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Isolation and Identification of Fish Infected with Bacteria in Some Farms of Babylon in Iraq

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Abstract

Fisheries is one of the main sectors on which some countries are increasingly dependent. Filling the food gap is an important resource in the agricultural resource base, but it is one of the neighborhoods affected by the agricultural activities. The bacterial disease agents were isolated from aquaculture in the Babylon governorate, Iraq, during December 2021 to the end of October 2022. A total of 65 specimens of *Cyprinus carpio* were infected (53%). Four bacterial diseases were diagnosed by clinical signs that appeared from severe infections of external and internal organs (fin rot, dropsy, bacterial gill disease, and vibriosis). Bacteria were identified according to their morphological characterization and using the Vitek 2 system, and they include Aeromonas *sobria, A. hydrophila, A. veronii, Streptococcus thoraltensis, Serratia ficaria, Serratia fonticola, Serratia odorifera,* and *Vibrio cholerae*. The results of the current study showed that the emergence of diseases in fish farms may be related to stress factors that fish suffer from as a result of dryness and scarcity of water, water quality, or the possibility of bacteria transfer either from water to fish or vice versa, or by handling.

Keywords: *Vibrio* spp., *Cyprinus carpio*, Fish pathogen, Vitek2, *Aeromonas* spp.

1. Introduction

Negative human beings are harmful to the aquatic environment, as its unsustainable exploitation led to the disruption of this resource (1). The world's focus has shifted towards fish to address the deficiency of animal protein, particularly in light of the increasing global population and depletion of natural resources (2). The world's fisheries reached 90.9 million tons in 2016, of which 11.6 million tons came from water. In the interior, in Haifa, the quantitative production of aquaculture was 80.0 million tons, and in Minya, 51.4 million tons at the level of inland water production. Aquaculture, especially fish farming, is one of the fastest-growing enterprises. Global fish production has increased dramatically over the past five decades, and aquaculture is an increasingly important option in animal protein production (3). To increase production, people typically raise fish at high densities in limited

places like earthen ponds and floating cages. In turn, this exposes the fish to stress factors like crowding, low oxygen levels, and water quality, all of which can negatively impact their health status. These factors increase the susceptibility of fish to disease, and stressors suppress the immune system (5). The largest family of freshwater fish in Iraq is the *Cyprinidae*, which also includes *C. carpio*. The species to which Iraq has most effectively adapted is the common carp, whether in fish farms or the majority of interior rivers. *C. carpio* is one of the most valuable fish species in the country's interior waters. It has an important place between the growth of the organism due to its omnivorous nature, quick growth, ease of keeping in enclosed spaces, viability in a wider range of water temperatures (3-35°C), significant tolerance against a lack of oxygen, disease resistance, high fertility, and ease of reproduction, and they reach sexual maturity at 3–4 years of age. It is also relatively tasty meat. The common carp has evolved to endure under a range of climatic and geographic circumstances (6, 7).

All living things, even common carp fish raised on farms and in the wild, are susceptible to these illnesses (8). Environmental problems, poor nutrition, and other factors cause non-infectious diseases, while harmful organisms in the environment cause infectious diseases. nutrition, genetic abnormalities, or both (9). Most pathogenic bacteria are naturally occurring saprophytes, acting as opportunistic pathogens that infiltrate the tissue of a fish host due to various disease processes. A few bacterial species appear to be obligatory parasites of fish, and they may be surviving for varying lengths of time in the environment. Bacterial disease is responsible for heavy mortality in both wild and cultured fish (10, 11). Generally, we categorize pathogenic diseases as bacterial infections due to their higher prevalence. They are frequently characterized by high death and morbidity rates as well as widespread adverse impacts on farmers, consumers, and the environment. The sickness may result from an imbalanced interaction between the disease-causing agents, the host fish, and the aquatic environment (pathogens). Unbalanced interactions facilitate stress and disease entry, thereby causing stress in the fish (9).

Without their hosts, bacteria can thrive in an aquatic environment. Additionally, bacterial illnesses have emerged as significant hurdles to aquaculture, particularly when the water is warm. Reports say that bacteria from at least 13 different genera can make aquatic animals sick. These genera include gram-positive bacteria like *Streptococcus*, *Lactococcus*, and *Renibacterium*, as well as gram-negative bacteria like *Aeromonas*, *Edwardsiella*, *Flavobacterium*, *Francisella*, Piscirickettsia, *Vibrio*, and *Yersinia*. Major pathogenic gram-negative and gram-positive bacterial species cause numerous illnesses and significant mortalities in farmed fish, contributing to bacterial infections (12, 13). Bacteria are particularly prevalent in aquatic environments, and the majority of bacterial disease pathogens are naturally occurring in water. Fish only produce disease when they experience stress due to poor environmental conditions, inadequate feed, and poor husbandry practices (14). Researchers have discovered that many fish harbor infectious microorganisms, potentially serving as the source or origin of human infection. Fish must be free of contagious diseases and have sustained nutritional value in order to ensure human health safety. The present study aimed to isolate and identify the bacterial flora isolated from fish in various ponds in the Euphrates District of the Babylon province, Iraq. Using morphological analysis and the VITEK 2 system, we also observed the prevalence and intensity of these bacteria on the fish.

2. Material and method

A number of 65 common carp fish belonging to *C. carpio* were gathered from four stations in the Euphrates District during the period from December 2021 to end of October 2022. Euphrates District is in the Babylon province, Iraq (**Figure 1**).

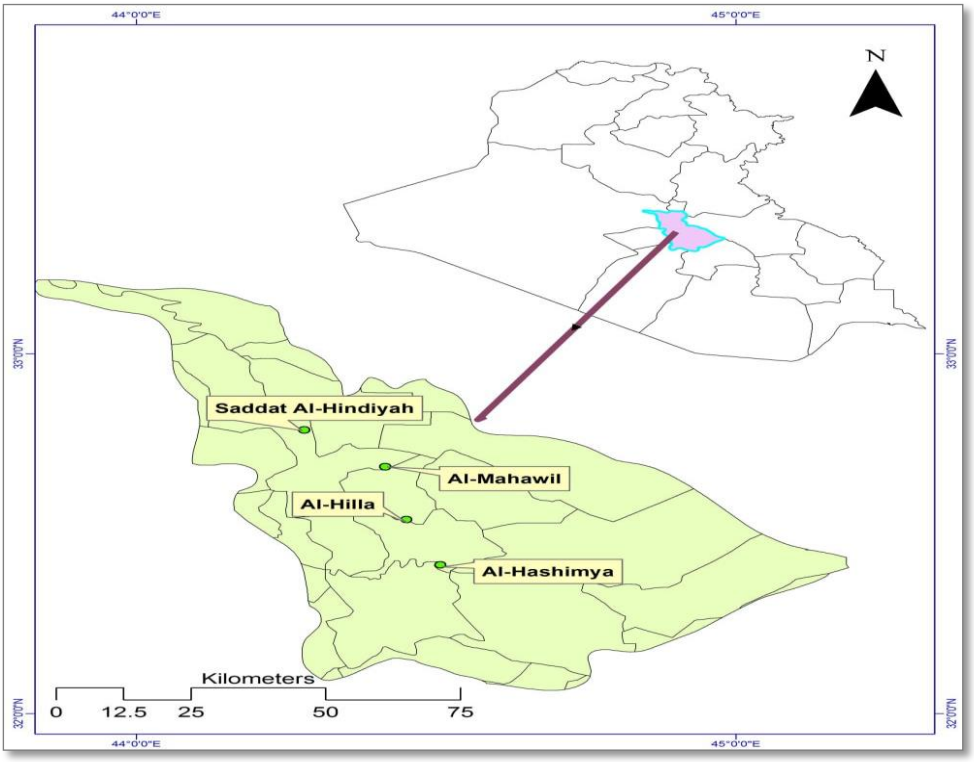

Figure 1. Sampling stations under study

The fish were captured by fishermen using cast nets and gill nets. Samples of fish weighted about (100–3000 g) and they were then brought alive to the lab at Baghdad University's Microbiology Lab for the isolation and identification of bacteria in a cold box filled with pond water. In this study, sterile swabs were used to collect fish skin, gills (particularly at lesion locations), and intestinal organs for bacterial isolations. Samples are cultured using the inoculating swab loop and Bunsen burners that have been lit. The nutrition agar media-containing Petri dish's lid is just cracked open. The appropriate body area of the fish is then gently touched with the tip of the inoculating swab loop before being streaked with TCBS, MaConckey, and nutritional agar. The bacterial colonies were characterized and identified by visual inspection after 24-48 hours of incubation at 37°C in the inverted posture **(Figure 2)**. Examined and noted were morphological traits like optical features, size, form, color, edge, and elevation. Gram staining reactions and a motility test were performed to identify the bacterial isolates from common carp fishes, and a VITEK2 **(Figure 3)** compact system was utilized to confirm the results (15). By reading each test every 15 minutes, the VITEK II device enables kinetic analysis. In order to record fluorescence, turbidity, and colorimetric signals, the optical system integrates multichannel fluorimeter and photometer values (16).

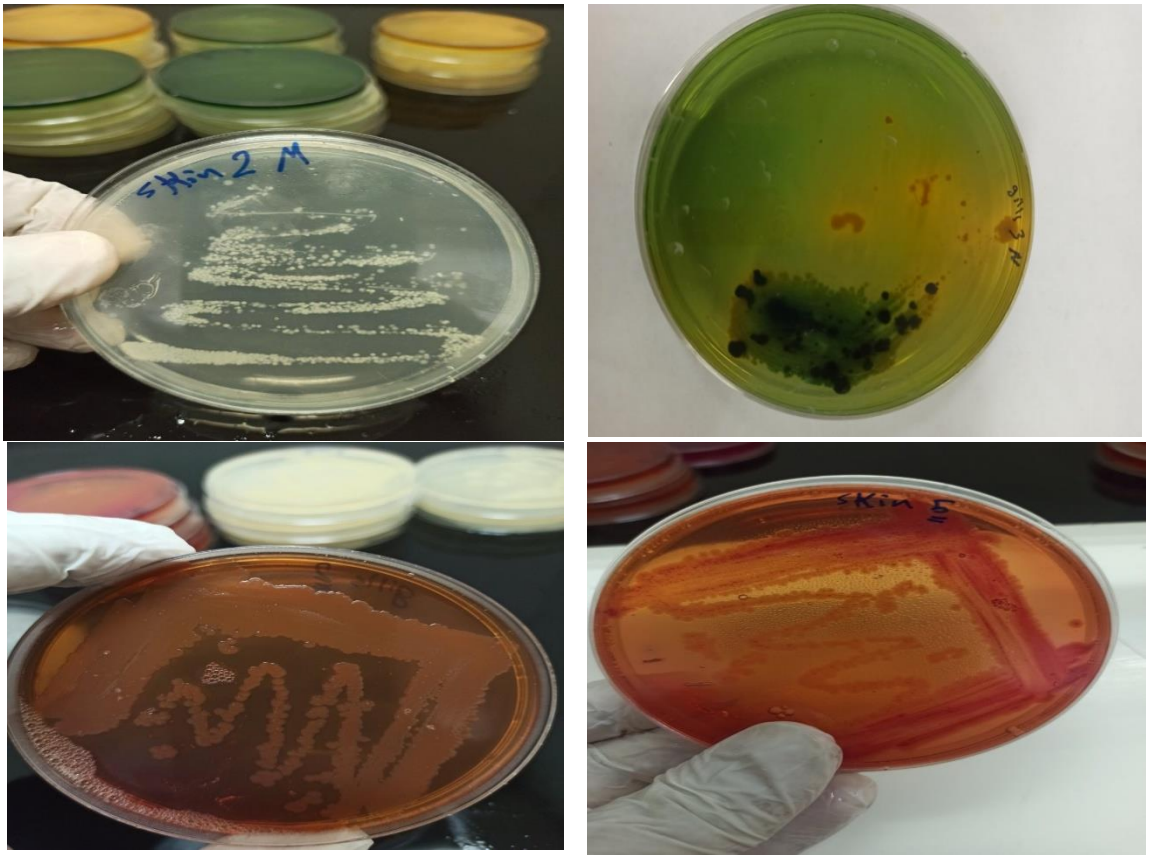

Figure 2. Bacterial isolate in nutrient agar, Macconkey and TCBS agar. Cultured media were incubated at 37°C.

Figure 3. VITEK 2 compact system.

3. Results

3.1 Water quality parameters

The study was carried out from December 2021 to end of October 2022. According to the results, fluctuations of the water quality parameters are found in all the aquacultures in all the sampling points **Table 1**. The mean temperature varied from 11°C to 32.4°C. Meanwhile, the mean of total dissolved solids was recorded to range from 540 to 950 mg/l. and the pH was relatively from 7.8 to 8.3.

Table 1. Physical measurements, type of fish farms and water source for each fish farm**.**

Station	Temp C (Mean)	total dissolved solid mg/l(Mean)	pH(Mean)	Fish farm Type	Water Source
Al- Mahawil	11.5-32.5°C (22)	540-950 (745)	7.8-8.3(8)	Terrestrial ponds	Shatt Al- Mahawil Rive
Saddat Al-Hindiyah	13.0-30.0°C (21.5)	620-780 (700)	7.8-8 (7.9)	Floating cages	Shatt Al- Hillah River
AL- Hillah	14.0-32.4°C (23.2)	650-740(695)	7.5-8 (7.7)	Terrestrial ponds	Shatt Al- Hillah River
AL- Hashimiya	16.0-32.5°C (24.2)	620-750 (680)	7.5-8 (7.7)	Terrestrial ponds	Shatt Al- Hillah River

3.2 Bacterial identification

A total number of 65 fish were sampled during the study. Seventy-five isolates were identified morphologically and subjected to gram staining and confirmed by the Vitek 2 system as follows: *Aeromonas sobria* (17) with probability 93%, *A. hydrophila* (19) with probability 95%, *A. veronii* (15) with probability 96%, *Streptococcus thoraltensis* (1) with probability 93%, *Vibrio cholera* (6) with probability 86%, *Serratia ficaria (1)* with probability 91%, *Serratia fonticola (*6) with probability *94%, Serratia odorifera* (6) with probability 90%.

In the present study many bacteria were isolated from water of aquaculture **(Table 2**).

Table 2. Bacterial isolated in the *Cyprinus carpio* fish in some station.

Studied farms.Stations	Bacteria
Al- Mahawil	*V. cholerae, Aer. hydrophila, Aer. Sobria Aer.veronii, Serratia ficaria, Strep .thoraltensis*
Saddat Al-Hindiyah	*Aer. hydrophila, Aer. Sobria, Aer.veronii, V.cholerae*
AL- Hillah	*Aer. sobria, Aer. Hydrophila, Serratia fonticola, Serratia odorifera*
AL- Hashimiya	*Aer. hydrophila, Serratia odorifera, Serratia fonticola*

We diagnosed four bacterial diseases of *Cyprinus carpio*: fin rot, dropsy, bacterial gill disease, and *vibriosis* **(Table 3)**. Symptoms of fin rot in fish include erosion, thinning of the fins, and darkening of the skin. The affected fins may appear ragged and emaciated, with a loss of healthy tissue. You should seek professional advice for proper diagnosis and treatment, as the severity of these symptoms may vary depending on the disease's stage and the type of fish affected. Bacterial gill disease, as illustrated, is another common fish disease with distinctive symptoms.

Table 3. bacterial isolate of *Cyprinus carpio*. isolated from the skin, gills and intestines of common carp fish

Bacteria	skin	Gills	Intestines
V. cholerae	-	+	-
Aer. hydrophila	+	+	+
Aer. Sobria	+	+	+
Aer.veronii.	+	+	+
Serratia ficaria	-	-	+
Serratia odorifera	+	+	+
Serratia fonticola	+	+	+
Strep .thoraltensis	-	+	-

(+): found**, (-):** not found

Fish affected by this disease may show signs such as congested gills, gill necrosis, and distorted gill capillaries. The gills may appear congested, indicating that they are struggling

to perform their respiratory functions properly. Upon necropsy, the gills may show signs of rot or decay. Additionally, the gills may exhibit distorted capillaries, a sign of damage and inflammation. Congested and enlarged kidneys, spleen, and exophthalmia were symptoms of dropsy disease. Some fish were found to have hemorrhages. Finally, vibriosis symptoms included bleeding around the belly, severe infections, and body ulcers, such as skin lesions that could be associated with hemorrhagic internal organs.

4. Discussion

The fish in a culture system are constantly subjected to a range of stressors, such as high stocking density, management, transportation, and poor water quality. On the other hand, when a stressful event occurs, fish immunity is reduced, making the fish more vulnerable to disease infection (17). Without regard to the water sources or kinds of aquaculture systems, it is clear from the results that temperature and salinity impacted the infected fish (**Table 1**). Furthermore, the frequent use of Oxytetracycline (1%) by farm managers can lead to the emergence of infections that are resistant to antibiotics and can infect both humans and domesticated animals (18).

In fish farming, water quality is a crucial component. The administration and maintenance of water quality, as well as the assessment of the water in fish ponds, are very important in raising fish as well as reducing stress, which is a significant factor that causes fish diseases. Fish activity depends on breathing, nutrition, and growth. Environmental changes can add stress to the fish (19). High concentrations of organic matter, high salinity, warm water (25 to 32°C), and a pH between 5 and 9 all promote the growth of *Vibrio* in water. The growth of this bacteria has been demonstrated to be inhibited by high pH (>9.5) and low salinity (20). Additionally, in the current investigation, the same Vibrio-friendly circumstances were seen. Stress is a condition in which an animal is unable to maintain a natural physiological state as a result of a variety of causes, such as the transportation and circulation of fish or the rise in the water load of microcellular (21). Because of the discharge of industrial, agricultural, and urban wastes, the aquatic environment is ultimately the recipient of more pollutants, and the change in water quality leads to sewerage water (22). Investigation of a few carp disorders at Al-Shamiya City, Iraq, revealed that parasite infections were the most common form of illness (20.7%), followed by diseases brought on by bacterial infections (14.4%) (23). High death rates of wild and farmed fish are largely caused by bacterial disease outbreaks, which also result in significant financial losses for fish farms. The most prevalent fish are susceptible to pathogenic bacteria from the families *Aeromonas*, *Vibrio*, and *Streptococcus*, which can lead to economically disastrous losses in aquaculture (24). The blue tilapia, Oreochromis aureus, is infected with the bacterium *Aer. hydrophila*, which affects the gills, liver, and intestine and causes histological abnormalities in the affected organs (25). Bacterial infection is typically linked to poor water quality, low dissolved oxygen, crowding, and improper handling. Birds can act as carriers for these pathogens. Infected fish typically exhibit bleeding or wounds along their bodies; to cure them, feed containing wild animal antibiotic in ponds that affect humans is used (26).

5. Conclusion

Fish bred in aquaculture facilities and fish that are raised in the wild are both susceptible to infectious illnesses brought on by the varied assortment of bacterial pathogens. These diseases may have been brought on by handling, improper diet, or exposing the fish to environmental conditions.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethics approval

All study animals were treated and handled in accordance with the necessary biosafety and security protocols. Before beginning this study, the Ethics Committee of the College of Science, Baghdad University, Ministry of Higher Education and Scientific Research, Iraq (Number 4118 A.P. on December 30, 2021) accepted the guidelines for the Care and Use of Laboratory Animals and the *Cyprinus carpio* Fish guide.

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