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Original Article

Virulence of bacteria isolates in Mesopotamian Himri, *Carasobarbus luteus*, from the Al-Diwaniya River, Iraq

Mohanad O. Al-Jubouri*, Sumod A. Salman, Noor R. Abady, Walaa F. Obaid, Marwah H. Ali

College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq.

Abstract: About 100 *C. luteus* were examined to determine the extent of the presence of disease-causing bacteria in the Diwaniyah River from April 2021 to March 2022. The bacteria were isolated using the VITEK2 system, and vital tests identified the species *Aeromonas hydrophila* and *Sphingobacterium thalpophilum*. *Aeromonas hydrophila* and *S. thalpophilum* were tested in vitro, revealing noticeable antibiotic susceptibility. Levofloxacin (≤0.22 µg/ml for *A. hydrophila* and ≤0.20 µg/ml for *S. thalpophilum*), as well as Ciprofloxacin (≤0.10 µg/ml) and Imipenem (≤0.23 µg/ml), demonstrated notable effectiveness. Resistance to Cefazolin was observed in *A. hydrophila* (≥69 µg/ml) and *S. thalpophilum* (≥70 µg/ml). The study highlights the presence of harmful bacteria in *C. luteus* from the river, specifically *A. hydrophila* and *S. thalpophilum*. The findings emphasize the critical importance of prudent antibiotic use and ongoing monitoring in aquaculture practices to mitigate risks and safeguard public health.

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Introduction

In the aquaculture sector, bacterial infections lead to financial losses. Aquatic environments, often characterized by high stocking densities and seasonal fluctuations in water quality, subject aquatic organisms to stress, making them susceptible to recurrent infections by opportunistic pathogens. These risks particularly impact commonly farmed species like common carp, *Cyprinus carpio*, especially from pathogenic bacteria such as Enterobacteriaceae. Among these pathogens, *Citrobacter* spp. stands out, having been isolated from fish, animals, humans, soil, water, and food waste, among other sources (Mhaisen et al., 2020).

The potential of *Citrobacter* spp. to cause diseases in fish has been extensively researched. Studies have focused on the pathogenesis induced by *C. freundii* in rainbow trout and the resistance mechanisms in antibacterial therapies for catfish. Stressful environments can lead to *Citrobacter* spp. infections in both humans and animals. Variations in environmental factors and stressors, such as rapid temperature fluctuations, overcrowded living conditions, low dissolved oxygen levels, and high ammonia levels, can trigger outbreaks of these infectious diseases (Ismi et al., 2019).

The analysis also examines the susceptibility of these bacteria to twelve additional antimicrobial drugs. Shedding light on the dissemination and resistance mechanisms of *Citrobacter* spp. is crucial to enhancing disease management practices and ensuring the sustainability of fish farming in the region. In the aquaculture sector, this investigation is significant (Mhaisen et al., 2020; Abady et al., 2022). Based on the above-mentioned background, this study aimed to investigate the virulence of bacteria isolates in Mesopotamian Himri, *Carasobarbus luteus*, from the Al-Diwaniya River, Iraq.

Materials and Methods

A total of 100 *C. luteus* were collected from the river between October 2021 and September 2022 (Fig. 1), ranging from 14 to 30 cm in total length and weighing between 60 to 370 g. The live fish specimens were immediately transferred to oxygenated pond water. Subsequently, the fish were transported to the

*Correspondence: Mohanad O. Al-Jubouri
E-mail: mohanad79@vet.uoqasim.edu.iq

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Figure 1. Map of field sampling sites in River.

laboratory at the University of Al-Qasim Green's College of Veterinary Medicine for further examination.

Ethical consideration: The current study has adhered to the accepted principles of ethical conduct set by the College of Veterinary Medicine, Al-Qasim Green University (No: 2023, 5/09/2023).

Bacterial isolation: Upon arrival at the laboratory, the fish were collected and dissected, and aseptic samples were obtained from the skin, gills, fins, and intestine using sterile loops. MacConkey agar medium was used for bacterial isolation. The infected plates were incubated at 37°C for 24 hours to promote bacterial growth.

Antibiotic Susceptibility Testing and Bacterial Identification: The Vitek 2 system was employed for bacterial identification and antibiotic susceptibility testing. A panel of 16 antibiotics, including Piperacillin/Tazobactam, Ampicillin, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Imipenem, Amikacin, Gentamicin, Nitrofurantoin, Ciprofloxacin, Levofloxacin, Tigecycline, and Trimethoprim/Sulfamethoxazole, was used for susceptibility testing. Following reculturing on MacConkey agar, the bacteria were incubated for a full day, and the data were analyzed using the Vitek 2 system (Tables 1, 2).

Table 1. Biochemical details of the isolated *Aeromonas hydrophila*/punctate (caviae).

Biochemical Details		Reaction	Biochemical Details		Reaction
2	APPA	+	3	ADO	_
4	PyrA	_	5	lARL	_
7	dCEL	_	9	BGAL	+
10	H2S	+	11	BNAG	_
12	AGL Tp	+	13	dGLU	+
14	GGT	_	15	OFF	_
17	BGLU	+	18	dMAL	_
19	dMAN	+	20	dMNE	+
21	BXYL	_	22	BAlap	_
23	ProA	+	26	LIP	+
27	PLE	_	29	TyrA	+
31	URE	+	32	dSOR	_
33	SAC	+	34	dTAG	_
35	dTRE	+	36	CIT	_
37	MNT	-	39	5KG	_
40	ILATk	_	41	AGLU	_
42	SUCT	_	43	NAGA	_
44	AGAL	_	45	PHOS	+
46	GlyA	_	47	ODC	_
48	LDC	_	53	IHISa	_
56	CMT	+	57	BGUR	_
58	O129R	_	59	GGAA	_
61	IMLTa	_	62	ELLM	+
64	ILATa	_			

Positive, + Negative, _

Results

Based on the results, *Aeromonas hydrophila* and *Sphingobacterium thalpophilum* were isolated from the examined *C. luteus* (Table 3). The study was conducted in the river between April 2021 and March 2022, a period characterized by notable variations in water quality indices (Fig. 2). Water temperature exhibited significant variability, ranging from a minimum of 11.2°C in February to a maximum of 33.8°C in August. Salinity values ranged from 0.50°C in April to 0.69°C in October, while oxygen levels varied from 4.5 mg/L in August to 9.0 mg/L in February. Although pH levels showed relatively minor fluctuations, with March recording the highest average value of 8 and August the lowest at 6.1, these variations are typical in the river.

Antibiotics have been extensively used worldwide as essential substances that either eradicate or halt the spread of infections. They are employed as growth

Table 2. Biochemical details of isolated *Sphingobacterium thalpophilum*.

Biochemical Details		Reaction	Biochemical Details		Reaction
2	APPA	+	3	ADO	_
4	PyrA	_	5	IARL	_
7	dCEL	_	9	BGAL	+
10	H2S	+	11	BNAG	_
12	AGL Tp	_	13	dGLU	+
14	GGT	_	15	OFF	_
17	BGLU	+	18	dMAL	_
19	dMAN	_	20	dMNE	_
21	BXYL	_	22	BAlap	_
23	ProA	+	26	LIP	+
27	PLE	-	29	TyrA	+
31	URE	+	32	dSOR	_
33	SAC	_	34	dTAG	_
35	dTRE	_	36	CIT	_
37	MNT	_	39	5KG	_
40	ILATk	_	41	AGLU	_
42	SUCT	_	43	NAGA	_
44	AGAL	_	45	PHOS	+
46	GlyA	_	47	ODC	_
48	LDC	_	53	IHISa	_
56	CMT	_	57	BGUR	+
58	O129R	_	59	GGAA	_
61	IMLTa	_	62	ELLM	_
64	ILATa	_			

Table 3. *Aeromonas hydrophila,* and *S. thalpophilum* isolated from the scale, fine, gills and intestines of *C. luteus*.

Bacteria	Scale	Fine	Gills	Intestine
A. hydrophila	+	-	-	-
S. thalpophilum	+	+	+	+

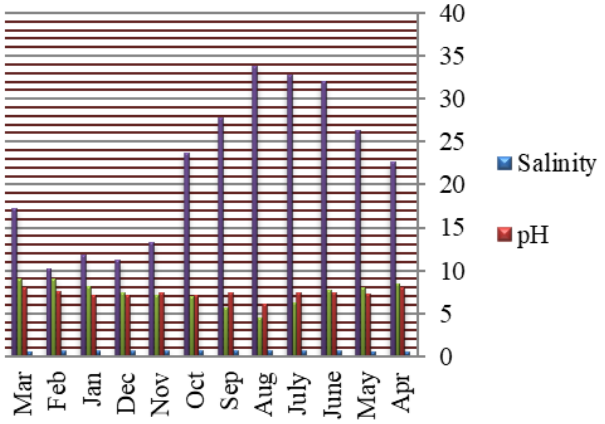

Figure 1. Monthly changes in water temperature, Do, salinity, and pH in the river.

promoters and for treating and preventing illnesses (Lulijwa et al., 2020). *Aeromonas hydrophila* and *S. thalpophilum* were tested in vitro, and the results demonstrated noticeable antibiotic susceptibility. Levofloxacin (≤0.22 µg/ml for *A. hydrophila* and ≤0.20 µg/ml for *S. thalpophilum*) also exhibited notable effectiveness, as did Ciprofloxacin (≤0.10 µg/ml) and Imipenem (≤0.23 µg/ml). Table 4 indicates that resistance to Cefazolin was observed in *A. hydrophila* (≥69 µg/ml) and *S. thalpophilum* (≥70 µg/ml).

Discussions

The study's findings raise serious concerns about public health regarding consuming *C. luteus* from the river, as they indicate the presence of bacteria, specifically *A. hydrophila* and *S. thalpophilum*. The study was conducted in the river between April 2021 and March 2022, coinciding with significant changes in water quality indices. In aquaculture systems, fish exposed to various stressors become highly susceptible to infections (Sheikh et al., 2022). The study observed a rise in bacterial numbers in water corresponding to elevated water temperatures between 25 and 32°C, an increase in organic topic, salinity, and a pH range of 5 to 9, aligning (Chankaew et al., 2023).

Table 4. Antibiotic susceptibility *A. hydrophila*, and *S. thalpophilum* isolated from the scale, fine, gills and intestines of *C. luteus*.

A. hydrophila					
Antimicrobial	**MIC***	**Interpretation**	**Antimicrobial**	**MIC***	**Interpretation**
Ampicellin	≥28	R	Imipeneme	≤0.23	R
Piperacellin/ Tazobactan	≤4	S	Amikacen	≤4	S
Cefazolin	≥70	R	Gentamicin	≤5	S
Cefoxetin	≤4	S	Nitrofurantion	44	R
Ceftazidime	≤15	S	Ciprofloxacin	≤0.10	S
Ceftriaxone	≤8	S	Levofloxacin	≤0.22	S
Cefepim	≤8	S	Tigecycline	3	R
Ertapinem	≤0.62	S	Trimethoprim/ Sulfamethoxazole	≤25	S
S. thalpophilum					
Ampicellin	≥22	R	Imipenem	≤0.23	R
Piperacellin/ Tazobactan	≤4	S	Amikacin	≤4	S
Cefazolin	≥76	R	Gentamicin	≤5	S
Cefoxitin	≤4	S	Nitrofurantion	44	R
Ceftazidime	≤15	S	Ciprofloxacin	≤0.10	S
Ceftriaxone	≤8	S	Levofloxacin	≤0.20	S
Cefepime	≤8	S	Tigecycline	3	R
Ertapenem	≤0.62	S	Trimethoprim/ Sulfamethoxazole	≤25	S

*MIC: Minimum Inhibitory Concentration (µg/ ml), S: Sensitive, R: Resistant.

These conditions likely contributed to the proliferation of bacteria detected in this study.

The susceptibility testing results revealed specific antibiotics' efficacy against *A. hydrophila* and *S. thalpophilum*. Notably, Ciprofloxacin (≤0.10 µg/ml) and Imipenem (≤0.23 µg/ml) exhibited excellent effectiveness, while Levofloxacin (≤0.22 µg/ml for *A. hydrophila* and ≤0.20 µg/ml for *S. thalpophilum*) also showed significant efficacy. Conversely, resistance was observed against Cefazolin (≥69 µg/ml for *A. hydrophila* and ≥70 µg/ml for *S. thalpophilum*) (Al-Jubouri et al., 2023). Prolonged use of antibiotics poses inherent risks, despite the potential for drug testing to reduce antibiotic dosages. These risks include the penetration of biological membranes and tissue antibiotics, leading to issues such as drug accumulation in fish tissues and environmental concerns. Previous research on *Aeromonas* spp. by Alwan et al. (2023) and Khulaif and Al-Charrakh (2023) indicated that *A. hydrophila* was sensitive to oxytetracycline, ofloxacin, azithromycin, norfloxacin, doxycycline, and chlortetracycline but resistant to amoxicillin, ampicillin, cefuroxime, flumequine, and erythromycin.

Researchers investigated the antimicrobial susceptibility test of 16 antimicrobials on *P. luteola*, *S. thalpophilum*, *S. lentus*, and *A. sobria*, which included Ampicillin, piperacillin/tazobactam, amikacin, cefazolin, ceftriaxone, ertapenem, gentamicin, cefoxitin, ciprofloxacin, imipenem, ceftazidime, levofloxacin, cefepime, tigecycline, nitrofurantoin, and trimethoprim-sulfamethoxazole. The antimicrobial susceptibility test was conducted using the Vitek II system. When examined in vitro on *P. luteola*, *S. thalpophilum*, *S. lentus*, and *A. sobria*, it was found to be resistant to cefazolin (>70, ≥70, ≥76, ≥68 µg/ml) (Alwan et al., 2023).

In a study on *S. thalpophilum* and *Streptococcus thoraltensis* from *Oreochromis aureus* (Steindachner, 1864) in Iraq, researchers tested different antibiotic combinations, recommending specific antibiotics including chloramphenicol, gentamicin, tetracycline, tazobactam, amikacin, and cefazolin for treating *S. thalpophilum* and *S. thoraltensis* infections in *O. aureus*. Intermediate efficacy was noted for amoxicillin, penicillin, and vancomycin (Hade et al., 2022). These findings underscore the critical importance of judicious antibiotic use and continuous monitoring in aquaculture practices to mitigate risks and protect public health.

Conclusion

In summary, this work highlights the presence of harmful bacteria in *C. luteus* from the river, specifically *A. hydrophila* and *S. thalpophilum*. Given the potential health risks associated with these bacteria, the study period from April 2021 to March 2022 was marked by significant variations in other water quality indicators crucial for aquatic ecosystems. The study findings emphasize the critical importance of prudent antibiotic use and ongoing monitoring in aquaculture practices to mitigate risks and safeguard public health. Continued vigilance and responsible management are essential to ensure the safety of aquatic ecosystems and consumers alike.

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