, the fish samples were opened from the left side to expose the liver. Following clinical examination, liver tissue was aseptically collected from the internal parenchyma of the liver using a sterile bacteriological loop, avoiding contact with the external liver surface, and inoculated into tubes containing 5 mL of Brain Heart Infusion (BHI) broth. The inoculated tubes were incubated at 25–30°C for 24–48 hours. Enriched broths were subsequently streaked onto TCBS agar, MacConkey agar, and 5% sheep blood agar plates. Plates were examined for colony formation after 24 and 48 hours. When consistent colonial morphology was observed across all media for a sample, a single colony was subcultured; in instances of mixed growth, representative, distinct colonies were selected.

Phenotypic analysis comprised Gram staining, oxidase, and catalase assays. Presumptive isolates were identified to the genus and species level and subjected to antimicrobial susceptibility testing utilizing the automated Phoenix™ microbiology system (Becton Dickinson, USA). Susceptibility profiles were interpreted according to established guidelines based on minimum inhibitory concentration (MIC) values. Multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antimicrobial classes.

Results

Macroscopic and Histological Examination

The examined *Mugil cephalus* specimens had a mean total length of 32.5 ± 4.2 cm and a mean body weight of 285 ± 65 g. A total of 87% (n=13/15) of the examined specimens displayed evident gross and microscopic lesions. External evaluation commonly showed mild lordosis and marbling of the gills, whereas internal findings included severe hepatic congestion with hemorrhages in the liver, swim bladder, and kidneys (Figure 1 A-C). Paleness of the liver and gills was also noted in several individuals (Figure 1D).

Histologically, the liver exhibited marked vascular congestion, diffuse granular and vacuolar degeneration of hepatocytes, and numerous pyknotic nuclei indicating cellular condensation and shrinkage (Figure 2 A-C). Leukocytes infiltration and activation of melano-macrophage centres were observed in 87% (n=13/15) of samples, while unidentified parasitic structures were observed in 80% (n=12/15) of specimens (Figure 2 D-F).

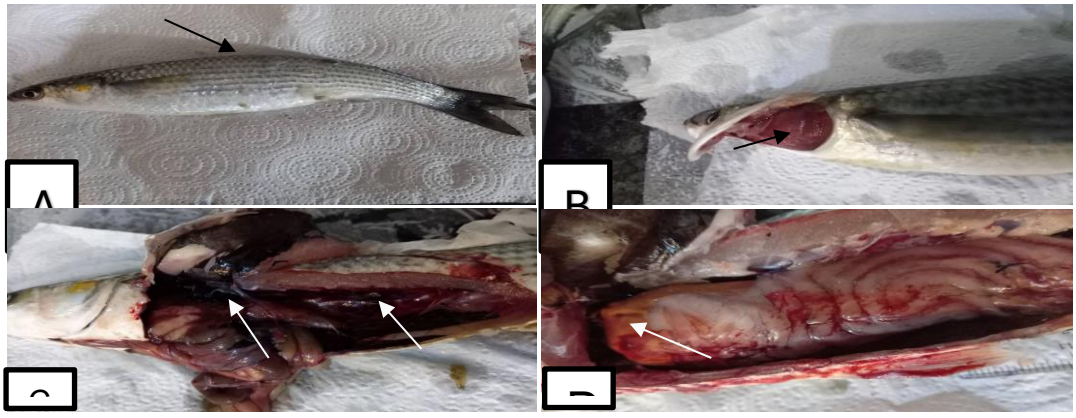

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Figure 1. Naturally infected *Mugil cephalus* fish collected from the Tripoli coast showing: (A) Lordosis (black arrow). (B) Marbling of gills (black arrow). (C) Severe congestion of the liver with hemorrhage in the kidney (white arrows). (D) Pale liver (white arrow).

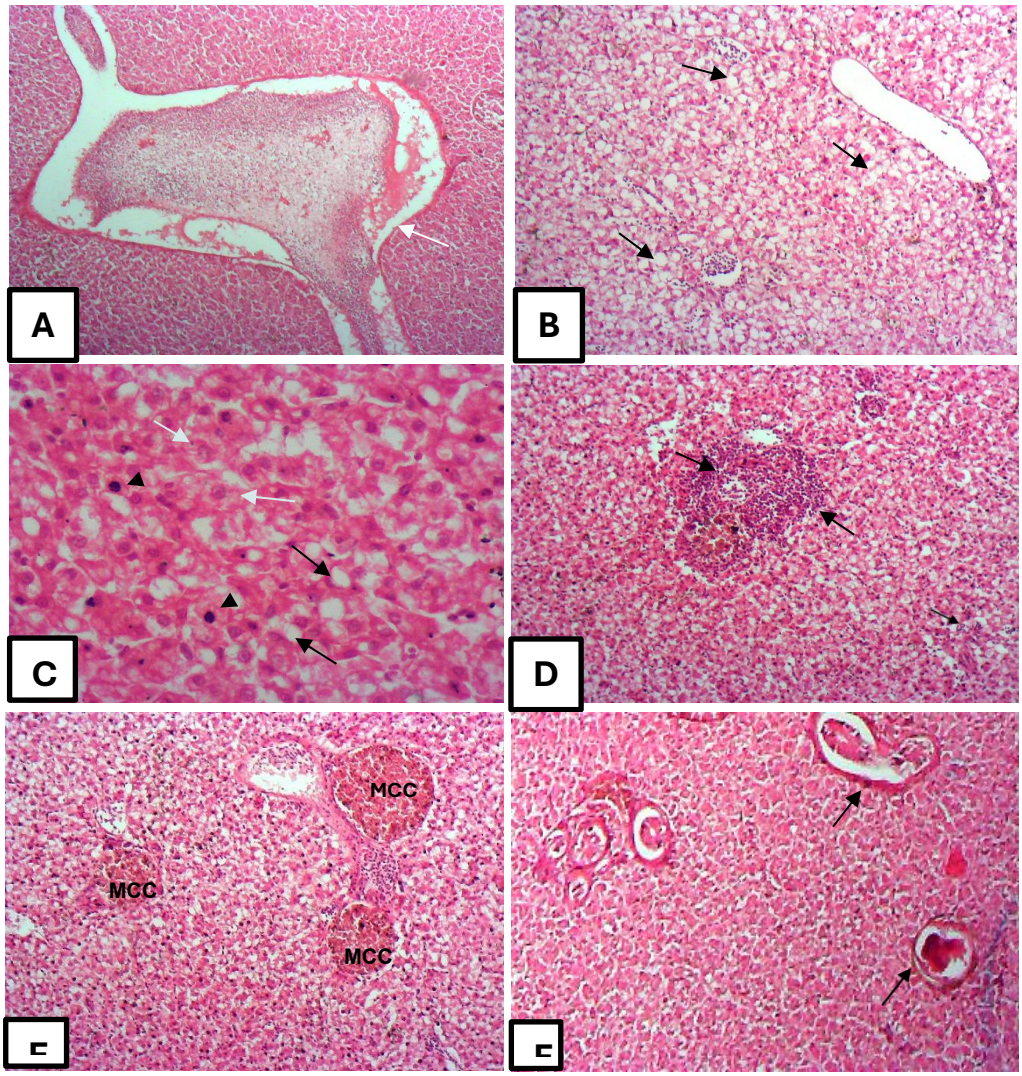

Figure 2. Histological sections of liver tissue of infected *Mugil cephalus* fish showing (A) marked congestion of blood vessels (white arrow); (B) diffuse vacuolar degeneration of hepatocytes (black arrows); (C) granular degeneration (white arrows), vacuolar degeneration (black arrows), and nuclear pyknosis (arrowheads); (D) leukocytes infiltration (black arrows); (E) activation of melano-macrophage centres (**MMCs);** (F) unidentified parasitic structures (black arrows) (incidental finding); (H&E stain: A&F x4; B, D&E x10; C x40).

Bacteriological Analysis and Antimicrobial Susceptibility Testing

Bacteriological examination showed uniform colonial morphology in all cultured samples in all media, yielding 15 Gram-negative isolates, predominantly *Aeromonas* and *Vibrio* species. Of these, 80% (n=12/15) of isolates demonstrated multidrug resistance.

Discussion

Gross pathological findings in the current investigation revealed mild lordosis, gill marbling, intense liver congestion, and multi-organ hemorrhages, which point to systemic disturbances commonly triggered by environmental toxins, nutritional imbalances, or highly transmissible viral or bacterial septicaemias that impair immune function and produce diffuse tissue injury [15].

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Pallor of the liver and gills observed in multiple specimens is a classic indicator of anemia or compromised blood flow [16]. When considered together with the other lesions, this sign reflects chronic physiological stress affecting the sampled population rather than an isolated acute event [17]. The presence of these abnormalities across different length and weight classes further indicates that the condition is not age-dependent [18].

Microscopic changes in the liver of *Mugil cephalus* strongly indicate ongoing exposure to toxicants, reflecting the species' constant contact with contaminated sediments and water [19]. Major hepatic alterations noted here include significant vascular congestion (seen in most samples), widespread vacuolar degeneration of hepatocytes, and pyknotic nuclei, which point to severe cellular damage and are similar to those observed in Arius maculatus under comparable polluted conditions (Kalaiyarasi *et al.*, 2017)[20]. Simultaneous leukocyte infiltration and marked activation of melano-macrophage centers (found in 87% of samples) indicate an active immune response and increased pigment storage for host defence [21].

The repeatability of these histopathological features across species and habitats confirms their value as dependable biomarkers. Analogous liver damage has been documented in *Mugil cephalus* from the Ennore Estuary and linked directly to heavy-metal pollution [22]. The present findings are consistent with reports in Oreochromis niloticus [23] and are supported by a broad literature on hepatic injury under varying environmental pressures [24]. Progressive structural deviations from initial vacuolation to terminal inflammatory infiltration illustrate the eventual overload of the liver's detoxification capacity in the face of persistent xenobiotic challenge.

The frequent detection of unidentified parasites within liver tissue (observed in 80% of specimens) adds another dimension to the observed health compromise. This observation is consistent with regional surveys in the Ain Ziana lagoon, where ectoprotozoan parasites were recorded in both *M. cephalus* and *Tilapia zillii* [1]. Such parasites likely intensify the physiological burden imposed by chemical pollutants, producing synergistic effects that further suppress host immunity.

Isolation of antibiotic-resistant bacteria, chiefly *Aeromonas* and *Vibrio* species, from the liver co-occurred with the inflammatory and leukocytic responses noted above. Of the 15 isolates recovered, 80% (n=12) demonstrated multidrug resistance to at least one agent in three or more antimicrobial classes. These results imply that the liver, while already compromised by chemical stressors [8], may also serve as a reservoir for bacterial persistence [10].

Studies in similar aquaculture systems have identified parasitic crustaceans as potential carriers that facilitate the spread of MDR strains, such as *Vibrio alginolyticus* [25]. This interaction between parasites and hosts highlights a complex relationship between parasitism and bacterial virulence that worsens in stressed marine ecosystems (Kumar *et al.*, 2024)[14]. The presence of MDR bacteria in a widely consumed species like *Mugil cephalus* presents a significant One Health concern, as fish populations can serve as reservoirs that spread resistant "superbugs" into the community, putting consumers and fishery workers at risk (Eid *et al.*, 2022)[26]. This environmental reservoir is especially concerning in the Libyan context, where hospital-acquired infections affect 13.7% of patients and carbapenem-resistant Gram-negative pathogens are already common in Tripoli hospitals [27,28].

According to recent investigations along the Tripoli coastline [13] The influx of untreated sewage during winter rain events introduces multidrug-resistant bacteria that, combined with seasonal thermal stress, trigger significant severe tubular necrosis, hyaline casts, glomerular atrophy, and interstitial mononuclear infiltration in kidney tissue, while splenic tissues exhibited marked activation of the melano-macrophage centres (MMCs), vascular congestion, and multifocal granuloma in *Mugil cephalus*.

Taken together, these data underscore the urgent need for coordinated strategies to monitor and mitigate the escalation of antimicrobial resistance. Effective management of these coastal ecosystems demands stricter oversight of antibiotic usage and strengthened biosecurity practices to interrupt transmission pathways linking the environment, aquatic life, and human populations.

Conclusion

The present study demonstrates significant systemic health compromise in *Mugil cephalus* populations along the Tripoli coastline, characterized by extensive gross and microscopic lesions and progressive hepatic deterioration that occurred independently of fish size. The concurrent isolation of multidrug-resistant Aeromonas and Vibrio species, alongside observed parasitic infestations, underscores a burgeoning 'One Health' challenge within this marine ecosystem. These findings necessitate the implementation of rigorous pollutant monitoring and antimicrobial regulatory frameworks to mitigate further environmental degradation and safeguard public health.

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Conflict of interest

The authors declare no conflict of interest.

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