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Pathogenic Bacteria Influencing 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. The bacteria isolated from all targeted organs showed that the potential pathogenic bacteria identified include *E. coli*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Staphylococcus* spp. In carp from this study, *E. coli* recorded the highest bacterial numbers in the skin and spleen compared of that *Pseudomonas aeruginosa* and *Staphylococcus* spp. 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 ± 0.14) ×106. The study illustrates the effect of different antibiotics on the isolated bacteria. The antibiotics used include chloramphenicol (C30), ceftazidime (KF30), cefoxitin (ZOX30), ticarcillin (TI30), oxacillin (OX30), doxycycline (DO20) and amikacin (AK30). This research investigates the pathogenic bacteria impacting common carp in floating cages within Diyala Governorate, Iraq. 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 implement measures to mitigate their overuse **(Imtiaz *et al*., 2024)**. 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

The study obtained 100 *Cyprinus carpio* fish samples from culturing in floating cages in Dayla Province from January to February 2024. Veterinary Medicine / Diyala University pathology laboratory received samples directly. Aseptically dissected fish components included Skin, Gills, Liver , Spleen, Kidney and Intestines. An incubated loop of soup was streaked at 37°C for 24 hours on various agars, including brain-heart infusion broth, blood agar MacConkey, and mannitol salt agar, for microbiological analysis.

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 culture of isolate were characterized use biochemically concepts from bergey's manually of formicative bacteriology **(Garrity *et al*., 2001)**. We used the API® 20 E system to confirm each strain.

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* recorded 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 recorded from the skin and gut. Additionally, *E. coli* was detected in the skin, gills, and intestine from 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.

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

Organs	Percentage 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

2- Total count of bacteria

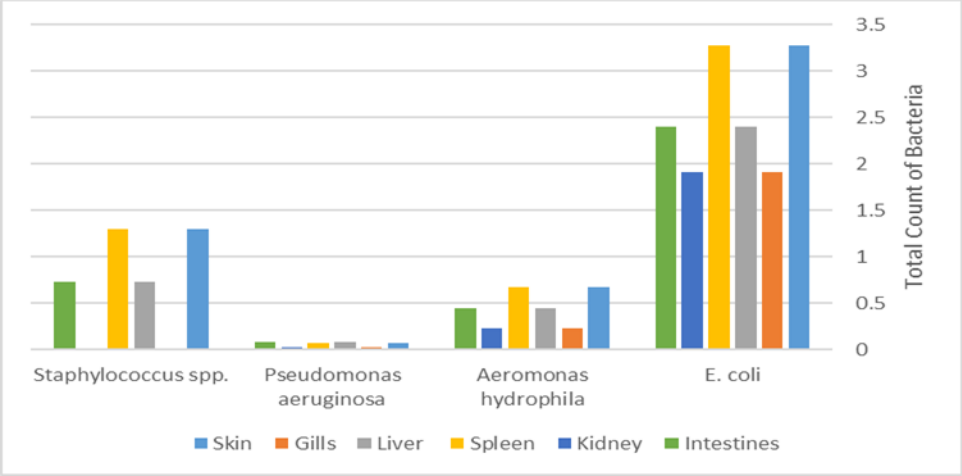
Maintaining sterile conditions is essential to prevent contamination. Samples were obtained from several areas of the fish, including the skin, gills, and internal organs. The total bacterial count in 1 ml of the sample was determined by utilizing the colony count and the inverse dilution ratio. The 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) × 10^6 in various organs.

Table 2. Total Count of Bacteria × 106 (CFU/ml) in different organs of the examination fish

Organs	**Total Count of Bacteria× 10^6**			
	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			

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



Skin: *E. coli* (3.27 ± 0.4), *Aeromonas hydrophila* (0.68 ± 0.4), *Pseudomonas aeruginosa* (0.07 ± 0.03), Staphylococcus spp. (1.3 ± 0.29). *E. coli* (1.91 ± 0.14), *Aeromonas hydrophila* (0.23 ± 0.11), *Pseudomonas aeruginosa* (0.03 ± 0.005), and Staphylococcus spp. were found in the gills. In the liver, *E. coli* (2.4 ± 1.01), *Aeromonas hydrophila* (0.45±0.09), *Pseudomonas aeruginosa* (0.08±0.04), and Staphylococcus spp. (0.73±0.05) were found. The spleen contained *E. coli* (3.27 ± 0.4), *Aeromonas hydrophila* (0.68 ± 0.4), *Pseudomonas aeruginosa* (0.07 ± 0.03), and Staphylococcus spp. The kidneys Aeromonas hydrophila: 0.23 ± 0.11, *Pseudomonas aeruginosa* (0.03 ± 0.005) and Staphylococcus spp. (0.02 ± 0.01). Intestines: *E. coli* (2.4 ± 1.01), *Aeromonas hydrophila* (0.45 ± 0.09), *Pseudomonas aeruginosa* (0.08 ± 0.04); Staphylococcus spp. (0.73±0.05). *E. coli* is most abundant in skin and spleen.

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 ± 0.14) ×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 resulting from organisms conveyed from fish **(Robinson, 2014). Mohammed (2024)** documented isolates (Table 2) in their investigation on the isolate and identification of harmful bacterial of fresh fish tissues. Furthermore, prior reports revealed the presence of numerous harmful bacterial isolations in the fish market, which would diminish the quality of fish accessible for local consumptions.

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). Distribution of inhibition zones Amikacin (AK30) and Doxycycline (DO20) are the most effective antibiotics since they have extensive inhibition zones against all types. The less effective chloramphenicol (C30) and cefoxitin (ZOX30) 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 those found by **Kane *et al.* (2024)**. Antibacterial treatments for pathogenic bacteria are frequently used for treating fish via medicated feed. Contamination of the external environment with these antibacterials mainly occurs through the leakage of waste food and feces. Additionally, the curative aquacultures practice could result in resistances of antibacteria in fish pathogens and environment **(Al-Shammari *et al.*, 2019)**. The resistances to antibacteria at the fresh water of fish cultures activities is more recent and limited **(Al-Shammari *et al.*, 2024)**. Currently, several disease that affect domestic fish stock are also a hazard to wild fish population **(Hiba *et al.*, 2020; Jumma, 2024).**

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

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

Types of antibiotics	Zone of inhibition (mm)			
	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 ± 0.32Ab	22.5 ± 0.13Aa	20 ± 0.70 Ab	24 ± 0.40 Aa
Oxacillin OX30	11 ± 0.58Bb	12.3± 0.10Ba	9 ± 0.15 Dd	15 ± 0.33 Bb
Ticarcillin TI30	9 ± 0.01Cb	10 ± 0.05Ca	11 ± 0.12 Cc	12 ± 0.25 Bb
Cefoxitin ZOX30	4 ± 0.22Ea	0 ± 0.08Fb	2 ± 0.10 Ef	5 ± 0.12 Ea
Ceftazidime KF30	8 ± 0.00Da	3.3 ± 0.16Eb	6 ± 0.05 Ef	7 ± 0.19 Da
Chloramphenicol C30	0.7± 0.10Ea	0.11 ± 0.41Fa	0.7 ± 0.05 Ga	0.8 ± 0.30 Fa
LSD(P<0.05)	0.82			

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.

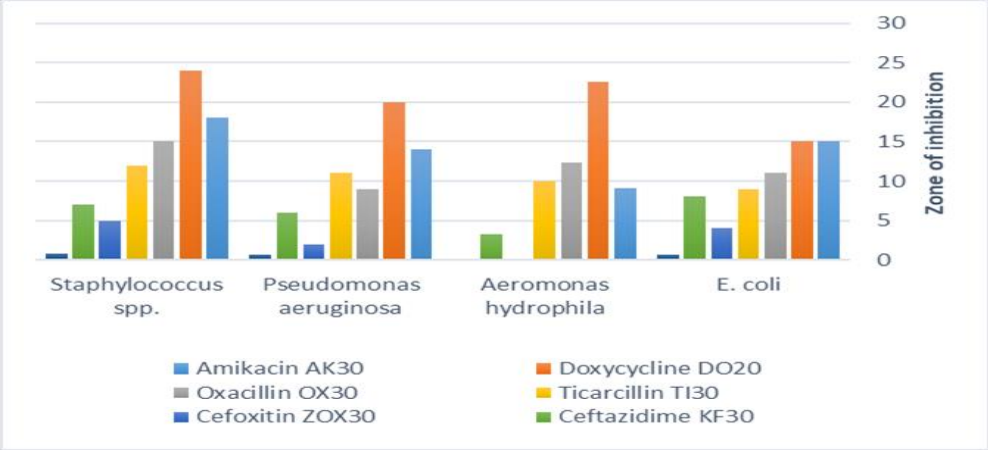


Fig. 2. The maximum and minimum inhibition zones observed with Doxycycline (DO20) against *Staphylococcus* spp.

Fig. (2). shows the maximum and minimum inhibition zones were observed with Doxycycline (DO20) against Staphylococcus spp., with the inhibition zone measuring approximately 24 mm, and the minimum inhibitory zone was recorded with Cefoxitin (ZOX30) against Aeromonas hydrophila, where the inhibition zone was zero mm, signifying its ineffectiveness against this bacterium. In view description of antimicrobial resistance varies among groups based on their perspectives. The microbiologist defines sensitive organisms as those lacking any resistance-conferring factors, whereas the clinical definition encompasses microorganisms that can be managed by therapeutically attainable concentrations of the medication **(Khalifa *et al.*, 2020; Shah *et al.*, 2024)**. An organism is considered sensitivte if the pharmacological achievable concentration of antibiotic is sufficient to suppress or eradicate the pathogens **(Abdullah *et al.*, 2024; Wani, 2024)**. **Wani (2024)** stated that bacteria antibiotic resistances which were observed soon after antibiotic introduction have been extensively studied. Steady increase in antibiotic resistances and decrease number of newer antibiotic appear to point to a post-antibiotic period during which treatments of infection would become increasingly difficult **(Shinu *et al.*, 2022; Aljoburi *et al.*, 2024)**. **Aguiar *et al.* (2024)** reported that bacterial isolates resistance to tetracycline, except for two isolates that demonstrated sensitivity to this antibiotic. The results aligned with the findings of **Papaleo *et al.* (2022)**, who confirmed that bacterial isolates exhibited sensitivity to the antibiotic rifampicin and resistance to ciproflaxin, with the exception of one antibiotic that is effective only against actively growing forms of bacteria.

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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