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Original Article

Histopathological alterations of intestines, liver, kidneys, and spleen in Common carp, *Cyprinus carpio* L. infected by *Staphylococcus lentus*

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Abstract: Staphylococcal infections and bacterial diseases significantly impact fish production and can lead to various fish diseases. However, the effect of infection on aquatic animals, including fish, has only recently received attention. This study examines bacterial Staphylococcal infection in the intestines, liver, kidneys, and spleen in Common Carp, *Cyprinus carpio*. Through a comprehensive review that explored the infection of fish pathogens, the interaction between pathogens and fish, clinical findings, and histological effects on the immune responses of internal fish organs. The histological examinations of infections by *Staphylococcus lentus* revealed notable effects, including necrosis and increased numbers of melanocytic phagocytes, indicative of enteritis.

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Introduction

Staphylococci are Gram-positive, spherical bacteria that are mostly coagulase-positive, belonging to the family of staphylococci and the order of Bacilli (Praja et al., 2023). They are typically found on the skin in a generalized form. Under a microscope, bacterial cells appear in characteristic clusters resembling grapes(Kim et al., 2018). *Staphylococci* are facultative anaerobic organisms. Recently, their pathogenicity and infection have garnered significant attention due to the increasing resistance of bacteria to various antibiotics and their ability to infect humans and animals. A type of pathogenic bacteria is considered opportunistic in animals, taking advantage of compromised immune systems to cause disease (Lozano et al., 2016).

Most studies have suggested that *Staphylococci* is a rare opportunistic pathogen in veterinary medicine; however, our study revealed that it causes many diseases and significant histological changes in fish, leading to numerous economic losses, especially *Cyprinus carpio* in Iraq. The pathogenesis of staphylococcal infection is attributed to virulence factors, including enzymes, toxins, and biofilms, which increase the microorganism's resistance to the host's immune system evasion (Lee et al., 2020).

Aquaculture is a rapidly growing industry that is crucial to ensuring global food security. *Cyprinus carpio* is the most widely consumed freshwater fish and is heavily cultivated in Iraq, which makes it a significant contributor to the country's food supply (Mahboub and Tartor, 2020). Therefore, this study aimed to provide a histological diagnosis of the stages of bacterial infection in the internal organs, viz. intestines, liver, kidneys, and spleen of *C. carpio.*

Materials and Methods

Sample collection: Healthy cultured *C. carpio* (n = 15, with an average body weight of 27.4±0.14 g) were selected for this study. Fish were sourced from the Shatt Al-Arab River.

Preparation of fish samples: Signs and symptoms related to infected fish were recorded after sampling. Live fish were euthanized, and their surfaces were disinfected with 70% ethanol. Aseptic dissection was performed by making a ventral incision on the fish's

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body using a sterile scalpel and scissors to obtain samples of organs, including intestines, liver, kidneys, and spleen. The organs were carefully removed, dissected, and placed in sterile Petri dishes for further processing and analysis.

Bacterial isolation and identification: Bacteria were isolated from the intestines, liver, kidneys, and spleen by aseptically collecting (1 g) of tissue from each organ using a sterile scalpel. The sample was mixed with 9 ml of sterile physiological saline solution in sterile test tubes. A serial dilution was prepared, and 0.1 ml of this solution was transferred to the transplant media using a pipette implanted by the diffusion method. Different culture media are used to isolate bacteria (Table 1). The media were incubated at 35 for 24 to 48 hours. Following colony growth, purification, and diagnosis were performed based on appearance, stained with Gram Stain, and their shape was distinguished by light microscopy. The bacteria were diagnosed using different culture media in terms of the shape of the colony and also were diagnosed using the Vitek 2.

Histopathology: To identify histopathological changes caused by experimental bacterial infection, fish exhibiting clinical signs were selected for examination, particularly during dissection after euthanasia. Due to blisters and bleeding in infected fish, the intestines, liver, kidneys, and spleen were carefully dissected from the collected fish, then fixed in 10% neutral formaldehyde, dehydrated using ethanol, and embedded in paraffin. The paraffin blocks were sectioned (5 µm) and stained with hematoxylin and eosin (H&E); later, the slides were examined under a light microscope to detect histopathological changes.

Results

The results revealed the successful isolation of bacteria from the kidney, spleen, intestine, and liver of injected fish *C. carpio*. Colonies appeared on the culture media 1-2 days after inoculation, characterized by small, white outgrowths with a diameter of approximately 1-1.5 cm. Positive colonies were observed at the center of the plates incubated at 20ºC for 24 hours. The isolated bacteria demonstrated their ability to infect fish experimentally, and our results confirmed the pathogenicity of the isolates. The isolated bacteria showed their ability to infect fish experimentally, and the current study's results confirmed the isolates' pathogenicity.

Table 1. The culture media used to isolate *Staphylococcus lentus*.

Bacteria	Media
Staphylococcus lentus	Nutrient agar MacConkey Blood agar

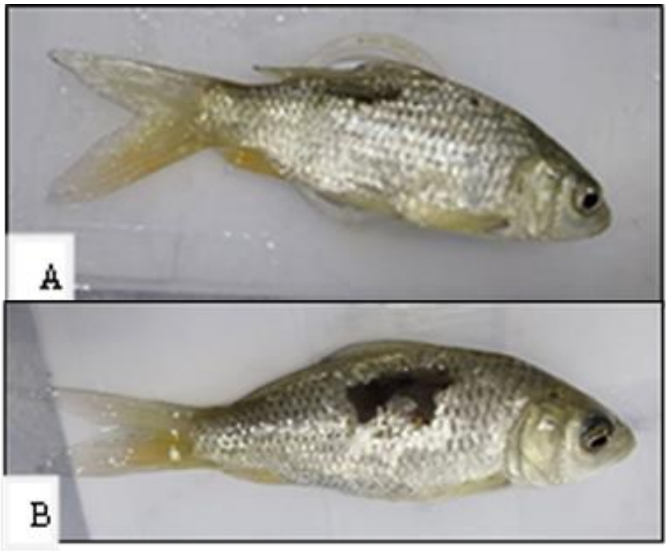

Figure 1. Clinical signs and symptoms of Cyprinus carpio infected by *Staphylococcus lentus*.

Quantitative analysis of the intestinal tissue infected with *Staphylococcus lentus* revealed a range of histopathological changes, including telangiectasia, epithelial lifting, epithelial hyperplasia, hyperplasia, necrosis, vasodilation with vascular hyperemia and cellular necrosis (Fig. 1A, B).

Histological examination of the infected intestine of *C. carpio* displays epithelial necrosis with sloughed necrotic debris in the intestinal lumen (Fig. 2A) and hyperplasia in goblet cells (Fig. 2B). Histological examination of the liver revealed significant changes in tissues infected with *S. lentus*, including melanomacrophages and necrotic pancreatic acini infiltrated with lymphocytes (Figs. 3A, B). Examination of *C. carpio* kidney infected with *S. lentus* showed epithelial cells (ED) and the tubular

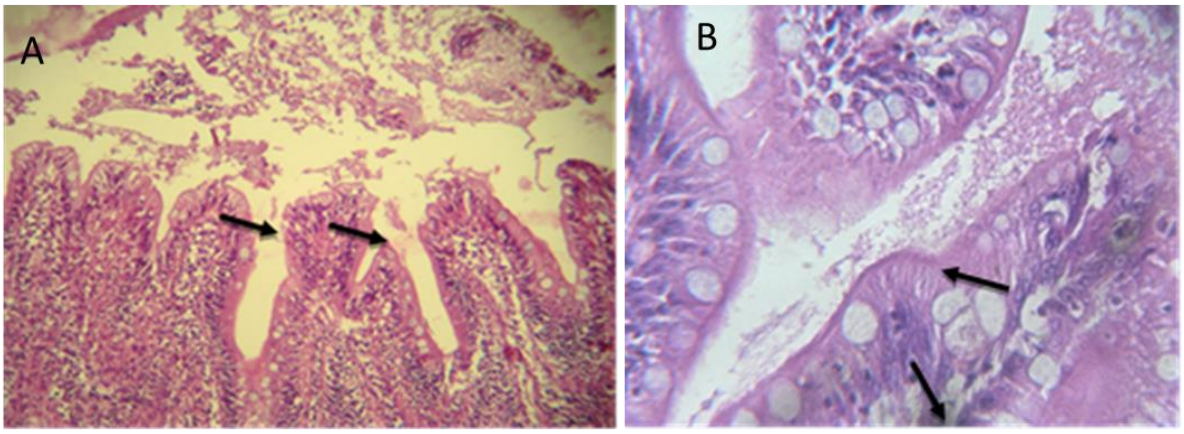

Figure 2. Photomicrograph of the infected intestine of *Cyprinus carpio* by *Staphylococcus lentus* (A) displays epithelial necrosis with sloughed necrotic debris in the intestinal lumen (arrows) (H&E, 200X) and (B) showing hyperplasia in goblet cells (arrows) (H&E, 400X).

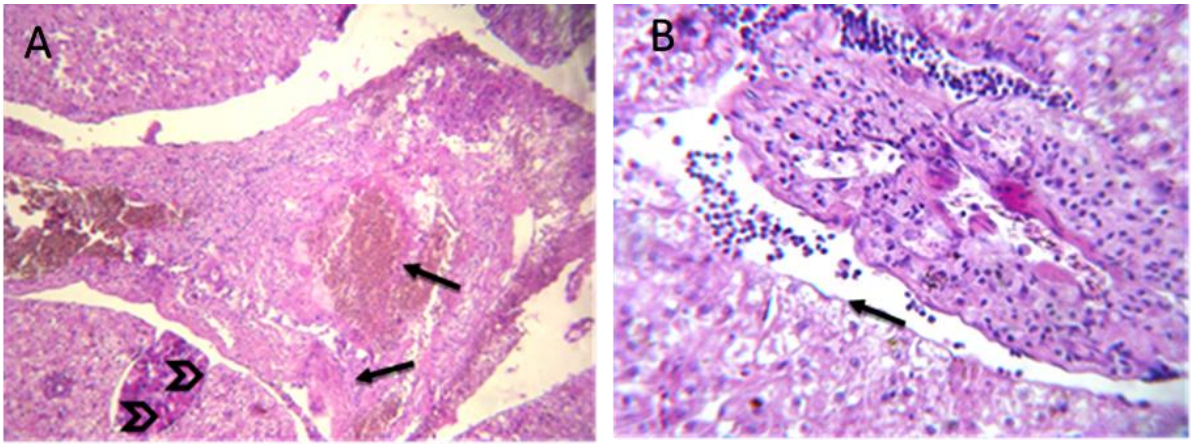

Figure 3. Photomicrograph of *Cyprinus carpio* liver infected with *Staphylococcus lentus* (A) showing melanomacrophages (arrow head) and necrotic pancreatic acini infiltrated with lymphocytes (arrow) (H&E, 200X), and (B) section in the liver (arrow) next to a small bile duct and surrounded with inflammatory cells including plasma cells and lymphocytes. The inflammation includes a collapsed area of parenchyma and present an adjacent nodule of hepatocytes (H&E, 400X).

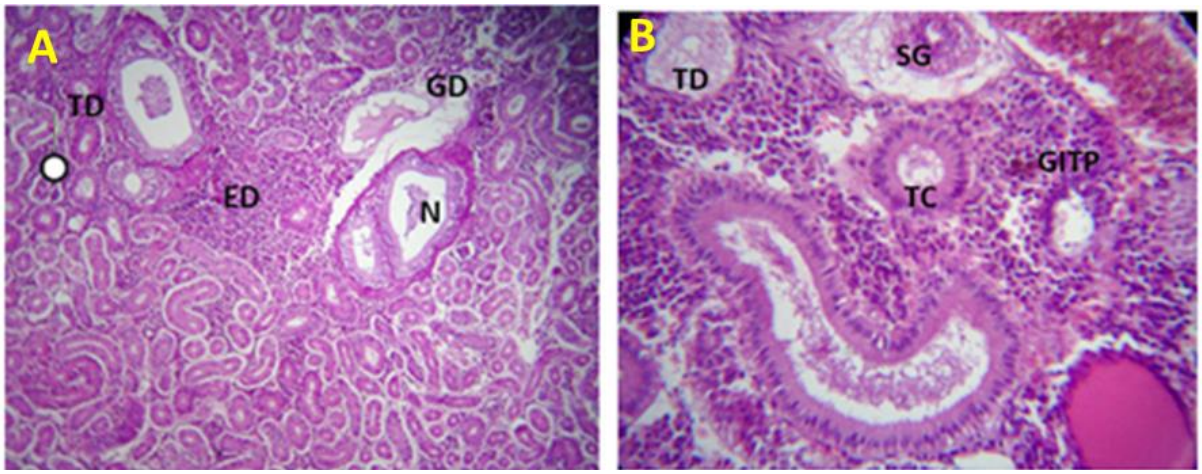

Figure 4. Histopathological changes in the kidney of *Cyprinus carpio* infected by *Staphylococcus lentus* (A) showing epithelial cells (ED), the tubular (TD), together with glomerular degeneration (GD), and necrosis (N) (H&E, 100X), and (B) displaying shrinking of the glomerulus (SG), increased per tubular space with the absence of bowmen's space, glomerulonephritis (GITP), tubules clogging (TC), and tubules degeneration (TD) (H&E, 400X).

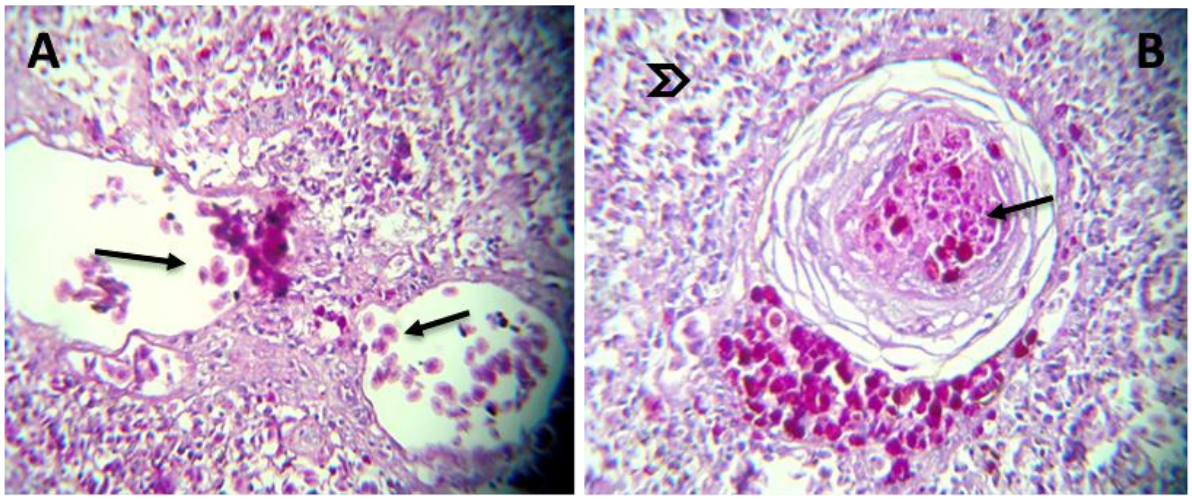

Figure 5. Photomicrograph of sections showing the spleen of *Cyprinus carpio* infected by *Staphylococcus lentus* (A) displaying degenerative changes in the wall of hepatic blood vessels addition to a small number of perivascular mononuclear leukocytic cellular infiltration (arrow) (H&E, 400X), and (B). showing necrosis and vacuolation of pancreatic acinar cells addition to deposition of hemosiderin pigment in some pancreatic acini (arrow), degenerative changes are presented in hepatocytes (arrow head) (H&E, 400X).

(TD), together with glomerular degeneration and necrosis (Figs. 4A, B). Histopathological changes in the spleen pancreas of *C. carpio* infected with *S. lentus* display degenerative changes in the wall of hepatic blood vessels and a small number of perivascular mononuclear leukocytic cellular infiltration. In addition, the deposition of hemosiderin pigment in some pancreatic acini and degenerative changes in hepatocytes were observed (Figs. 5A, B).

Discussions

Bacterial pathogenesis diseases in freshwater fish are closely linked to environmental conditions, diet, poor maintenance, seasonal changes, and physiological conditions. Water pollution by various chemicals and sudden fluctuations in temperature and pH values also significantly increase disease susceptibility and infections mediated by microorganisms. *Staphylococcus* is a Gram-positive bacterium of pathological fish origin and poses a significant challenge to fish farming worldwide. They are commonly found on healthy human and animal skin and mucous membranes. Their pathogenetic and infectious mechanisms have garnered significant attention recently due to their resistance, particularly among the spore-forming staphylococci. As opportunistic pathogens, *Staphylococcus* species can cause diseases in fish (Watts et al., 2017).

The growing demand for high-protein food has led to an expansion in fish production, with fish farming being the most productive technique to meet this demand. However, large-scale fish farming can subject fish to stressful conditions, compromising their immune status and making them more susceptible to infectious diseases. These infectious diseases pose the greatest threat to aquaculture development, resulting in negative impacts on production. The objective of studying *C. carpio* is to prevent the administration of treatment, control, and eradication during outbreaks of infectious diseases caused by environmental conditions or stress from farmed fish by adjusting external and internal sources of stress and minimizing risks. There are inevitably some problems related to external and internal environmental changes of fish growing under metabolic stress caused by loss of nutrients and toxic substances and affecting the endocrine system. Among these challenges, infectious diseases remain aquaculture's most significant limiting factor (Mohammed, 2024).

In the current study, histological examination of the liver and intestines revealed significant changes in tissues infected with *S. lentus*, indicative of bacterial infection. Pathological signs appeared in the tissues across different groups, characterized by vacuolation resulting from cell death and degeneration of liver

cells. This led to an abnormal tissue appearance, with the formation of vacuoles and necrotic areas in the liver tissue (Pier and Ramphal, 2005; Jasim and Al-Sudani, 2019). Coagulative necrosis typically results from infarction (lack of blood flow from an obstruction causing ischemia) and can occur in any cells except those in the brain. The heart, kidneys, adrenal glands, and spleen are common sites for coagulative necrosis. Affected cells become dry, hard, and white with a jelly-like appearance in necrotic tissues while preserving cellular structure for a few days. Coagulation occurs due to protein breakdown and denaturation (Chong, 2022). It effectively stimulates the immune response against inflammatory pathogens and stimulates macrophages, pivotal cells in the innate antimicrobial immune system. The outbreak of bacterial diseases affects the health status of fish, especially vulnerable ones.

The histopathological changes observed in the spleen in this study, likely caused by a bacterial infection, are consistent with those reported by Fernandez-Bravo and Figueras (2020) in common carp with marked liver cell necrosis. This necrosis resulted from the biological interaction between invading bacteria and the host's immune defense, leading to cell death due to a failed blood supply. The liver cell injury and the subsequent blood supply failure (cell death) were caused by the antibody-antigen interaction between invading bacteria and the host's immune defense (Baldissera et al., 2019; Al-Haider et al., 2019). The spleen exhibited a pronounced hemorrhaged distribution of the lymphatic follicles in the white pulp, possibly due to increased defensive cells indicating immunostimulation. This immunostimulation was likely a result of experimental injury and the onset of pathogenic symptoms on the fish surface, leading to histological changes in the spleen characterized by macrophage aggregation and degeneration (Srichaiyo et al., 2020).

Conclusion

In conclusion, the histopathological results indicated that the bacteria infected the skin and penetrated internal organs, including the intestines, liver, and spleen. Based on these findings, the authors recommend a future study to develop diagnostic methods for detecting pathological bacteria in Iraq, contributing to improved disease management and prevention in aquaculture.

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