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Pathogenic Bacteria Influencing the Common Carp (*Cyprinus carpio* L.) Farming in Floating Cages in Dayla Province

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ABSTRACT

Bacterial infections that arise naturally in the environment of fish present a major challenge for fish farming in Iraq and globally. The objective of this study was to isolate pathogenic bacteria from common carp that are raised in floating cages and subsequently sold in the local market of Diyala Province. The local examination involved counting 100 fish and collecting samples from them. A dissection was conducted on each fish in the laboratory, isolating bacterial strains from different regions including the skin, gills, liver, spleen, kidney and intestines. Bacteria were identified through microscopic examinations and biochemical tests to pinpoint potential isolates, subsequently confirmed using the API® 20 E system for each strain. Bacteria isolated from all targeted organs revealed the presence of potential pathogenic species, including Escherichia coli, Aeromonas hydrophila, Pseudomonas aeruginosa, and Staphylococcus spp. In carp from this study, E. coli was found in the highest numbers, particularly in the skin and spleen, while P. aeruginosa and Staphylococcus spp. were detected in lower quantities. The maximum bacterial count in fish organs is found in the skin and spleen (3.27 \times 106), while the minimum count is observed in the gills and kidneys (1.91 \pm $(0.14) \times 106$. The effect of different antibiotics was illustrated on the isolated bacteria. The antibiotics used include Chloramphenicol (C30), Ceftazidime (KF30), Cefoxitin (ZOX30), Ticarcillin (TI30), Oxacillin (OX30), Doxycycline (DO20) and Amikacin (AK30). In this research, the pathogenic bacteria impacting the common carp in floating cages within Diyala Governorate, Iraq, were investigated. Sensitivity tests indicated significant resistance rates to commercial broad-spectrum antibiotics, underscoring their prevalence, impact on fish health, and implications for management strategies.

INTRODUCTION

Bacteria in aquaculture result in significant economic losses (Sánchez et al., 2022). Seasonal variations in water quality and stock conditions induce recurrent infections in common carp (Alrudainy & Jumma, 2016; Hossain et al., 2022). Fish are vulnerable to multiple bacterial infections, some of which are thought to be saprophytic and can induce disease (Dinev et al.,

2023). Microbes are found in diverse environments, including the gills, skin, and internal organs (Alam *et al.*, 2023; Hussein & Jumma, 2024). The efficacy of antibiotics in treating bacterial infections guides treatment strategies (Al-Bayati *et al.*, 2020; Milijasevic *et al.*, 2024). The excessive use of antibiotics can lead to bacterial resistance, complicating treatment. Additionally, antibiotics may harm fish livers, alter fish tank ecology, and reduce essential bacteria necessary for ecosystem health (Oday *et al.*, 2024; Dhyani *et al.*, 2025). Prior to the application of antibiotics in aquaculture, it is essential to assess the associated risks and to implement measures to mitigate their overuse (Imtiaz *et al.*, 2024). Additionally, antibiotic residues in fish meat pose risks to food safety and consumer health (Alwan & Al-Bayati, 2020; Dhyani *et al.*, 2025).

MATERIALS AND METHODS

Sampling

A total of 100 *Cyprinus carpio* (common carp) samples were collected from floating cage culture systems in Diyala Province between January and February 2024. The samples were immediately transported to the Pathology Laboratory at the College of Veterinary Medicine, Diyala University. Fish were aseptically dissected, and samples were taken from the skin, gills, liver, spleen, kidneys, and intestines. For microbiological analysis, an inoculating loop of broth culture was streaked onto various media—including brain-heart infusion broth, blood agar, MacConkey agar, and mannitol salt agar—and incubated at 37°C for 24 hours.

Bacteria identification

Gram's staining, phenotypic features (colonial morphology, microscopic appearance) and biochemical tests were used to identify bacteria using identification keys from **Mondal** *et al.* (2010) research. Pure bacterial isolates were characterized using biochemical methods based on the principles outlined in Bergey's Manual of Systematic Bacteriology (Garrity *et al.*, 2001). To confirm the identity of each strain, the API® 20E system was used.

Study susceptibility testing using 7 antibiotics

The susceptibility or resistance of the isolates to antibiotics was determined based on measuring the diameter of the growth inhibition zone (mm) around the disc. The measurements were made accurately and the results were extracted for comparison with the approved standards (Ataee *et al.*, 2011).

Zone of inhibition

This measurement reflects the effectiveness of each antibiotic against the bacteria tested, with a larger zone indicating greater antibacterial activity.

RESULTS AND DISCUSSION

1. Bacteria identification

The analysis demonstrates the bacterial diversity present in various regions of the examined fish, as illustrated in Table (1). *Aeromonas hydrophila* is the primary bacterium linked to skin

infections, accounting for 60% of positive cultures, indicating a significant risk for these infections. *Pseudomonas aeruginosa* shows a prevalence of 33%, alongside a notable occurrence of *Staphylococcus* spp. at 55%. *E. coli* was detected in the gills at a rate of 20%, whereas in liver *E. coli* was detected at a rate of 10%. The spleen demonstrates a varied bacterial composition, comprising *E. coli* (12%), *Aeromonas hydrophila* (22%), *Pseudomonas aeruginosa* (15%), and *Staphylococcus* spp. (12%). The intestines show a notable prevalence of *Aeromonas hydrophila* (75%) and *Pseudomonas aeruginosa* (55%), alongside *E. coli* (33%) and *Staphylococcus* spp. (22%). The current investigation confirmed the results of **Tesfaye** *et al.* (2018), demonstrating that *Pseudomonas* spp. was recovered from the skin and gut, while *E. coli* was recovered from the skin, gills, and intestine of common carp. Aquaculture quality is influenced by numerous chemical and bacteriological parameters, as water quality directly impacts fish productivity and health (**Hildah** *et al.*, 2019). The aquatic environment ultimately receives a multitude of contaminants due to the discharge of industrial, agricultural, and municipal waste, leading to alterations in water quality and the presence of sewage (Al-Shammari *et al.*, 2024).

Table (1) shows that bacteria primarily affect the intestines and the gills demonstrate a modest infection rate.

Organ	Percentages of bacteria %					
	E. coli	Aeromonas hydrophila	Pseudomonas aeruginosa	Staphylococcus spp.		
Skin	10	60	33	55		
Gills	20	30	10	22		
Liver	10	15	14	45		
Spleen	12	22	15	12		
Kidney	11	55	12	15		
Intestines	33	75	55	22		

Table 1. The analysis of percentage of bacteria % in different organs of the examination fish

2. Total count of bacteria

Maintaining sterile conditions is essential to prevent contamination. Samples were obtained from several parts of the fish, including skin, gills, and internal organs. The total bacterial count in 1ml of the sample was determined by utilizing the colony count and the inverse dilution ratio. The bacterial colonies were enumerated using a bacterial counting apparatus, which recorded the total number of colonies, resulting in the total bacterial count per milliliter (Andrews, 1992). Table (2) illustrates the total number of bacteria (CFU/ml) $\times 10^6$ in various organs.

Organ	Total count of bacteria× 10 ⁵					
	E. coli	Aeromonas hydrophila	Pseudomonas aeruginosa	Staphylococcus spp.		
Skin	3.27±0.4Aa	0.68±0.4Ab	0.07±0.03Ac	1.3±0.29Ab		
Gills	1.91±0.14Ba	0.23±0.11Ab	0.03±0.005Ab	0.02±0.01Bb		
Liver	2.4±1.01Ca	0.45±0.09Abc	0.08±0.04Ac	0.73±0.05Ab		
Spleen	3.27±0.4Aa	0.68±0.4Ab	0.07±0.03Ac	1.3±0.29Ab		
Kidney	1.91±0.14Ba	0.23±0.11Ab	0.03±0.005Ab	0.02±0.01Bb		
Intestines	2.4±1.01Ca	0.45±0.09Abc	0.08±0.04Ac	0.73±0.05Ab		
LSD 0.05			0.68			

Fable 2. Total count of bacteria >	× 106 ((CFU/ml)) in different	organs of the	examination fish
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The bacterial counts (mean \pm SD, log CFU/g) isolated from different organs of *Cyprinus carpio* are summarized as follows:

- Skin: E. coli (3.27 ± 0.40), Aeromonas hydrophila (0.68 ± 0.40), Pseudomonas aeruginosa (0.07 ± 0.03), Staphylococcus spp. (1.30 ± 0.29)
- **Gills**: *E. coli* (1.91 ± 0.14), *Aeromonas hydrophila* (0.23 ± 0.11), *Pseudomonas aeruginosa* (0.03 ± 0.005), *Staphylococcus* spp. (value not provided)
- **Liver**: *E.* coli (2.40 \pm 1.01), Aeromonas hydrophila (0.45 \pm 0.09), Pseudomonas aeruginosa (0.08 \pm 0.04), Staphylococcus spp. (0.73 \pm 0.05)
- **Spleen**: *E.* coli (3.27 \pm 0.40), Aeromonas hydrophila (0.68 \pm 0.40), Pseudomonas aeruginosa (0.07 \pm 0.03), Staphylococcus spp. (value not provided)
- **Kidneys**: Aeromonas hydrophila (0.23 ± 0.11), Pseudomonas aeruginosa (0.03 ± 0.005), Staphylococcus spp. (0.02 ± 0.01); E. coli not detected
- Intestines: *E. coli* (2.40 ± 1.01), *Aeromonas hydrophila* (0.45 ± 0.09), *Pseudomonas aeruginosa* (0.08 ± 0.04), *Staphylococcus* spp. (0.73 ± 0.05)

Among all sampled organs, *E. coli* was the most abundant bacterium, particularly in the skin and spleen.



Fig. 1. Total number of different bacteria $\times 10^6$ (CFU/ml) in the fish's body organs, with each type represented by a different color

The maximum bacterial count in fish organs is found in the skin and spleen (3.27×106) , while the minimum count is observed in the gills and kidneys $(1.91 \pm 0.14) \times 106$. Fish diseases may

arise from microbial pollution in freshwater or contamination during harvesting activities (**Raufu** *et al.*, 2024; **Bashar** *et al.*, 2025). Human illnesses result from organisms conveyed from fish (**Robinson**, 2014). Mohammed (2024) documented the bacterial isolates (Table 2) of their study on the isolation and identification of harmful bacteria from fresh fish tissues. Additionally, previous reports have highlighted the presence of various pathogenic bacterial isolates in fish from local markets, which may reduce the quality and safety of fish available for consumer use.

3. Antibiotic sensitivity

Table (3) demonstrates antibiotic inhibition zones against E. coli, Aeromonas hydrophila, Pseudomonas aeruginosa, and Staphylococcus spp. The antibiotics are Chloramphenicol (C30), Ceftazidime (KF30), Cefoxitin (ZOX30), Ticarcillin (TI30), Oxacillin (OX30), Doxycycline (DO20), and Amikacin (AK30). For the distribution of inhibition zones, Amikacin (AK30) and Doxycycline (DO20) were recorded as the most effective antibiotics since they have extensive inhibition zones against all types. The less effective antibiotics are Chloramphenicol (C30) and Cefoxitin that (ZOX30), as they do not suppress bacterial strains. E. coli and Aeromonas hydrophila show significant inhibition, while Staphylococcus spp. and Pseudomonas aeruginosa show varying inhibition with more powerful antibiotics. The results are consistent with the findings of Kane et al. (2024), who reported that antibacterial treatments are commonly administered to fish through medicated feed. However, contamination of the external environment with these antibacterials often occurs due to the leakage of uneaten feed and fish feces. Furthermore, curative practices in aquaculture may contribute to the development of antibacterial resistance in both fish pathogens and the surrounding environment (Al-Shammari et al., 2019). Antibacterial resistance in freshwater fish culture systems is increasingly reported, although it remains poorly understood (Al-Shammari et al., 2024). At present, several diseases affecting farmed fish stocks also pose a threat to wild fish populations (Hiba et al., 2020; Jumma, 2024).

	Zones of inhibition (mm)				
Type of antibiotic	E. coli	Aeromonas hydrophila	Pseudomonas aeruginosa	Staphylococcus spp.	
Amikacin AK30	15± 0.74Aa	9.1 ± 0.33Db	14 ± 0.50 Ca	18 ± 0.20 Aa	
Doxycycline DO20	$15 \pm 0.32 Ab$	$22.5\pm0.13Aa$	$20\pm0.70\;Ab$	$24\pm0.40~\mathrm{Aa}$	
Oxacillin OX30	$11 \pm 0.58Bb$	12.3± 0.10Ba	9 ± 0.15 Dd	15 ± 0.33 Bb	
Ticarcillin TI30	$9\pm0.01\text{Cb}$	10 ± 0.05 Ca	11 ± 0.12 Cc	$12 \pm 0.25 \text{ Bb}$	
Cefoxitin ZOX30	4 ± 0.22Ea	$0\pm0.08 Fb$	2 ± 0.10 Ef	5 ± 0.12 Ea	
Ceftazidime KF30	$8 \pm 0.00 \text{Da}$	3.3 ± 0.16 Eb	6 ± 0.05 Ef	7 ± 0.19 Da	
Chloramphenicol C30	0.7± 0.10Ea	0.11 ± 0.41 Fa	$0.7\pm0.05~\mathrm{Ga}$	$0.8\pm0.30\ Fa$	
LSD(P<0.05)			0.82		

Table 3. Zone of inhibition (mm) of *Pseudomonas aeruginosa, Aeromonas hydrophila, E. coli* and *Staphylococcus* spp. to antibiotics

Statistical Significance: The LSD (P<0.05) value indicates the least significant difference, which is useful for determining if differences between the means of groups are statistically significant.

It is noted in Table (3) the sensitivity of *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *E. coli* and *Staphylococcus* spp. to various antibiotics, as measured by inhibition zones (in mm) and statistically significant differences (LSD, P < 0.05).



Fig. (2) shows that the largest inhibition zone was observed with Doxycycline (DO20) against *Staphylococcus* spp., measuring approximately 24mm. In contrast, the smallest inhibition zone—measuring 0 mm—was recorded with Cefoxitin (ZOX30) against *Aeromonas hydrophila*, indicating complete resistance and the antibiotic's ineffectiveness against this bacterium.

It is important to note that the interpretation of antimicrobial resistance can vary based on disciplinary perspectives. From a microbiological standpoint, a sensitive organism is one that lacks resistance-conferring mechanisms. Clinically, however, sensitivity is defined by whether a microorganism can be effectively treated with drug concentrations achievable in the body through standard therapeutic dosing (**Khalifa** *et al.*, **2020**; **Shah** *et al.*, **2024**). An organism is considered sensitive if the pharmacologically achievable concentration of an antibiotic is sufficient to suppress or eliminate the pathogen (**Abdullah** *et al.*, **2024**; **Wani**, **2024**). Wani (2024) also noted that bacterial resistance to antibiotics was observed soon after their initial introduction and has been extensively studied. The steady increase in antibiotic resistance, coupled with a decline in the development of new antibiotics, suggests the potential onset of a "post-antibiotic era"—a period in which treating infections becomes increasingly difficult (Shinu *et al.*, **2022; Aljoburi**, **2024**).

Aguiar *et al.* (2024) reported widespread resistance to Tetracycline among bacterial isolates, with the exception of two isolates that remained sensitive. These results are consistent with the findings of **Papaleo** *et al.* (2022), who observed that most bacterial isolates were sensitive to Rifampicin but resistant to Ciprofloxacin, except for one antibiotic that was effective only against actively growing bacterial cells.

CONCLUSION

Pathogenic bacteria provide a considerable risk to the aquaculture of common carp in floating cages throughout Diayla Province. The research findings have indicated the necessity for

efficient techniques to control fish health and avert bacterial infections in aquaculture. Antibiotics having limited potency, such as Cefoxitin and Chloramphenicol, are not advised for use against examined bacteria.

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