Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(2): 1179 – 1185 (2025)
www.ejabf.journals.ekb.eg

Histopathological Study of the Common Carp (*Cyprinus carpio* L.) Infected by *Pseudomonas aeruginosa*

Entisar SH. Hashim[1], Nadia A. H. Al- Shammari[2]*, Habeeb M. H. Alsudani[3] , Khalidah S. Al-Niaeem[4]

[1]Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Iraq
[2]Department of Natural Marine Science, College of Marine Sciences, University of Basrah, Basrah, Iraq
*Corresponding Author: nadia.ali.alshmeri@gmail.com

ARTICLE INFO
Article History:
Received: Jan. 28, 2025
Accepted: March 9, 2025
Online: March 23, 2025

Keywords:
Histopathological,
Liver,
Pseudomonas aeruginosa,
Common carp

ABSTRACT

This study focused on isolating and diagnosing the pathogenic bacteria in the fish and examining their histological effects on the internal organs, including the liver, kidneys, and intestines of the common carp (*Cyprinus carpio* L.). In the intestine, necrosis was observed, with dissection of the mucous layer and infiltration of neutrophil cells in the submucosal layer. The liver exhibited hepatocellular vacuolar degeneration with bile duct dilatation and degeneration of its lining. In kidneys, a renal tubule necrosis was observed with epithelial shedding and infiltration of macrophages and neutrophils in the tissue between the tubules. *Pseudomonas aeruginosa* has been identified as a causative agent through histological methods, and its impacts on the fish health have been documented.

INTRODUCTION

Pseudomonas aeruginosa, known as a Gram-negative that is aerobic-facultative and anaerobic with rod-shaped bacteria, causes disease in plants, animals, humans **(Al Shammari *et al*., 2024)**. Bacterial diseases are one of the most common pathogens in farmed fish (**Austin & Austin, 2007**). The infections of these bacteria can cause damage to the liver, kidneys, and spleen, aligned with symptoms such as loss of appetite, lost scales, lost tail fin, ulcers, or even skin bleeding. Untreated, the symptoms can cause significant fish mortality due to the imbalance between pathogens and the host fish **(Al Shammari *et al*., 2023)**. The common carp (*Cyprinus carpio*) is widely used as an animal model in biomedical research due to fish being ideal for studying disease mechanisms **(Megarani *et al*., 2020)**. *C. carpio* is a popular species in Basra aquaculture and has been extensively studied as a model for fish disease in Iraq due to their relative sensitivity and ability to survive in heavily polluted environments. Furthermore, the common carp (*Cyprinus carpio*) is often used as a biomarker for water quality in pathology **(Sanoesi *et al*., 2020; Abduljabbar *et al*., 2025)**. This study aimed to experimentally investigate the pathological and histopathological changes caused by *Pseudomonas aeruginosa* infection in *C. carpio* by focusing on examining the pathological anatomy of infected organs (liver,

kidneys, and intestine) and understanding the histological pathogenesis of *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

1. Fish study

A total of 16 infected common carp (weighing 12.45-16.2g) were collected from the Garmat Ali River in Basrah, Iraq, and then transported to the Laboratory of Fish Diseases, Department of Natural Marine Science, College of Marine Sciences, University of Basrah, during 2023.

2. Histopathology

Tissues (liver, intestine, and kidney) were collected from both infected and control fish and were then fixed with 10% buffered formalin for 48 hours. The tissue processing technique was conducted according to the method of **Humason (1972)**. The tissues were dehydrated in a graded ethanol series and were cleared with xylene, then embedded in paraffin. Blocks were sectioned (5-7μm thickness) by using a rotary microtome. The sections were mounted on glass slides and were then deparaffinized in xylene, hydrated in alcohol, and stained with Hematoxylin and Eosin (H&E) for general histological examination. Photomicrographs of the stained sections were done by using a light microscope, and the gills, kidney, and liver tissues were photographed by using a digital camera (optical camera), and analyzed at a total magnification of 400x.

3. Isolation and identification of bacterial strains

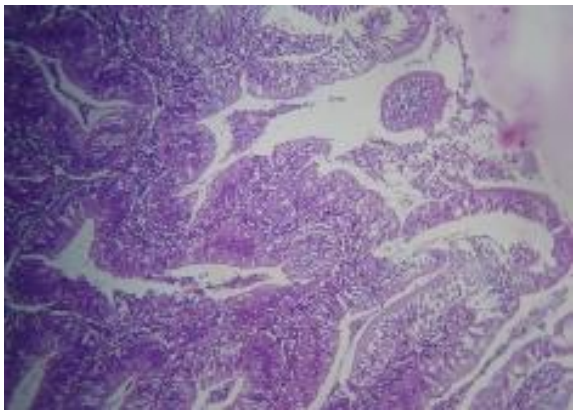
Bacteria were isolated from the liver, intestine, and kidneys of infected fish, which exhibited symptoms of bacterial disease that included ulcers and skin bleeding. The bacterial strains were identified by using Vitek II.

RESULTS

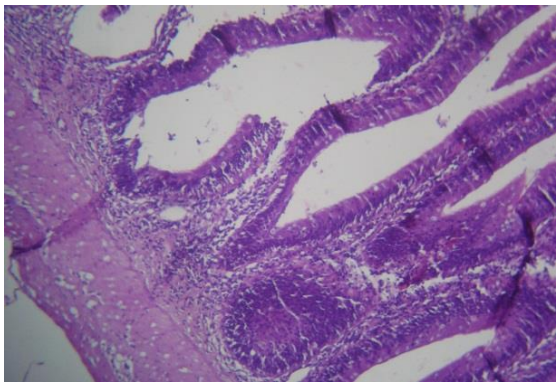
The histological effects of *Pseudomonas aeruginosa* on fish intestines, kidney, and liver were examined. Fig. (1) displays the histological section of the liver.

Histopathological changes in *C. carpio* caused by *Pseudomonas aeruginosa*, a pathogenic bacterium isolated and identified by Vitek 2, were observed in the liver, kidney, and intestine. The pathological states are shown in Figs. (1, 2). The internal organs of C. *carpio* exhibited synthetic pathological changes, as depicted in Fig. (1), including (A) the presence of necrosis with dissection of the mucous layer with infiltration of neutrophil cells in the submucosal layer; (B) the inflammatory cells in the submucosal layer and the presence of degeneration and necrosis; (C) the hepatic cells showing hepatocellular vacuolar degeneration with bile duct dilatation and degeneration of its lining **(Tattiyapong *et al.,* 2020)**, and (D) infiltration of large neutrophil and macrophage cells. Fig. (2) illustrates a histological section of the kidney of common carp with *P. aeruginosa*, where (E) kidney necrosis of renal lining epithelial tubule and

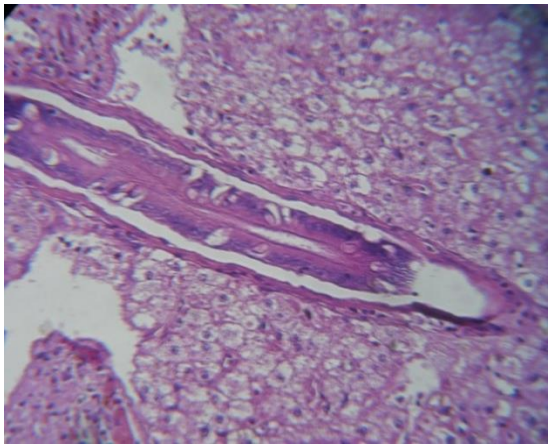
degeneration with eosinophilic other lining epithelial cell, collecting duct occurred; (F) the occurrence of clusters of macrophages, melanocytic macrophages, and lymphocytes between the degenerated renal tubules, and (G) kidney renal tubule necrosis with epithelial shedding with infiltration of macrophages and neutrophils in the tissue between the tubules.



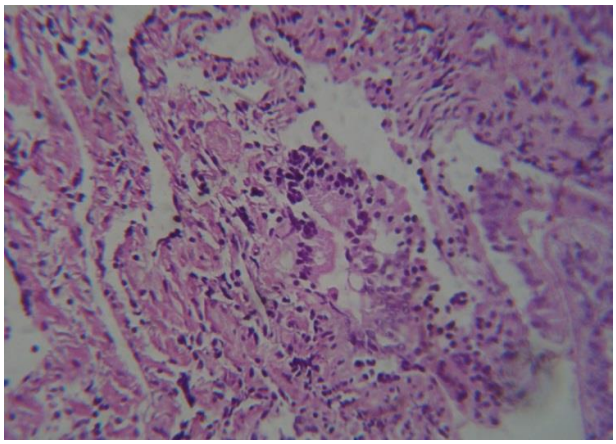
(A) Intestine of the species showing the presence of necrosis with dissection of the mucous layer with infiltration of neutrophil cells in the sub mucosal layer (400×) H&E



(B) Intestine showing the inflammatory cells in the sub mucosal layer and the presence of degeneration and necrosis. (400×) H&E

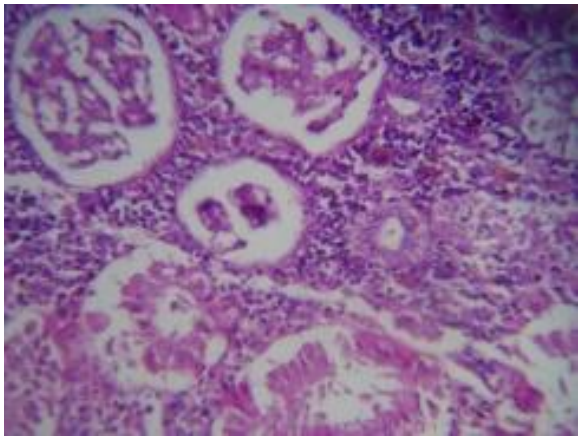


(C) Hepatic cells showing hepatocellular vacuolar degeneration with bile duct dilatation and degeneration of its lining (400×) H&E

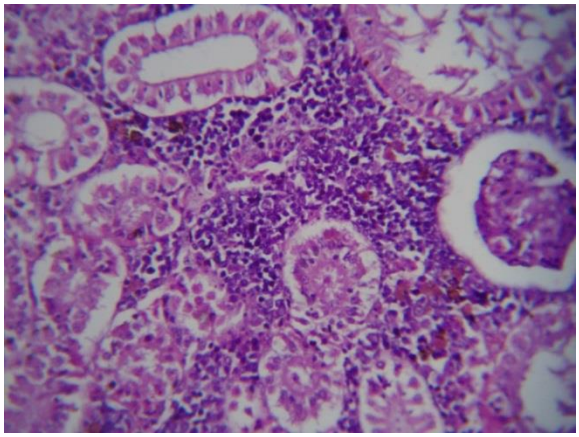


(D) Hepatic cells showing infiltration of large neutrophil and macrophage cells (400×) H&E

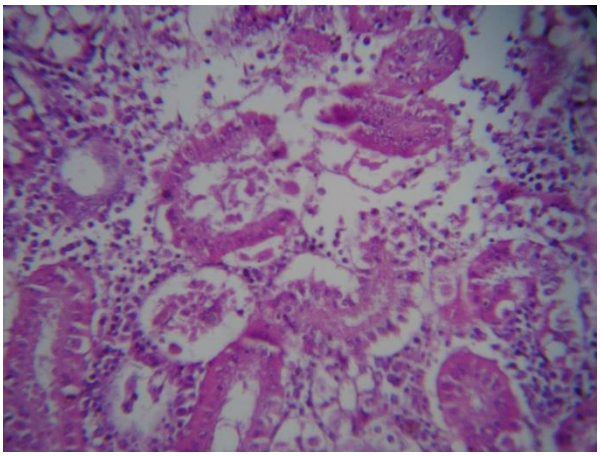
Fig. 1. Photomicrograph (H& E) of intestine & liver of common carp infected by *P. aeruginosa* (400×)



(E) Kidney necrosis of renal lining epithelial tubule and degeneration with eosinophilic other lining epithelial cell, collecting duct (400×) H&E



(F) Kidney the occurrence of clusters of macrophages, melanocytic macrophages and lymphocytes between the degenerated renal tubules (400×) H&E



(G) Kidney renal tubule necrosis with epithelial shedding with infiltration of macrophages and neutrophils in the tissue between the tubules

Fig. 2. Photomicrograph (H& E) of kidney of common carp infected by *P. aeruginosa* (400×)

DISCUSSION

The body of the fish has a high resistance to antibacterial molecules to eliminate bacterial infection **(Al-Shammari, 2021)**. The resistance varies from one fish to another by maintaining internal balance, but as a result of this resistance, the immunity is weakened. Therefore, the current study mainly aimed at isolating the pathogen causing this pathogen, which is widespread in the Iraqi waters in Basrah.

Histological anatomy is a useful tool to identify various effects of the pathogen on living organisms. Histological biomarkers reflect the general state of the health of fish in water **(Moon *et al*., 2012)**. The liver acts as the main organ of detoxification in fish. Hence, the changes observed in the liver of aquatic animals reflect aquatic contamination, as indicated by the precense of pathological tubercles. The liver is a very sensitive tissue and is a delicate organ for assessing the impact of any pollutant on fish, as shown in Fig. (1). Presently, studies on pathogens such as bacteria T showed changes in the normal structure of the liver, such as dilated blood vessels and paranasal sinuses, congestion of dead cells, and grunt. Similar changes in the liver cells of carp liver with dilated sinuses have been observed due to the loss of structural proteins **(Abdel Rahman *et al*., 2022)**.

In examining the kidney, the results revealed that the kidneys of fish consist of a glomerulus, tubes, and blood vessels. The structure of the kidneys in carp specimens revealed the presence of congestion, as shown in Fig. (2).

The ulcers were observed under the skin in some fish (Fig. 1). In advanced stages of the infection, it was detected that dermal necrosis turned into muscle necrosis, and it reached vertebrae by deepening **(Ashiru *et al*., 2011)**.

Infection stimulates physiological stress as the high levels of cholesterol and triglycerides in those affected have a significant effect on blood glucose. The increase in these parameters is due to the fat mobilization in the liver **(Alsudani *et al*., 2024)**.

CONCLUSION

Bacterial diseases are a major challenge for the aquaculture industry. Due to the dangers and low effectiveness of chemicals, it is necessary to find an effective treatment without side effects for these common diseases. The study identifed the important bacterial species responsible for causing diseases, particularly due to the spread of bacterial diseases in Basra.

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