

Original Article

Diagnosis and antibiotic resistance of some *Aeromonas* species isolated from *Planiliza abu* at Al-Diwaniya River, Iraq

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Abstract: This study aimed to investigate the presence of *Aeromonas* species and their antibiotic resistance isolated from *Planiliza abu* collected from Al-Diwaniya River, Iraq. A total of 100 *P. abu* were examined to determine the presence of *A. hydrophila* from April 2021 to March 2022. The bacteria were isolated and identified using the VITEK2 system. Vital tests determined the species *Aeromonas sobria*, *A. hydrophila*, and *A. veronii*. Antibiotic sensitivity testing with 16 antibiotics using a VITEK2 system. The results showed these bacteria are sensitive to Levofloxacin and Ciprofloxacin.

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Introduction

One of the important factors affecting fish production is disease-related mortality. The widespread use of antibiotics to control disease may be due to the spread of antibiotic-resistant bacterial strains and to weakened immune systems in animals (Pavaraj et al., 2011; Brogden et al., 2014; Allameh et al., 2016). Many factors make fish more susceptible to bacterial infections, such as wounds or scratches, increased fish density in the area, the presence of certain fish metabolic products, nutrient deficiencies, changes in temperature, low dissolved oxygen levels, and Immunosuppression. All of these factors contribute to an increase in the rate of bacterial infection. Fish are infected with bacteria; the role of these infections varies from primary to secondary causes; that is, when fish become immunologically weak, they are susceptible to various pathological conditions (Alobaidi and Al Dabaagh, 2012; Aravenaromán et al., 2014).

Improper water quality is one of the factors contributing to the spread of bacterial and fungal diseases in fish, thereby reducing fish production.

Many bacteria are also found in the aquatic environment (Hassan et al., 2017; Jauneikaite et al., 2018; Hakeem et al., 2022). *Aeromonas hydrophila* is a heterotrophic, Gram-negative, rod-shaped bacterium that is mainly found in warm climates. These bacteria can be found in fresh or brackish water. It is a facultative anaerobic bacterium. Congestion and hemorrhage on the abdominal wall and fins are obvious clinical signs, in addition to erosion of scales on all surfaces of the body, and after death, severe congestion in the internal organs, ascites in the abdominal cavity, and swelling of the kidneys and spleen (Aboyadak, 2015; Janda and Abbott, 2010).

The family Mugilidae, an economically important group of fish, comprises 78 species (Coad, 2017; Jorfipour, 2022). Among them, *Planiliza abu*, a freshwater member of this family, is found in Iraq, Syria, Pakistan, Turkey, and Iran (Turan et al., 2004; Mouludi-Saleh et al., 2021; Eagderi et al., 2022; Çiçek et al., 2023). This study aimed to investigate the presence of *Aeromonas* species and their antibiotic resistance isolated from *P. abu* collected from Al-Diwaniya River, Iraq.

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Materials and Methods

A total of 100 *Planiliza abu* were collected from the AL-Diwaniya River, Iraq, from April 2021 to March 2022 (Fig. 1). The total length of the collected fish was measured, and their TL ranged between 12.6 and 23.5 cm, and the weight was 22-60 g. In addition, water parameters of the sampling stations were recorded during the study period.

The live fish, in oxygenated water, were transported to the laboratory of the College of Veterinary Medicine at Al-Qasim Green University. Samples for bacterial isolation were taken using sterile swabs from scales, gills, and intestines. Bacterial identification and antibiotic susceptibility were determined using the Vitek 2 system (Tables 1, 2, 3).

Results and Discussions

The results of this study revealed the isolation and identification of three *Aeromonas* species: *A. hydrophila*, *A. sobria*, and *A. veronii* from the examined *P. abu*. *Aeromonas sobria* was isolated from the gills of *P. abu* for the first time in Iraqi freshwaters (Table 4). Motile *Aeromonas* septicemia causes major health problems in fish farms (Cipriano, 2011). Infectious disease outbreaks are usually caused by a change in environmental conditions (stress), temperature fluctuation (sudden), overcrowding, poor quality of water, decreased dissolved oxygen, and increased levels of ammonia are the common factors associated with the disease (Ko et al., 1998).

The results of the current study showed that water quality at the studied stations fluctuates (Fig. 2). Temperature reached its highest value in August (33.8°C) and its lowest in February (10.2°C). Regarding DO, the highest value was 9.0 mg/L during February, and the lowest value was 4.5 mg/L in August. Salinity ranged from 0.5‰ in April until it was 0.69‰ in October. pH reached its highest level of 8 in March and its lowest level of 6.1 in August.

Fish that are constantly exposed to a broader range of environmental changes become more susceptible to disease and bacterial infections. This is because most fresh water is contaminated with sewage and is a source of bacterial pollution, especially in areas near



Figure 1. Map of sampling locations in the Diwaniyah River, Iraq.

residential areas, due to wastewater discharge and the lack of treatment; therefore, the water must be treated. Hence, changing environmental factors along with improper water quality make their inhabitant, especially fish species, susceptible to *Aeromonas* infection, as freshwater, which is considered a carrier of most types of pathogens, such as bacteria (Rijnsdorp et al., 2009; Amal et al., 2015; Albert and Ranasangan, 2013).

Antibiotics have been the most widely used substances worldwide over the past 50 years. It is a group of organic or chemical compounds that inhibit the growth or kill bacteria (Lulijwa et al., 2020). Ibrahim et al. (2014) showed that these bacteria are widely distributed in freshwater environments, and the reason may be that freshwater, especially that with an organic load, is a natural habitat for *A. hydrophila*. In the current study, *A. hydrophila*, *A. sobria*, and *A. veronii* were sensitive to Levofloxacin and Ciprofloxacin (Table 5), whereas the remaining antibiotics were resistant. These results show that the bacteria are resistant to antibiotics routinely used in aquaculture but are sensitive to newer generations of antibiotics (Hade et al., 2022; Alwan et al., 2023; Al-Jubouri et al., 2023). Indiscriminate use of antibiotics by non-aquaculture professionals can lead to the emergence of antibiotic-resistant pathogenic bacterial

Table 1. Biochemical details of isolated *Aeromonas sobria*.

Biochemical Details		Reaction	Biochemical Details		Reaction
2	APPA	+	3	ADO	-
4	PyrA	-	5	IARL	-
7	dCEL	+	9	BGAL	+
10	H ₂ S		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, -

Table 2. Biochemical details of isolated *Aeromonas veronii*.

Biochemical Details		Reaction	Biochemical Details		Reaction
2	APPA	+	3	ADO	-
4	PyrA		5	IARL	
7	dCEL	+	9	BGAL	+
10	H ₂ S		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, -

Table 3. Biochemical details of isolated *Aeromonas hydrophila/punctate*(caviae).

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	-			

Positive, +; Negative, -

Table 4. The infected organs of fish by *Panaliza abu*.

Bacteria	Scale	Fine	Gill	Intestine
<i>A. hydrophila</i>	+	-	-	-
<i>A. sobria</i>	-	-	+	-
<i>A. veronii</i>	-	+		+

strains in fish, which are then transmitted to consumers. Wamala et al. (2018) showed that the misuse and overuse of antibiotics in fish farms may lead to antibiotic resistance in fish and the aquatic environment. In conclusion, the extensive use of antibiotics leads to the emergence of antibiotic-resistant bacterial strains, so specialists should use them sparingly.

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Table 5. Antibiotic susceptibility of *Aeromonas hydrophila*, *A. sobria*, and *A. veronii* isolated from *Panaliza abu* collected from the Diwaniyah River.

<i>A. hydrophila</i>					
Antimicrobial	MIC*	Interpretation	Antimicrobial	MIC*	Interpretation
Ampicillin	>= 26	R	Imipenem	<= 0.22	R
Piperacillin/Tazobactan	<= 4	S	Amikacin	<= 4	S
Cefazolin	>= 73	R	Gentamicin	<= 4	S
Cefoxitin	<= 4	S	Nitrofurantion	44	R
Ceftazidime	<= 14	S	Ciprofloxacin	<= 0.12	S
Ceftriaxone	<= 7	S	Levofloxacin	<= 0.22	S
Cefepime	<= 8	S	Tigecycline	3	R
Ertapenem	<= 0.59	S	Trimethoprim/Sulfa methoxazole	<= 23	S
<i>A. sobria</i>					
Antimicrobial	MIC*	Interpretation	Antimicrobial	MIC*	Interpretation
Ampicillin	>= 20	R	Imipenem	<= 0.23	R
Piperacillin/Tazobactan	<= 4	S	Amikacin	<= 3	S
Cefazolin	>= 73	R	Gentamicin	<= 5	S
Cefoxitin	<= 4	S	Nitrofurantion	46	R
Ceftazidime	<= 13	S	Ciprofloxacin	<= 0.11	S
Ceftriaxone	<= 8	S	Levofloxacin	<= 0.21	S
Cefepime	<= 8	S	Tigecycline	3	R
Ertapenem	<= 0.60	S	Trimethoprim/Sulfa methoxazole	<= 24	S
<i>A. veronii</i>					
Antimicrobial	MIC*	Interpretation	Antimicrobial	MIC*	Interpretation
Ampicillin	>= 20	R	Imipenem	<= 0.23	R
Piperacillin/Tazobactan	<= 4	S	Amikacin	<= 3	S
Cefazolin	>= 73	R	Gentamicin	<= 5	S
Cefoxitin	<= 4	S	Nitrofurantion	46	R
Ceftazidime	<= 13	S	Ciprofloxacin	<= 0.11	S
Ceftriaxone	<= 8	S	Levofloxacin	<= 0.21	S
Cefepime	<= 8	S	Tigecycline	3	R
Ertapenem	<= 0.60	S	Trimethoprim/Sulfa methoxazole	<= 24	S

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