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Prevalence and Antibiotic Resistance in *Aeromonas* species Isolated from Common Carp (*Cyprinus carpio* L.) Cultivated in Floating Cages at Al-Hilla River

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Abstract: The aim of this study is describe the case of ulcer and hemorrhaging (red-sore disease) in a common carp (*C. carpio*) that is cultivated in floating cages at Al-Hilla river and to finding efficient antibiotic treatments. A total of 144 specimens of the common carp were examined for ulcer with hemorrhaging during the period from December 2017 till the end of November 2018. In this study, red-sore disease was found in 45 specimens. This disease is caused by the infection of Motile *Aeromonas* Septicemia (MAS). *Aeromonas hydrophila*, *A. sobria* and *A. veronii* were isolated from the skin, gills and intestine of common carp. *Aeromonas* species were identified with a different percentage in common carp as the follows: *A. hydrophila* (58.76%), *A. sobria* (31.83%) and *A. veronii* (43.3%). Antibiotic susceptibility test of 12 antibiotics (Piperacillin/ Tazobactan, Amikacin, Cefazolin, Gentamicin, Cefoxitin, Ciprofloxacin, Ceftazidime, Levofloxacin, Cefepime, Tigecycline, Imipenem and Trimethoprim/ Sulfamethoxazoleby) showed variable resistances for *Aeromonas* species. *Aeromonas* species were identified and examined for antibiotic susceptibility using the VITEK 2 system.

Keywords: Fish, *Aeromonas* spp., antibiotic susceptibility, Iraq.

Introduction

Aeromonas species cause a wide spectrum of disease syndromes among warm and cold blooded animals, including fish, reptiles, amphibians, mammals and humans (Janda & Abbott, 2010). The members of *Aeromonas* spp. belong to the family Aeromonadaceae comprise Gram negative, nonspore forming and motile bacilli or coccobacilli rods with rounded ends (Skwor *et al*., 2014). It is cause most serious infectious disease known as Motile *Aeromonas* Septicemia (MAS), associated with high mortality rates without symptoms in per acute phase but in acute phase, skin and fin ulcers appear on the external surface of fish with ascites in the abdomen and exophthalmia (Bondad *et al*., 2005; Austin & Austin, 2012; Skwor *et al*., 2014; Al-Niaeem *et al*., 2015). The disease

can produce significant losses in aquaculture industry because of reduced growth in survived cases (Pachanawan *et al*., 2008; Buján *et al*., 2010). The *Aeromonas* spp. consist of 27 species, but some such as *A. hydrophila*, *A. allosaccharophila*, *A. jandaei*, *A. piscicola*, *A. salmonicida*, *A. schubertii*, *A. bestiarum*, *A. sobria*, *A. veronii*, *A. dhakensis* and *A. caviae* are considered pathogenic to animals and humans (Beazhidalgo & Figueras, 2013; Aravenaromán *et al*., 2014; Li *et al*., 2019).

Outbreaks of the disease and its influence on the survival, persistence and ability of *Aeromonas* to cause infection are usually related with the change of environmental state, such as transfer the fish to poor water quality, stress, mishandling, changing of temperature, dioxide levels, overcrowding, high nitrite and carbon (Dixon, 1993; Aoki, 1999; Al-Taee *et al*., 2017).

Because, there is a little information pertains to *Aeromonas* in the fish farms particularly in Iraq. The aim of the present to describe a case of hemorrhaging and skin ulcer in common carp, *C. carpio* cultivated in floating cages in Al-Hilla river and the susceptibility pattern of bacteria to 12 antimicrobial drugs.

Materials & Methods

A total of 144 fish of common carp were sampled from four farms of floating cages at Al-Hilla, the first and second stations, before the city center and the third and fourth stations, after the city center. During the period December 2017- November 2018. The length of fish ranging between 23.8- 37.16 cm and the weight was 261.66- 693.3 gm. The live fishes were transported to oxygenated pond water. Before transferred to the laboratory in College of Veterinary Medicine, University of Al-Qasim Green.

The collected fishes were dissected and bacterial swabs were taken aseptically using a sterile loop from skin, gill and intestine. For isolation of bacteria, MacConkey Agar medium was used. The inoculated plate was incubated at 37 °C for 24 h. Bacteria were identified and antibiotic susceptibility (Piperacillin/ Tazobactan, Amikacin, Cefazolin, Gentamicin, Cefoxitin, Ciprofloxacin, Ceftazidime, Levofloxacin, Cefepime, Tigecycline, Imipenem and Trimethoprim/ Sulfamethoxazoleby using the VITEK 2 system.

Results & Discussion

The results of this study showed that *A. hydrophila*, *A. sobria* and *A. veronii* isolated and identified from the four floating cages in Al-Hilla river are a dangerous and may be unhealthy for public health when consumed. *Aeromonas* species were identified with different percentages for common carp, including *A. hydrophila* (58.76%), *A. sobria* (31.83%) and *A. veronii* (43.3%) (Table 1). The Motile *Aeromonas* Septicemia is one that causes main disease problems in the carp farm (Cipriano, 2011). Outbreaks of the infectious disease are usually caused the changing of environmental state and stress. Fluctuation of temperature (sudden), crowding, poor water quality, low dissolved oxygen, high ammonia levels are the common factors related with MAS (Ko *et al*., 1998).

The study was conducted from December 2017 till the end of November 2018 in which there are fluctuations in water quality parameters in the aquaculture in the studied stations (Table 2). The mean of temperature fluctuated from 10.7 °C to 32.9°C. the mean of salinity was recorded ranged from 460 to 580 PSU, ammonia was recorded ranged from 1.3 to 2.8 PSU. The pH value was relatively from 6.4 to 8.1. In culture system of fish that

always exposed an assortment of stresses become over sensitive to disease infection (Rijnsdorp *et al*., 2009; Albert & Ransangan, 2013).

The growing of bacteria in water increased by rising levels of water temperature from 25 °C to 32°C, organic matters, salinity and pH 5-9 (Kiriratnikom *et al*., 2000). These levels of growing bacteria were observed in the current study.

Gram negative bacilli, *A. hydrophila A. sobria* and *A. veronii* were isolated from the skin, gills and intestine form common carp (Fig. 1 & table 3).

Table (1): Percentage of isolated *Aeromonas* spp.

Station	*A. hydrophila* (%)	*A. sobria* (%)	*A. veronii* (%)
1	11.76	5.88	9.80
2	15.60	8.26	11.0
3	14.40	7.69	11.5
4	17.0	10.0	11.0
Total	58.76	31.83	43.3

Table (2): The water parameters of studied stations.

Station	Temp (°C) Min-max (mean)	Salinity (PSU) Min-max (mean)	Ammonia ($mg.l^{-1}$) Min-max (mean)	pH Min-max (mean)
	Range (mean)			
1	11.9 - 32.7 (22.66)	480 - 560 (517.5)	1.3-1.8 (1.62)	6.8 - 8.1 (7.36)
2	10.7 - 32.9 (22.9)	460 - 570 (516.66)	1.4-1.9 (1.69)	6.5 - 8.1 (7.33)
3	13 - 32.6 (23.8)	480 - 580 (530.8)	2.0-2.8 (2.42)	6.4 - 7.6 (7.09)
4	12.5 - 31.2 (22.86)	460 - 560 (513.33)	0.7-1.4 (1.21)	7.1 - 7.9 (7.35)

Biochemical characterization of Motile *Aeromonas* Septicemia using the VITEK 2 system was given in tables (4-6). It has been noticed by some researchers that *Aeromonas* spp. causes skin lesions with hemorrhagic in fresh water fish and cultured fish (Aguilera *et al*., 2005; Beazhidalgo & Figueras, 2013; Skwor *et al*., 2014; Gao *et al*., 2016; Li *et al*., 2019).

The best antibiotics susceptibility were Tigecycline, Ceftazidime, Cefepime and Gentamicin, when tested in vitro on *A. hydrophila* while, it was resistant to Cefazolin and Cefriaxone (Table 7).

In vitro, the more antibiotic susceptibility to *A. sobria* were Ciprofloxacin, Levofloxacin, Tigecycline, Gentamicin, Cefazolin and Cefepime

whereas *A. sobria* had intermediately resistant to Imipenem (Table 8). The antibiotic susceptibility in vitro were more effect on *A. veronii* Ciprofloxacin, Levofloxacin, Tigecycline, Gentamicin, Cefazolin and Cefepime (Table 9).

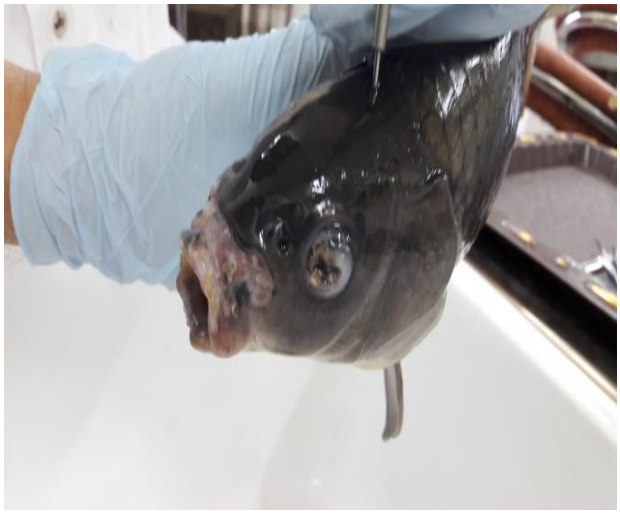
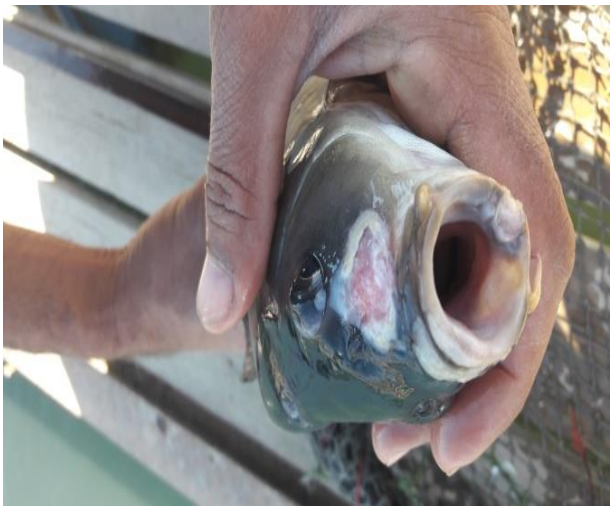
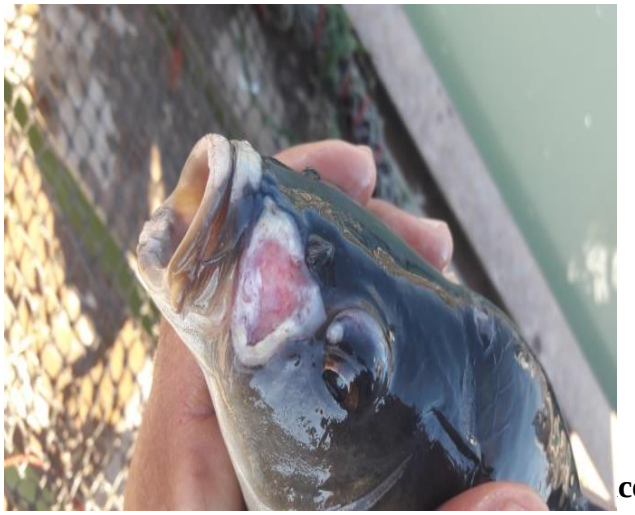
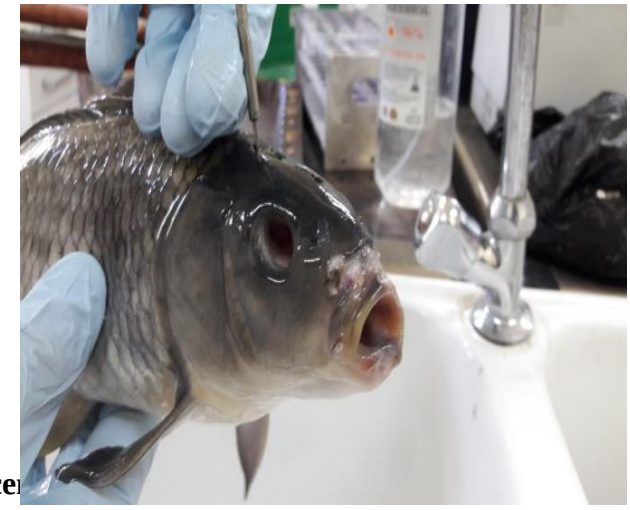

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Table (3): The infected organs of fish by *Aeromonas* spp.

Month	Skin			Gills			Intestine		
	A. hydrophila	*A. sobria*	*A. veronii*	*A. hydrophila*	*A. sobria*	*A. veronii*	*A. hydrophila*	*A. sobria*	*A. veronii*
December 2017	-	-	-	-	-	-	+	-	-
January 2018	+	+	-	-	+	-	-	+	-
February	-	+	-	-	+	-	-	+	-
March	+	+	-	+	-	-	+	+	+
April	+	-	+	+	-	+	+	-	+
May	+	-	+	+	-	+	+	-	+
June	+	-	+	+	-	+	+	-	+
July	+	+	+	-	+	+	-	+	-
August	+	-	+	-	-	+	-	-	+
September	+	-	+	+	-	+	+	-	+
October	+	-	+	-	-	+	+	-	+
November	-	-	+	-	+	+	+	-	+

+: Positive, -: Negative

Table (4): Biochemical features of isolated *A. hydrophila*.

Biochemical features		Reaction	Biochemical features		Reaction
2	APPA	-	3	ADO	-
4	PyrA	-	5	IARL	-
7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+
12	AGLTp	-	13	dGLU	+
14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+
19	dMAN	+	02	dMINE	+
01	BXYL	-	00	BAlap	-
03	ProA	-	26	LIP	+
07	PLE	-	09	TyrA	+
31	URE	-	30	dSOR	+
33	SAC	+	34	dTAG	-
35	dTRE	+	36	CIT	-
37	MNT	-	39	5KG	-
42	ILATk	-	41	AGLU	-
40	SUCT	+	43	NAGA	-
44	AGAL	-	45	PHOS	+
46	GIyA	-	47	ODC	-
48	LDC	-	53	IHISa	-
56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+
61	IMLTa	-	60	ELLM	+
64	ILATa			-	

+: Positive, -: Negative

Table (5): Biochemical features of isolated *A. sobria*.

Biochemical features		Reaction	Biochemical features		Reaction
2	APPA	-	3	ADO	-
4	PyrA	-	5	IARL	-
7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	+
12	AGLTp	-	13	dGLU	+
14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+
19	dMAN	+	02	dMINE	+
01	BXYL	-	00	BAlap	-
03	ProA	+	26	LIP	-
07	PLE	-	09	TyrA	+
31	URE	-	30	dSOR	-
33	SAC	+	34	dTAG	-
35	dTRE	+	36	CIT	+
37	MNT	-	39	5KG	-
42	ILATk	-	41	AGLU	-

40	SUCT	+	43	NAGA	+
44	AGAL	-	45	PHOS	-
46	GIyA	-	47	ODC	-
48	LDC	-	53	IHISa	-
56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	+
61	IMLTa	-	60	ELLM	+
64	ILAT	-			

+: Positive, -: Negative

Table (6): Biochemical features of isolated *A. veronii*.

Biochemical features		Reaction	Biochemical features		Reaction
2	APPA	-	3	ADO	-
4	PyrA	-	5	IARL	-
7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+
12	AGLTp	-	13	dGLU	+
14	GGT	-	15	OFF	+
17	BGLU	+	18	dMAL	+
19	dMAN	+	02	dMINE	+
01	BXYL	-	00	BAlap	-
03	ProA	+	26	LIP	-
07	PLE	-	09	TyrA	+
31	URE	-	30	dSOR	-
33	SAC	+	34	dTAG	-
35	dTRE	+	36	CIT	+
37	MNT	-	39	5KG	-
42	ILATk	-	41	AGLU	-
40	SUCT	+	43	NAGA	-
44	AGAL	+	45	PHOS	-
46	GIyA	-	47	ODC	+
48	LDC	-	53	IHISa	-
56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	+
61	IMLTa	-	60	ELLM	+
64	ILATa	-			

+: Positive, -: Negative

Table (7): Antibiotic susceptibility of *A. hydrophila*.

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Piperacillin/ Tazobactan	<= 4	S	Amikacin	<= 2	S
Cefazolin	>= 64	R	Gentamicin	<= 1	S
Cefoxitin	32	R	Ciprofloxacin	1	S
Ceftazidime	<= 1	S	Levofloxacin	1	S
Cefepime	<= 1	S	Tigecycline	<= 0.5	S
Imipenem	4	S	Trimethoprim/ Sulfamethoxazole	<= 20	S

MIC*:* Minimum Inhibitory Concentration (µg.ml^{-1}), S*:* Sensitive, R: Resistant.

Table (8): Antibiotic susceptibility of *A. sobria*.

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Piperacillin/ Tazobactan	16	S	Amikacin	<= 2	S
Cefazolin	<= 4	S	Gentamicin	<= 1	S
Cefoxitin	<= 4	S	Ciprofloxacin	<= 0.25	S
Ceftazidime	<= 1	S	Levofloxacin	0.5	S
Cefepime	<= 1	S	Tigecycline	<= 0.5	S
Imipenem	8	I	Trimethoprim/ Sulfamethoxazole	<= 20	S

MIC*:* Minimum Inhibitory Concentration (µg.ml^{-1}), S*:* Sensitive, R: Resistant, I: Intermediate.

Table (9): Antibiotic susceptibility of *A. veronii*.

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Piperacillin/ Tazobactan	20	S	Amikacin	<= 2	S
Cefazolin	<= 4	S	Gentamicin	<= 1	S
Cefoxitin	<= 4	S	Ciprofloxacin	<= 0.5	S
Ceftazidime	<= 1	S	Levofloxacin	0.5	S
Cefepime	<= 1	S	Tigecycline	<= 0.5	S
Imipenem	8	I	Trimethoprim/ Sulfamethoxazole	<= 20	S

MIC*:* Minimum Inhibitory Concentration (µg.ml^{-1}), S*:* Sensitive, R: Resistant, I: Intermediate.

Recently, there are many processes for treating *Aeromonas* spp. (primarily *A. hydrophila*) that infected in cultivated fish, one of them is antimicrobial drugs, it is the common antibiotic for controlling the aquatic bacteria, so antimicrobial sensitivity experiments are crucial for an efficient therapy (Skwor *et al*., 2014).

High allergy of drug examination in various organisms is feasible technique that can clearly decrease antibiotic dosage (Skwor *et al*., 2014; Li *et al*., 2019). In the current study, the Motile *Aeromonas* Septicemia was intensely susceptible to Tigecycline, Ciprofloxacin and Levofl-oxacin. Therefore, it suggests may be that these drugs are convenient to treat Motile *Aeromonas* Septicemia. On the other hand, there are some risks for using antibiotics, firstly, the medicine can penetrate the biological membrane and tissues, for example, it can pass through blood vessels in the brain, secondly, using antibiotics for a long time causes the resistance of bacteria, drug accumulation in fish tissues and environmental problems (El-Bouhy *et al*., 2011; Li *et al*., 2019).

About 90% of *A. hydrophila*, were susceptible to Ceftazidine, Moxalactam, Aztreonam, Cefepime, Amikacin, Fluoroquinolones and Imipenem, but it was more resistant to Trimethoprim-Sulfameth- oxazole, Tetracycline, Aminoglycosides and Cephalosporins (Chopra, & Roberts, 2001; Soltan *et al*., 2016). Guz & Kozinska (2004) found that about 21 isolates of *A. sobria* and *A. hydrophila* which isolated from common carp were 100% were sensitive to Oxolinic acid, Trimethoprim- Sulphamides,

Cloramphenicol, Flumequine, Norfloxacin, Perfloxacin, lincomycin and resistant to Penicillin.

Nawaz *et al*. (2006); Adanir & Turutoglu (2007) and Hassan *et al*. (2017) confirmed that the resistance to Oxytetracycline, Penicillin, Amoxicillin and other antibiotics may be attributed to genes which recently discovered as responsible for this antibiotic resistance in genus *Aeromonas*.

Conclusion

Aeromonas spp. may cause hemorrhages with ulcers in common carp. Extensive and uncontrolled use of antimicrobial drugs may generate an multiple resistance for antimicrobial. So, the test of antimicrobials susceptibility in genus *Aeromonas* infection and other bacterial infections must be under intensive in cultivated fish. The water should be orderly changed and stress and overcrowding in the fish population should be avoided to prevent the infection.

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Ethical approval: all applicable national and international guidelines for the care and use of animals were followed.

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