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Sensitivity and Antibiotic Resistance of *Aeromonas spp.* Isolated from Some Fish Farms in Basrah, Iraq

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Abstract

This study sought to isolate and identify *Aeromonas species* and assess their antibiotic resistance. The inquiry centered on 56 dubious fish contaminated with *Aeromonas* germs from Basra. The fish weighed between 20 and 44 grams and were thereafter placed in individual tanks. This research was undertaken at the Fish Breeding and Nutrition Laboratory within Fish and Marine Resources, College of Agriculture, University of Basra. Specimens were obtained from the ulcerated dermal regions, kidneys, liver, and spleen. A homogenous bacterial isolation was subsequently conducted and confirmed leading to identification of Gram-negative bacteria. ID-GN demonstrated that clinical isolates, including *A. hydrophila, A. sobria, and A. caviae*, aligned with the fundamental biochemical assays and employed by Vitec 2. The Clinical and Laboratory Standards Institute (CLSI, 2021) was used to evaluate the antibiotic susceptibility of Aeromonas isolates responsible for carp infections using the Kirby-Bauer disk diffusion method. We are looking at how resistant three types of bacteria are to oxytetracycline, erythromycin and ampicillin after exposed to them. The results show differing levels of resistance to various antibiotics *A. hydrophila* has an 11% resistance to oxytetracycline, 33% to erythromycin and a significant 95% to ampicillin. *A. sobria* exhibits a 10% resistance to oxytetracycline, 45% to erythromycin and 75% resistance to ampicillin. *A. caviae* shows 8% resistance to oxytetracycline, 50% to erythromycin and 77% resistance to ampicillin. The mean antimicrobial resistance (MAR) for three *Aeromonas species* studied is around 80 percent to ampicillin with moderate resistance to erythromycin observed in fifty percent of cases. Oxytetracycline demonstrates the greatest sensitivity and a low resistance rate of ten percent among the isolates.

Keyword: Species of Aeromonas, Cyprinus carpio L., Bacterial Sensitivity, Antimicrobial resistance, MAR Index

I. Introduction

In fish farming (Bakhtiari *et al.* 2019), bacterial infections provide a significant challenge.Most commonly, Aeromonas bacteria are the primary cause of high rates of illness and death in common carp (*C. carpio*) (Pattanayak *et al.*, 2020). This has a very serious consequence to human beings and other animals (Nhinh *et al.*, 2021).Antimicrobial resistance (AMR) refers to the emergence of bacterial defense mechanisms against antibiotics (Marston *et al.*, 2016).The management of these diseases is becoming more complex, and higher dosages mean higher costs (Gronde *et al.*, 2017).Genetic mutations have been found to exert a significant influence on the development and dissemination of bacterial resistance mechanisms in a number of animal species. In domestic animals, antibiotic resistance is constantly happening (Christaki *et al.*, 2017).Antibiotic-resistant infections are ever more widespread and, with such limited effective options for treatment available, it represents a problems of global proportion (Darby *et al.*, 2023).By the year 2050, Brüssow (2024) suggests that the search for novel antibiotics or the upgrading of current ones will be a necessity if effective antibiotics for disease management are to continue being available. This research sought to establish the resistance of *Aeromonas spp.* in common

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carp (*C. carpio*) commercially grown in Basra, Iraq against the three most frequently used antibiotics: penicillin, erythromycin, and oxytetracycline.A number of isolates were found to demonstrate moderate levels of antibiotic resistance.Among Aeromonasspecies, namely *A. hydrophila*, *A. sobria*, and *A. caviae*, there were different degrees to which they were susceptible to various antibiotics. The resistance rates and multi-drug resistance (MAR) index for comparative reference are key measures in the evaluation of antibiotic resistance among various strains of Aeromonas bacteria. The MAR Index seeks to measure the relative resistance levels of particular strains to a variety of antibiotics, giving much information about the antibiotics' efficacy (Tang *et al.* 2021). Both of these indicators have significant implications for aquiculture and veterinary medicine. They help us chart courses to effectively combat bacterial infections which attack aquatic organisms, and perpetuate the health and survival of our aquatic species-a matter important in both food securities worldwide and human health (Nogueira and Botelho, 2021).

II. Materials and methods

Environmental factor of the study area:

Materials and methods Sites of investigation: This study has been carried out with selected 56 fish species *C. carpio*, the specimens in its weight range between ±44g / (20) cm to (December - March, 23 January consuming Karma Ali -- Basra/ Iraq), which was obtained fresh from private fisheries suspected infected by disease organ Aeromonas species . Fish samples were taken aseptically to study facility sending in sealed containers with ice.

Preparation of fish samples collection and transportation:

According to Inyang-Etoh *et al.*, (2024), the collected fishes were dissected and bacterial swabs were taken aseptically using a sterile loop from skin, gills and intestine were collected for bacteriological examination.

Isolation pathogenic bacteria from infected *C. carpio* and samples collection:

The MacConkey agar medium was used to isolate bacteria, according to Tolouei *et al.*, (2021). For twenty-four hours, the inoculation plate was incubated at 37 °C. Gram stain, Oxidase, Catalase, Motility, Hemolysis, Indole, Methyl Red, Lysine decarboxylase, Ornithine decarboxylase, Arginine dehydratase, Urease production, Hydrogen sulfide production, Tryptophan deaminase, Nitrate reduction, Citrate utilization, Glucose, Sucrose, Mannitol, and Lactose (acid production) were among the biochemical tests that were carried out. Sun *et al.*, (2021) state that the VITEK 2 system is utilized to identify bacteria.

Susceptibility testing of bacteria to antibiotics;

Kirjy-Bauer disc diffusion (Kyeremeh, 2010) very few common antibiotics reached the maximum growth inhibition level when evaluated by using disc diffusion technique on Muller-Hinton Agar, excluding a high number of strains from susceptibility data. We performed an antibiotic susceptibility test using oxytetracycline (30 µg), erythromycin (15 µg) and ampicillin (10 µg). The inhibitory zone was measured 24 hours after checking for the results, in duplicate following the manufacturer's instructions (Bubonja *et al.*,2008). Susceptibility was defined as the absence of growth on solid medium containing each antimicrobial agent (Benkova *et al.*, 2020). The inhibitory zones were measured on the agar plate and classified as susceptible (S), intermediate(I) or resistant(R). Azzam-Sayuti *et al.* (2021) defined that MAR index had a helpful role to assess how much the measured level of antibiotic resistance was in bacterial strain for suitable treatment methods and good management of antibiotics so this is recommended. The results showed in Table 2 as a bar chart representing the mean resistance rates for each of these antibiotics. Such an approach reveals important information on bacterial response to antibiotic resistance, adding value in designing treatment regimes.

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III. Results and discussion

The environmental parameters:

like temperature, dissolved oxygen concentrations, and ammonia levels, have played a major role in the proliferation of infectious diseases within aquatic ecosystems. Besides, the water quality of Karma Ali River exhibited some significant variations, ranging from 20.52 ± 2.64 °C in January to 25.01 ± 0.11 °C in February. This is fundamental for fish health, since water temperature is mostly less variable compared to atmospheric temperature. Salt concentrations, dissolved oxygen, and pH levels also varied over the two-month period. Aeromonas is one of the most significant pathogens in carp aquaculture and plays a major role in the development of disease problems. Bacterial growth in the water is based on temperatures ranging between 25 °C and 32 °C, organic matter, salt concentration, and pH between 5 and 9. For those reasons infectious diseases can be spread between water-living hosts.

Fig (2): The fish displayed hemorrhagic lesions, abdominal distension, and exophthalmia.

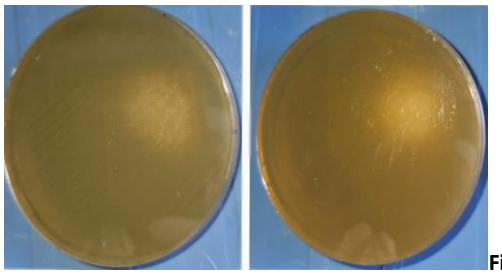

Fig (1) : *Aeromonas spp.* They produced yellow colonies on Remiler-Shots medium (R-S medium).

Isolation and Identification of Aeromonas from *Cyprinus carpio*:

Post-mortem studies of naturally infected Cyprinus carpio exhibited dark body pigmentation, exophthalmia, poor balance, frayed tail and fins, scale loss, skin discoloration, moderate ascites, petechial hemorrhages, and deep ulcers as shown in Figure 2 . The fish exhibited congestion in the spleen, kidneys, ovaries, and liver, along with a dilated gallbladder and hemorrhagic, inflammatory intestines. Aeromonas species infections induce changes including altered body pigmentation, opaque membranes, exophthalmia, raised scales, eroded opercula, erythema, ulcers, body edema and erosions of the gills, fins, and tail.

The bacteriological study:

showed positive results for the Aeromonas bacteria type, as bacterial swabs were collected from several fish organs (kidneys, liver, intestines, gills), revealing numerous bacterial isolates. The analysis of the affected fish tissues was conducted based on cultural characteristics, where the samples were placed on nutrient agar and TSA (Tryptic Soy Agar). Growth characteristics on BHI agar, triple sugar iron agar, urea agar, blood base agar and MacConkey agar. The isolates exhibited morphological and biological characteristics as shown in Table 1.

The cultural and biochemical characteristics of the isolates classified as Aeromonas were identified through white, convex, round colonies with entire edges, 2 mm in diameter, on convex white TSA agar base medium. They showed positivity for oxidase and catalase. They fermented glucose and sucrose. The absence of fermentation of inositol, rhamnose, mannitol, and sorbitol indicates the presence of Aeromonas species Table 1. The VITEK 2 system identified the bacteria, and the ID-GN test for Gram-negative bacteria indicated that the clinical isolates included *A. hydrophila*, *A. sobria* and *A. caviae* which is consistent with the preliminary biochemical tests and morphological characteristics. The current study investigated the spread of bacteria showing varied

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percentages of Aeromonas species in inflected *common carp* as *A. hydrophila* (65%), *A. sobria* (30%) and *A. caviae* (5%).

Table 1: The cultural and biochemical characteristics and morphological characteristics of the isolates Aeromonas species.

Test and Reaction	*A. hydrophila*	*A. sobria*	*A. caviae*
Percentages%	65	30	5
Gram stain	-	-	-
Morphology	Short rod	Short rod	Short rod
Oxidase	+	+	+
Catalase	+	+	+
Motility	+	+	+
Hemolysis	β	-	-
Indole	+	+/-	+
Methyl red	+	-	-
Lysine decarboxylase	+	+	-
Ornithine decarboxylase	+/-	+/-	-
Arginine dihydrolase	+/-	+	+
Urease production	-	-	-
Production Hydrogen sulfide	+	-	-
Tryptophan deaminase	+	+	+
Nitrate reduction	+	+	+
Citrate utilization	-	-	-
Glucose	+	+	+
Sucrose	+	+	-
Mannitol	+/-	-	-
Lactose (acid production)	-	-	+
Colony characters	On TSA white minor convex On blood agar hemolysis		

Results of Comparative resistance rates (%) of *Aeromonas species*:

The study evaluated the antibiotic resistance of *Aeromonas spp.*, especially *A. hydrophila*, *A. sobria* and *A. caviae* isolated from carp, with emphasis on their resistance to oxytetracycline, erythromycin and ampicillin as shown in Fig. 3. Resistance to oxytetracycline was the lowest among all groups, with resistance rates of 11%, 10% and 8%. The resistance rates to erythromycin of 33%, 45% and 50% indicated moderate susceptibility across all *Aeromonas spp.*, with *A. caviae* showing greater resistance to erythromycin. The study found that all bacterial species showed significantly increased resistance to ampicillin, especially *A. hydrophila*. The ampicillin resistance rates were recorded at 95%, 75% and 77% as shown in Figure 1.

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Results of the multiple resistance index (MAR index) for *Aeromonas spp.*:

The results of the study showed in chart 2 that the average MAR index represents the average index of multiple resistance against antibiotics (MAR) for the three drugs based on the results of laboratory analyses. Ampicillin the resistance rate was 100%, which means that the majority of the isolated bacterial strains showed significant resistance to the antibiotic. Erythromycin has a moderate susceptibility score of around forty percent, placing it second, signifying substantial resistance, but less pronounced than that of ampicillin. Oxytetracycline exhibits the lowest resistance rate, around 20%, signifying that this antibiotic retains considerable efficacy against the isolated isolates.

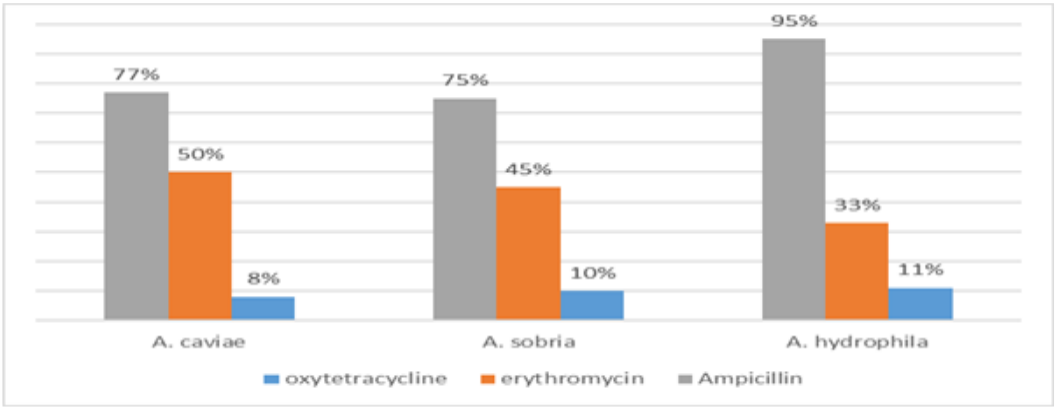

Chart (2) shows the percentage resistance (%) of *A. spp*. with a resistance to oxytetracycline, erythromycin

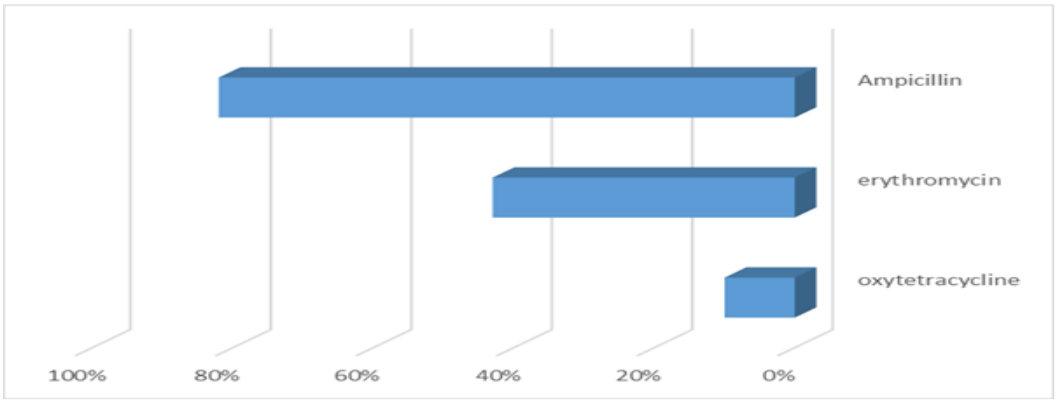

Chart 3: Resistance (%) rates of *A. spp.* to oxytetracycline, erythromycin and ampicillin.

IV. Discussion

The Variation in environmental conditions recorded in the Karma Ali River was convergence in many water parameters and the level of variables was within the acceptable range. Hoang *et al.*, (2020) classified this water as warm based on its temperature classification. Temperature is a critical climatic factor influencing fish health in aquatic ecosystems, despite the fact that water temperatures exhibit less variability than air temperatures. Nonetheless, it significantly influences the vitality of fish (Mohammed *et al.*, 2024). Aeromonas is a primary pathogen responsible for significant disease issues in carp aquaculture (Mayrhofer *et al.*, 2020). Some researchers have observed that *Aeromonas spp.* induces hemorrhagic skin lesions in freshwater and farmed fish (Majeed, *et al.*, 2023). In a fish culture system consistently subjected to various pressures, The proliferation of bacteria in water

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escalated with increasing water temperatures from 25 °C to 32 °C, as well as with organic matter, salt, and pH values ranging from 5 to 9 (Farhan and Shareef, 2024).

These consequences correspond with (Varalakshmi *et al.*,2022) discoveries that *Aeromonas species* infections induce changes including altered body pigmentation, opaque membranes, exophthalmia, raised scales, eroded opercula, erythema, ulcers, body edema and erosions of the gills, fins, and tail. The fish exhibited hemorrhagic lesions, abdominal distension, and exophthalmia, indicative of hemorrhagic sepsis attributed to *Aeromonas spp.* the skin and muscles may be linked to bacterial infections and virulence factors, influencing pathogenic potential.

V. Conclusions

A. hydrophila, it is also vaulted to induction of multi drug resistance due to misuse (overuse) antimicrobial drugs in Carp that leads hemorrhaginsand ulceration (10). Rigorous antimicrobial susceptibility testing in aquaculture settings is part of the prevention. The study reported the prevalence of multidrug resistant Aeromonas isolates in ponds and majority of these showed resistance to two or three antibiotics. In this regard, all isolates were resistant to ampicillin (Tables 1 and 2) but displayed different patterns of resistance to erythromycin. Aeromonas species are the infectious bacterial agents that threaten fish production, and this investigation helped to enrich his knowledge in terms of recognizing more about Aeromonas diverse multidrug-resistant (MDR) strains found among freshwater tilapia farms. Multidrug Aeromonas spp. greater than 20–80 % of the isolates exhibiting a Multiple Antibiotic Resistance (MAR) index was isolated from antibiotic usage prevalent farms. Fish culture settings are more likely to produce various source with multiple antibiotic resistances, resulting in impaired aquaculture productivity. Therefore, it can safely be said that the drug is not correct and must be controlled at all stages of administration. While the MAR Index provides a broad assessment of multi-resistance and measures resistance pressure, comparing per drug-resistant rates offer detailed information on individual antibiotic attractiveness that directs effective treatment strategies in aquaculture and veterinary medicine.

Conflict of interest

No

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Ethical approval

This paper complied with all ethical regulations on fish and their care set forth by national and international organizations.

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