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Investigation and Identification of the Most Bacterial Infection in the Invasive Nile Tilapia Fishes in Euphrates River Banks, Al-Najaf Province, Iraq

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ABSTRACT

This study was conducted to identify and diagnose the major bacterial pathogens affecting the Nile tilapia (*Oreochromis niloticus* L.) collected from the banks of the Euphrates River in Al-Najaf Province. A total of forty live Nile tilapia showing disease symptoms were sampled between November and December 2024. Samples were collected from the skin, gills, and internal organs, including the liver, spleen, kidney, and gastrointestinal tract (GIT). The samples were cultured on different media, and the results revealed the presence of *Escherichia coli*, *Aeromonas* spp., *Staphylococcus aureus*, and *Salmonella* spp. Pathological changes observed in the affected fish included necrotic and skin ulcers, widespread hemorrhagic spots, exophthalmia (eye protrusion), visceral congestion, and pale liver coloration. The findings confirmed that members of the Enterobacteriaceae family are the most prevalent bacteria in the Nile tilapia. Moreover, *Salmonella* spp. were found to be passively carried by fish and are capable of causing a wide range of diseases, including bacteremia and gastroenteritis. In addition, the study demonstrated that *E. coli* is associated with serious health disorders in the Nile tilapia. Importantly, the consumption of diseased fish poses significant risks to public health, including food deterioration, food poisoning, and food intoxication.

INTRODUCTION

Tilapias are among the most important warm-water fish species used in aquaculture production **(Kumar & Engle, 2016)**. Owing to their adaptability and tolerance to diverse ecological and environmental conditions, tilapia—originally native to Africa—have been introduced to tropical, subtropical, and temperate regions worldwide to support capture fisheries **(El-Sayed, 2013; Al-Shammari & Al-Niaeem, 2025)**. Globally, about 70 species of tilapia are known, with the Nile tilapia (*Oreochromis niloticus*) being the most widely farmed species. It is generally recognized that concerted

efforts are required to preserve and improve the genetic quality of farmed tilapia stocks **(Bentsen *et al.*, 2012)**.

In Africa, tilapia farming was first documented in Kenya in 1924, after which it spread throughout the continent **(Shrestha *et al.*, 2018; Adeleke *et al.*, 2020)**. In Iraq, the Nile tilapia was first reported in the Shatt al-Arab River, making it the third cichlid species recorded in the country, alongside *Tilapia zillii* and *O. aureus* **(Al-Faisal & Mutlak, 2014; Bashar *et al.*, 2025)**. Their ability to tolerate harsh climatic conditions is one of the key reasons for their successful spread. Nile tilapia are particularly suitable for warm-water aquaculture due to their rapid growth, resilience to stress and disease, broad salinity tolerance, short generation time, herbivorous/omnivorous feeding habits, ability to reproduce in captivity, and early acceptance of artificial feeds after yolk-sac absorption **(Ng & Romano, 2013; Jumma, 2024)**.

Despite these advantages, bacterial infections remain one of the most significant challenges in tilapia aquaculture, causing severe economic losses and posing zoonotic risks to consumers **(Plant & La Patra, 2011; Hamouda *et al.*, 2019)**. Most infections are caused by opportunistic bacteria. While Gram-positive pathogens have been reported in recent years, Gram-negative bacteria remain the dominant group **(Najem *et al.*, 2020; Mohamed *et al.*, 2021)**. Pathogens affecting tilapia include *Flavobacterium* spp., *Edwardsiella* spp., *Lactococcus* spp., *Enterococcus* spp., *Micrococcus* spp., *Streptococcus* spp., *Vibrio* spp., *Aeromonas* spp., and *Pseudomonas* spp. **(Abu-Elala *et al.*, 2020)**.

In addition to bacterial pathogens, tilapia are known to impact ecosystems by outcompeting native fish species for food and habitat, leading to competitive displacement and reduced biodiversity. This study therefore aimed to evaluate both the distribution of bacterial diseases in the Nile tilapia and the ecological implications of tilapia introduction in the study region.

MATERIALS AND METHODS

Subject of study

This study was conducted on the diseased Nile tilapia (*Oreochromis niloticus*) collected from the banks of the Euphrates River in Al-Najaf Province during the period from November to December 2024. The aim was to diagnose and investigate bacterial infections affecting the sampled fish.

Sample collection

A total of forty diseased Nile tilapia were collected from the Euphrates River. Samples were obtained from the skin, gills, and internal organs, including the kidney, spleen, liver, and gastrointestinal tract (GIT).

Bacterial culture

Bacteriological examinations were performed on all collected samples. Swabs were taken aseptically from the liver, kidney, spleen, stomach, intestine, and gills, and inoculated into nutrient broth. Cultures were incubated at 30°C for 24–48 hours. Pure bacterial isolates were obtained through sub-culturing onto selective media, including MacConkey agar, Mannitol salt agar, and Xylose Lysine Deoxycholate (XLD) agar, for differentiation and identification of bacterial species.

RESULTS AND DISCUSSION

Database description of fishes sampling

The Nile tilapia (*Oreochromis niloticus*) specimens were collected by local fishermen from the banks of the Euphrates River in Kufa, Al-Najaf Province, during the period from November to December 2024 (Fig. 1).

Fig. 1. Photographs of the Nile tilapia fishes sampled from the Euphrates River banks

Clinical signs and macroscopic findings

Macroscopic lesions in the liver, gills, skin, spleen, kidney, and gastrointestinal tract (GIT) of the Nile tilapia affected by bacterial disease are shown in Fig. (2). The fish exhibited extensive ulcerative necrosis, darkened skin with erosions on the caudal fin, external scattered hemorrhagic patches, and exophthalmia. On necropsy, the liver, spleen, and kidney were swollen, accompanied by abdominal distension. The kidney appeared congested, while the liver showed marked pallor.

Fig. 2. The Nile tilapia fish (*Oreochromis niloticus*) infected with bacterial disease: erosion in caudal fin and external scattered hemorrhagic patches and exophthalmia. Upon necropsy, the liver spleen and kidney swollen and the abdominal distension

Microbiological findings

Tissue samples from the skin, gills, liver, kidney, spleen, and gastrointestinal tract (GIT) were cultured on blood agar, MacConkey agar, Xylose Lysine Deoxycholate (XLD) agar, and Mannitol salt agar to identify bacterial species. Reisolation and identification of bacteria were achieved from organs showing gross lesions.

On MacConkey agar, *Aeromonas* spp. colonies appeared pale, indicating the inability of the bacteria to ferment lactose. *Staphylococcus aureus* grew on Mannitol salt agar, producing golden-yellow colonies, which reflected mannitol fermentation. *Salmonella* spp. produced slightly red colonies with black centers on XLD agar, indicating xylose fermentation and hydrogen sulfide production. In contrast, *Escherichia coli* colonies appeared pink to red on MacConkey agar, confirming their ability to ferment lactose (Fig. 3).

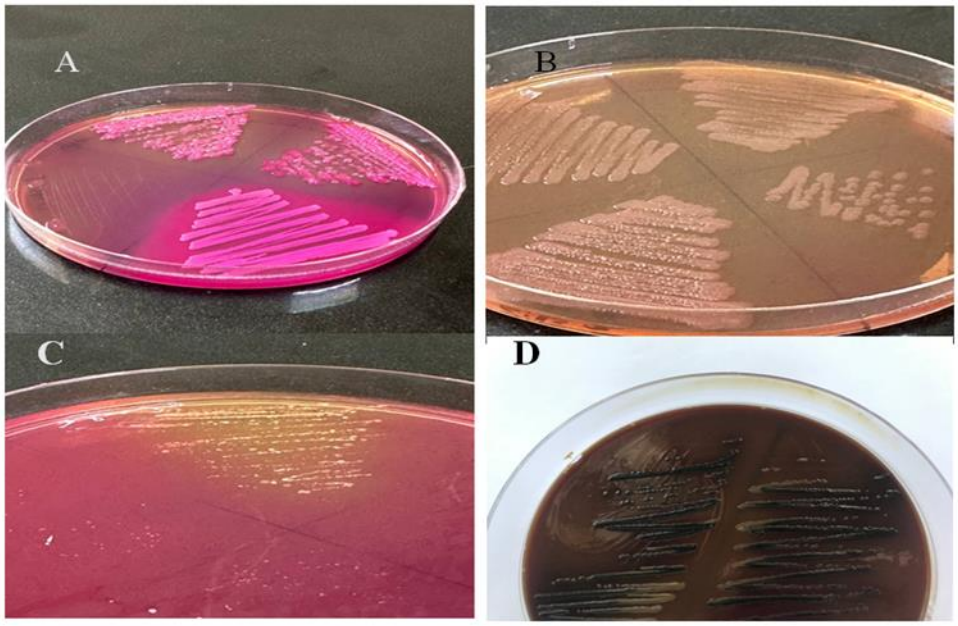

Fig. 3. A, B- *E. coli* produces pink colonies on Macconky agar, *Aeromonas* spp. produce pale colonies on Macconky agar after 24 hours. **C-** *S. aureus* produces yellow golden colonies on MS agar. **D-** *Salmonella* spp. produces slightly red color with black center colonies on XLD medium

Bacterial species isolated from the Nile tilapia

The bacteriological examination revealed the presence of both Gram-negative bacteria, including *Escherichia coli*, *Aeromonas* spp., and *Salmonella* spp. (Table 1), as well as the Gram-positive bacterium *Staphylococcus aureus* (Table 2). The incidence rates of bacterial infection among the forty diseased Nile tilapia samples were as follows: *E. coli* – 16 isolates (40%), *Aeromonas* spp. – 20 isolates (50%), *Salmonella* spp. – 5 isolates (12.5%), and *Staphylococcus aureus* – 7 isolates (17.5%).

Table 1. Gram negative bacteria isolated from the Nile tilapia fish

Bacterial species	**Culture media**	**Colony appearance**	**Fermentation Indicator**	**No (%) incidence of bacteria**
Escherichia coli	MacConkey agar	Pink to red colonies	Lactose fermentation (+)	16 (40%)
Aeromonas spp.	MacConkey agar	Pale colonies	Lactose fermentation (-)	20 (50%)
Salmonella spp.	XLD agar	Slightly red colonies with black center	Xylose fermentation (+)	5 (12.5%)

Abbreviation: XLD Xylose lysine deoxy cholate agar.

Table 2. Gram- positive bacteria isolated from the Nile tilapia fish

Bacterial species	**Culture media**	**Colony appearance**	**Fermentation Indicator**	**No (%) incidence of bacteria**
Staphylococcus aureus	Manitol salt agar	Golden yellow colonies	Mannitol fermentation (+)	7 (17.5%)

Table (3) illustrates the incidence rates of bacteria isolated from the internal organs of the infected Nile tilapia. *Escherichia coli* was detected in all sampled organs, with the highest incidence in the gastrointestinal tract (GIT) (6 isolates; 15%), followed by the spleen (4; 10%), liver (3; 7.5%), kidney (2; 5%), and gills (1; 2.5%). Similarly, *Aeromonas* spp. was most frequently isolated from the gills (7; 17.5%), followed by the liver (4; 10%), spleen (3; 7.5%), kidney (2; 5%), and GIT (4; 7.5%). *Salmonella* spp. was isolated only from the gills (3; 7.5%) and GIT (2; 5%). In contrast, *Staphylococcus aureus* was recovered primarily from the spleen (5; 12.5%) and to a lesser extent from the kidney (2; 5%).

Table 3. Incidence rate of bacteria isolated from organs of the Nile tilapia

Organs	***Escherichia coli***	***Aeromonas* spp.**	***Salmonella* spp**	***Staphylococcus aureus***
Gills	+ 1 (2.5%)	+ 7 (17.5%)	+ 3 (7.5%)	-
Liver	+ 3 (7.5%)	+ 4 (10%)	-	-
Spleen	+ 4 (10%)	+ 3 (7.5%)	-	+ 5(12.5%)
Kidney	+ 2 (5%)	+ 2 (5%)	-	+ 2 (5%)
GIT	+ 6 (15%)	+ 4 (7.5%)	+ 2 (5%)	-

Abbreviation: + positive, - negative.

Tilapia fish (*O. niloticus*) are found in the Euphrates and Tigris rivers, as well as in marshes and ponds. After carp, they are considered a secondary fish source for consumers in Iraq. In recent years, their population has increased due to their ability to tolerate a wide range of environmental conditions, such as water scarcity, changes in water pH, and rising salinity levels. Additionally, tilapias are known to feed on fingerlings and smaller fish, further contributing to their ecological impact.

Disease management in tilapia aquaculture is considerably more challenging compared to carp. Tilapia predominantly inhabit open-water systems, including rivers and natural ponds, where environmental variables cannot be controlled and pathogen exposure is inevitable. In contrast, common carp are typically reared in controlled cage culture environments that facilitate disease management **(Gu *et al.*, 2015)**. Moreover, carp (*Cyprinus carpio*) are intensively cultured in Iraq and represent the most preferred species for human consumption. However, this intensive production system makes them

particularly susceptible to disease outbreaks, including viral diseases and secondary bacterial infections **(Kane *et al.,* 2025)**.

Fish are significant in the human diet because they contain essential proteins, vitamins, and minerals necessary for health. Since fish are vital to human existence, they are often considered the most appropriate bioindicators in aquatic environments **(Najem *et al.,* 2024)**. The study's findings showed that fish affected by bacterial infections displayed hemorrhages on the skin, swollen organs, pale livers, congested kidneys, and erosions at the nuchal region (Fig. 2). These results align with Gomez Gil, who reported external abnormalities such as skin damage and fin erosion. Other studies noted loosening scales, congested gills, eye cloudiness, abdominal edema, and general body reddening **(Gil, 2007; Sabah *et al.,* 2019)**.

The current study found that the liver, spleen, and kidney of infected fish were swollen, with abdominal distension. Additionally, the kidneys were congested, and the livers appeared pale (Fig. 2). These results agree with findings that Nile tilapia infected with bacterial diseases suffered from enlarged and darkly swollen kidneys, splenomegaly, enlarged livers with enlarged gallbladders, and in some cases, pale livers **(Mohammd *et al.,* 2019; Ayoub *et al.,* 2021)**.

Important preliminary information on pathogens affecting fish along the Euphrates River bank is provided by the bacterial isolates detected in diseased tilapia in this study. The bacteria recovered from the infected Nile tilapia belong to several genera, including Gram-negative and Gram-positive bacteria. These isolates are known primary and secondary pathogens that infect tilapia (Table 3). The study revealed that bacterial cultures from fish samples included *E. coli*, *Salmonella* spp., *Aeromonas* spp., and *Staphylococcus aureus* (Fig. 3). These results are consistent with previous studies, which reported that certain bacteria are opportunistic and infect fish under favorable conditions. Many bacteria are commensal to fish and other aquatic organisms or exist freely in the environment **(Kassa & Mitiku, 2021; Sabah *et al.,* 2024)**. Fish that are immunosuppressed by stress are particularly vulnerable to opportunistic bacterial infections **(Gomez *et al.,* 2013)**.

Tilapia fish infections can be caused by a variety of pathogenic bacteria, including *Pseudomonas* spp., *Aeromonas* spp., *Vibrio* spp., *Flavobacterium* spp., *Edwardsiella* spp., *Lactococcus* spp., *Enterococcus* spp., and *Micrococcus* spp. **(Dong *et al.,* 2017)**. Human and animal waste can contaminate cultured water, and tilapias prepared in contaminated environments often carry *E. coli* **(James *et al.,* 2001)**.

The study found that *E. coli* was present in all isolated organs of the tilapia fish: GIT 6 (15%), spleen 4 (10%), liver 3 (7.5%), kidney 2 (5%), and gills 1 (2.5%) (Table 3). The Nile tilapia infected with *E. coli* showed hemorrhages in the gills, kidney congestion, and necrosis in the liver and GIT **(Aly *et al.,* 2012)**. In the present study, the incidence rate of *Aeromonas* spp. was 7 (17.5%) in gills, 4 (10%) in liver, 3 (7.5%) in spleen, 2 (5%) in kidney, and 4 (7.5%) in GIT (Table 3). *Aeromonas* species, particularly *A.*

hydrophila, are commonly isolated from various organs of diseased tilapia, including the gills, spleen, liver, intestine, and kidneys. They cause gill damage and inflammation, leading to respiratory distress, as well as splenomegaly, liver necrosis, kidney congestion, and intestinal damage **(Ibrahem *et al.*, 2008)**.

The current study revealed the isolation of the Gram-positive bacterium *Staphylococcus aureus* from the spleen (5; 12.5%) and kidney (2; 5%) of infected tilapia (Table 3). These results are consistent with the study of Gaafar, who reported methicillin-resistant *Staphylococcus aureus* (MRSA), leading to significant morbidity and mortality rates in the Nile tilapia bred in Egypt **(Gaafar *et al.*, 2015)**. MRSA was also isolated from Malaysian tilapia for the first time in aquaculture **(Atyah *et al.*, 2010)** and linked to morbidity and mortality in the cultured Nile tilapia in northern Egypt **(Soliman *et al.*, 2014)**. Infected tilapia displayed skin hemorrhages and dorsal erosions. Postmortem examination revealed enlarged gallbladders, pale livers, congested gills, and enlarged kidneys and spleens **(Soliman *et al.*, 2014)**. MRSA is a serious human and animal pathogen, and its emergence as a fish pathogen has raised zoonotic concerns **(Salam *et al.*, 2020; Ziarati *et al.*, 2022)**.

The study also showed that *Salmonella* spp. was isolated from gills (3; 7.5%) and GIT (2; 5%) (Table 3). *Salmonella* in freshwater is typically linked to fecal contamination at fish harvesting sites **(Bibi *et al.*, 2015)**. Fish can excrete *Salmonella* as passive carriers without showing symptoms **(Araújo *et al.*, 2024)**. Globally, *Salmonella* is the second leading cause of foodborne illness **(Lamichhane *et al.*, 2024)** and is responsible for diseases in humans, including gastroenteritis, bacteremia, and enteric fever. Most human salmonellosis cases each year are caused by consuming undercooked eggs, shellfish, and fish **(Awuor *et al.*, 2011)**.

CONCLUSION

The Nile tilapia are considered a significant exotic invasive species in fish aquaculture in Iraq. This study suggests that tilapia have a negative impact due to their competitive displacement of other fish species, which reduces native fish populations. Furthermore, fish affected by bacterial diseases may pose serious public health risks, including food spoilage, food intoxication, and food poisoning. Therefore, the findings of the present study provide a starting point for controlling and preventing bacterial infections affecting Nile tilapia and other local fish species within Iraq's aquaculture systems. Further studies are needed to confirm the isolation of these bacteria using genetic techniques. Additionally, due to water scarcity, the intensive culture of the Nile tilapia is expected to expand in Iraq in the coming years.

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