



Bacterial Fish Diseases in some Semi-close Aquaculture Systems in Basrah Province, Iraq

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Abstract : The object of this study was to detect the bacterial species that founded in infected common carps *Cyprinus carpio* in semi-closed systems in two districts of Basrah province. Bacterial gill disease, skin ulcer disease and intestine inflammatory disease were recorded. In the present study, *Aeromonas sobria* (38.46) was the predominant species, followed by *Citrobacter freundii* (29.23), *Vibrio cholerae* (21.53) and *Serratia fonticola* (10.76%). Identification of the isolates was carried out depending on the morphology of colony, specific media and identification using VITEK 2 system (Biomerieux- USA). Five antibiotic discs were used for antibiotic sensitivity test by the disk diffusion method. *A. sobria* was sensitive to Nitrofurantion, *V. cholerae* was sensitive to Ampicillin, *C. freundii* was resistance for all antibiotics while *S. fonticola* was sensitive to Gentamicin. This study represented the first investigation in Iraq on bacterial isolates from semi- closed system.

Keywords: Bacteria, Fish, Diseases, Aquaculture.

Introduction

experience. As aquaculture continues to become more intensive and expands into new frontiers, fish health problems are likely to become more significant (Buller, 2004). Bacterial gill disease is one of the most influent problem which faces aquaculture in the world. For example, in freshwater aquaculture, bacterial septicaemia is considered to be one of the most important diseases in history of Chinese aquaculture (Qi, 2002). The present study was designed to detect bacterial fish diseases in semi-enclosed

In recent years, closed and semi-enclosed aquaculture systems had been used in Iraq due to water shortage. There are many examples of recirculation systems operating without any disease problems at all (Bregnballe, 2015). This type of culture requires good knowledge and high training and it's not available in all projects that were established in Basrah Province. So, most projects face different problems and the farmer preferred the semi-enclosed aquaculture system. This means that the health problems will certainly be present due to bad management and lack of

(India), Nitrofurantion 300, Gentamicin 10, Tetracycline 30, Erythromycin 15 and Ampicillin 10 as shown in tables (3, 4, 5 and 6).

Results

Signs of diseases

The infected fishes swam slowly and tried to make bubbles near the water surface and this is the normal reaction of the fish when the level of oxygen is low in water, but in this case, the problem is related to the decrease of the efficiency of breathing due to the crash of gill plates. It was noted that infected fish head was slightly above the horizontal line of the body during swimming. The fishes in the acute stages lose the reaction to any stimuli and swam so quietly that they can be held by hand. Prevalence of infection in both locations was very high (more than 90%) and there are mortality 10-30 fish per day.

Clinical identification: In this study, three type of bacterial diseases were identified. Bacterial gill disease (B.G.D.) and skin ulcer disease were recorded in both locations while intestine inflammatory disease (I.I.D.) was recorded in Abu Alkhaseeb district only (table 1). Identified bacterial species of B.G.D. were *Vibrio cholerae* Pacini, 1854 and *Aeromonas sobria* Popoff & Veron, 1981 in Abu Al-Khaseeb district while *Citobacter freundii* (Braak, 1928) Werkman & Gillen, 1932 and *V. cholerae* in Shatt Al-Arab district. For the skin ulcer disease, the pathogen was *A. sobria* only in both districts. Pathogens of intestine inflammatory disease were *Serratia fonticola* Gavini, Ferragut, Izard, Trinel, Leclerc, Lefebvre & Mossel, 1979 and *C. freundii* (Table 1) in Abu Al-Khaseeb district only.

In the present study, *A. sobria* (38.46%) was the predominant species, followed by *C. freundii* (29.23%), *V. cholerae* (21.53%) and *S. fonticola* (10.76%) (Table 2). These

aquaculture system in Basrah province and identify their pathogens.

Materials & Methods

Common carps were collected during the period from February to March 2018 from two infected fish farms (semi-closed system) in two locations of Basrah province, Iraq (Shatt Al-Arab and Abu Alkhaseeb districts).

Fish samples (100-250 g) were transported to the laboratory in chill condition. The samples were morphologically inspected at first and after that, gills and other organs were cut and examined under a dissecting microscope (Optika SZM-1) to detect signs of diseases.

One gram of infected tissue was taken from the gills and the intestine and homogenized in nine ml of sterile normal saline solution using a sterilized glass homogenizer (Brand- Germany). One millilitre of solutions were serially diluted (10^{-1} to 10^{-7}). An amount of 0.1 ml of the serial dilutions were inoculated onto nutrient agar (N.A.), trypticase soy agar (TSIA) and thiosulfate citrate bile sucrose salt agar (TCBS) from Hi media (India). The plates were incubated at 37°C for 24 h (N.A. and TSIA) and for 48h (TCBS). One growth of colonies was transported to new media at the same temperature and time above. Isolated bacteria were morphologically identified and distinguished to Gram positive and negative. Pure growths cultured on N.A. at 37°C for 24 h after that were picked to bacterial identification using VITEK 2 system (Biomerieux- USA).

Five antibiotic discs were used for antibiotic sensitivity test, performed by the disk diffusion method according to CLSI (2016) guidelines using antibiotic discs on Mueller-Hinton agar (MH) of Hi media

4), *V. cholerae* was sensitive to Ampicillin (Table 5), *C. freundii* was resistant for all antibiotics (Table 6) while *S. fonticola* was sensitive to gentamicin (Table 6).

species were identified by VITEK 2 system and the probability was high (Table 3). Antibiotic sensitivity test showed that *A. sobria* was sensitive to Nitrofurantion (Table

Table (1): Diseases and bacterial pathogens of the two districts.

Locations	Diseases	Bacterial pathogens
Abu Al-Khaseeb	B.G.D.	<i>V. cholerae</i> , <i>A. sobria</i>
	Skin ulcer	<i>A. sobria</i>
	Intestine inflammatory disease	<i>S. fonticola</i> , <i>C. freundii</i>
Shatt Al-Arab	B.G.D.	<i>C. freundii</i> , <i>V. cholera</i>
	Skin ulcer	<i>A. sobria</i>
	Intestine inflammatory disease	-

Table (2): Number and percentage of isolated bacteria.

Bacterial Species	No.	Isolated bacteria (%)
<i>Aeromonas sobria</i>	25	38.46
<i>Citrobacter freundii</i>	19	29.23
<i>Vibrio cholera</i>	14	21.53
<i>Serratia fonticola</i>	7	10.76

Table (3): Number of bacterial isolates diagnosed and the probability of the diagnosis by using VITEK 2 system.

Bacterial species	No.	Probability (%)	Time per h	Unidentified bacteria
<i>Aeromonas sobria</i>	25	95	5	11
<i>Citrobacter freundii</i>	19	97	5.75	
<i>Vibrio cholera</i>	14	94	5	
<i>Serratia fonticola</i>	7	95	4	

Table (4): The value of resistance of *Aeromonas sobria* to antimicrobial agents.

Type of antibiotics	Concentration (mcg.g ⁻¹)	Type of resistance	Diameter of inhibition zone (mm)
Nitrofurantoin	300	S	25
Gentamicin	10	R	-
Tetracycline	30	R	7
Erythromycin	10	R	11
Ampicillin	25	R	-

Table (5): The value of resistance of *Citrobacter freundii* to antimicrobial agents.

Type of antibiotics	Concentration (mcg / g)	Type of resistance	Diameter of inhibition zone (mm)
Nitrofurantoin	300	R	10
Gentamicin	10	R	8
Tetracycline	30	R	12
Erythromycin	10	R	10
Ampicillin	25	R	-

Table (6): The value of resistance of *Vibrio cholerae* to antimicrobial agents.

Type of antibiotics	Concentration mcg.g ⁻¹	Type of resistance	Diameter of inhibition zone (mm)
Nitrofurantoin	300	R	-----
Gentamicin	10	R	15
Tetracycline	30	R	7
Erythromycin	10	R	10
Ampicillin	25	S	23

Table (7): The value of resistance of *Serratia fonticola* to antimicrobial agents.

Type of antibiotics	Concentration mcg.g ⁻¹	Type of resistance	Diameter of inhibition zone (mm)
Nitrofurantoin	300	R	16
Gentamicin	10	S	19
Tetracycline	30	R	9
Erythromycin	10	R	10
Ampicillin	25	R	-----

Table (8): Species identification of *Aeromonas sobria* isolates by the VITEK 2.

2	APPA	+	3	ADO	-	4	PyrA	-	5	IARL	-	7	d CEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGL Tp	+	13	d GLU	+	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	d TAG	-	35	d TRE	+	36	CiT	+	37	MNT	-	39	SKG	-
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	0129R	+	59	GGAA	+	61	IMLTa	-	62	ELLM	+	64	ILATa	-			-

Table (9): Species identification of *Citrobacter freundii* isolates by the VITEK 2.

2	APPA	+	3	ADO	-	4	PyrA	+	5	IARL	-	7	d CEL	-	9	BGAL	+
10	H2S	+	11	BNAG	+	12	AGL Tp	-	13	d GLU	+	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	d TAG	-	35	d TRE	+	36	CiT	+	37	MNT	-	39	SKG	+
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	0129R	+	59	GGAA	+	61	IMLTa	-	62	ELLM	+	64	ILATa	-			-

Table (10): Species identification of *Vibrio cholerae* isolates by the VITEK 2.

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	d CEL	-	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGL Tp	-	13	d GLU	+	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	d TAG	-	35	d TRE	+	36	CiT	-	37	MNT	-	39	SKG	-
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	0129R	-	59	GGAA	+	61	IMLTa	-	62	ELLM	+	64	ILATa	-			-

Table (11): Species identification of *Serratia fonticola* isolates by the VITEK 2.

2	APPA	-	3	ADO	+	4	PyrA	-	5	IARL	+	7	d CEL	+	9	BGAL	+
10	H2S	+	11	BNAG	+	12	AGL Tp	-	13	d GLU	+	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	d TAG	+	35	d TRE	+	36	CiT	-	37	MNT	-	39	SKG	-
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	0129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			-

Discussion

The semi-enclosed aquaculture system in Basrah province has been used as an alternative to the closed systems due to the lack of sufficient experience for farmers to manage with the closed systems, which can avoid wastes problem, increase ammonia and decrease the dissolved oxygen content. The health problems in farms that rely on closed and semi-closed systems were recorded in 10 farms with a total area of 1233.2 m² with different deaths (Jassim, 2019). This study is the first attempt to identify the most important health problems facing this type of culture. The bacterial infections recorded during the study (bacterial gill disease, skin ulcer disease and intestinal inflammation disease) are due to the poor health management that contributed to the infection. The fishes were transported in improper ways and with high densities. Farmers also significantly reduce water level in the ponds weekly for the purpose of cleaning the tank which was originally designed in a way that does not ensure the disposal of fish wastes, which caused stress and decrease immunity contributed to the incidence of such diseases.

Bacterial gill disease and skin ulcer disease recorded in Shatt Al-Arab and Abu Al-Khaseeb farms are due to of above conditions and also due to the use of corrupted floating feed stored in high moisture conditions which, in turn, was the main reason to the infection of fishes with inflammatory of intestine in Abu Al-Khaseeb farms.

A. sobria (38.46 %) was the predominant species (Table 2) being isolated from skin of fishes in both districts while it was found in gill of fishes in AbuAl-Khaseeb farms (Table 2). Many studies recorded this bacterium from different organs of various

fish species and the infection was related with changes in water quality. *A. sobria* was isolated from the liver, kidneys, spleen and skin lesions of farmed perch fish (Wahli *et al.*, 2005). Also, it was recognized from *C. carpio* by Kozińska *et al.* (2002), *Garra rufa* by Majtán *et al.* (2012) and *Labeo rohita* by Dar *et al.* (2016). Barzani & Mustafa (2016) recognized *A. sobria* in intestine of fishes and water of aquarium in Erbil province, Iraq. Al-Hisnawi (2016) identified this bacterium from *Liopropoma santi* and water of a local river in Kerbala city, Iraq.

The second species of bacteria, *C. freundii* was isolated from the gills and intestine. Because of *C. freundii* isolated from different species of fishes and caused health problems in intestine, gills and skin of fishes so, recorded it living in bad water quality was expect as in this study . Al-Obaidi & Al-Dabbagh (2012) isolated it from intestine of *C. carpio* which collected from Tigris river passing through Mosul city, Iraq. Abid & Al-Hamdani (2016) recorded this species as one of the causative agents of ulcerated skin lesion of *C. carpio* which cultured in Sulaimani province, Iraq. Al-Hisnawi (2016) identified it in the intestinal mucosa and gills of *L. santi* from a local river in Kerbala city, Iraq. Hammood & Ibrahim (2018) identified this bacterium in gut of *Alburnus mossulensis*, *C. carpio* and *Garra elegans* from Tigris river in Baghdad city.

V. cholerae (21.53 %) was isolated from the gills and considered as pathogen of fishes. Identification of this species in the two districts in the same organ refers to its importance as an infectious agent. This species was recorded either with *A. sobria* or with *C. freundii*. Al-Tae *et al.* (2017) recorded six species of *Vibrio* which included

V. cholerae in farmed common carps from different districts of Basrah city. Al-Hussainy *et al.* (2017) isolated this bacterium from frozen and fresh fishes which were collected from fish market of Basrah city and Thi Qar province. Hammood & Ibrahim (2018) identified this species in gut of *A. marmid* and *G. elegans*.

S. fonticola (10.76%) was isolated from intestine of fishes in Abu Al-Khaseeb district. This bacterium has a wide distribution in aquatic environments and also regarded as a significant human pathogen (Carneiro *et al.*, 2013). Reporting this species as pathogen for fishes was scarce. Ture *et al.* (2018) recorded this bacterium as pathogen of whiting (*Merlangius merlanguseuxinus*) in the Eastern Black Sea coast of Turkey. Recording of this bacterium is considered here as the second record as pathogen of fishes in Iraq after that of Majeed *et al.* (2016).

A. soberia was sensitive for Nitrofurantoin and this result is in agreement with Barzani & Mustafa (2016). *C. freundii* was resistance for all antibiotic. *S. fonticola* was sensitive to Gentamicin. Results of the present study about the sensitivity of above species are in agreement with Al-Obaidi & Al-Dabagh (2012) and Stock *et al.* (2003). *V. cholerae* was sensitive for Ampicillin while many studies mentioned this species as resistance for this antibiotic. However, some studies reported it as a sensitive such as of Ali *et al.* (2019). It is important to mention that treatment of diseased fishes which recorded in this study by using oxytetracycline was excellent and the healing achieved 100%.

Conclusions

Three types of diseases were recorded in semi-enclosed fish culture systems and four species of bacteria were identified. The results

of this study confirm that the emergence of diseases is caused by mismanagement and that these diseases undoubtedly will appear in other farms due to lack of experience among farmers.

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