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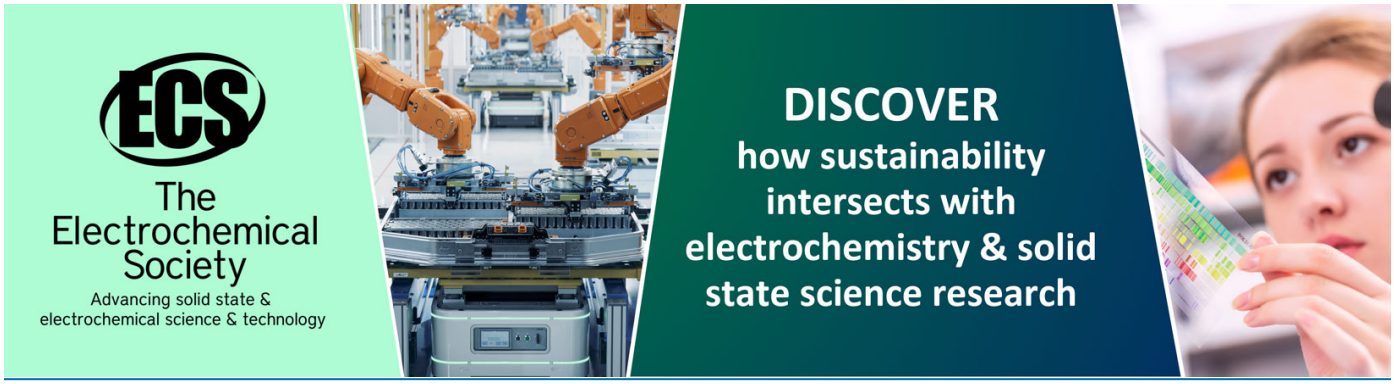

Isolation and treatments of Aeromonas hydrophila and Staphylococcus lentus implicated in the seasonal autumn mortalities of farm-raised Cyprinus carpio, Basrah governorate, Iraq.

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Abstract. Aeromonas hydrophila and Staphylococcus lentus are a widespread bacterial species in the aquatic environment, isolate from different aquatic environments, and it is a responsible for bacterial disease of Hemorrhagic Septicemia in freshwater fish, in addition to other species. Furthermore, the main reason for its spread is not following proper farming systems and food quality and lack of oxygen and intensive farming play a key in the spread of this type of bacterial. The presence of bacteria as a real indicator of severe water contamination and this underlines the presence of high numbers of fish mortalities domain as accompanies this shortage pollution biomarkers of fish including dissolved oxygen and this has been confirmed by many international studies which the most important. The samples were collected from the common carp farms north of Basrah city. A total of 30 live infected fishes was collected, during the autumn season of the year 2019, samples were placed in ice box and transferred to the laboratory of department marine vertebrate marine science center and to Al Noor vet. lab. Within 3hrs. Fish weights and lengths from l50 -350 gm, and 15-35cm respectively. Bacterial samples were taken from the skin, gills and intestines using a sterile scalpel, samples were homogenized in 9 ml of sterile normal saline solution, one milliliter aliquots of the homogeneous solutions were serially diluted (10-1 to 10-7), aliquots of 0.1 ml of the serial dilutions were inoculated on tryptic soya agar (TSA) (Hi media- India). Nutrient agar (Hi media- India) in duplicate using the spread plate method and the plates were incubated at 37°C for 24-48 h. Then after, Bacteria were identified using the VITEK II system. The bacterial sensitivity testing used following Bauer method, through the use of a sterile swab of vaccination on a predefined inoculate a plate of Mueller Hinton agar. Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 35 C for 24 h (temperatures over 35 C). After overnight incubation, using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts. Five antibiotics used in the study were: ampicillin 20mg (Amp), Erythromycin 15 mg (Eritrea), Florfenicol 10 mg (Flo), Enofloxacin 10mg (Eno), Neomycin 30mg (Neo). The amount of processed fish for therapeutic course treatment was 2000 fish which kept in a clay bottom pond with size of 100x 10 m, with depth 2 m. The treatments were done

by adding 10% concentration of Enrofloxacin in fish feed directly with ratio with a percentage of 15 mg/kg of cultures fish for five days. The current study included isolating and treating of two species of bacterial, Aeromonas hydrophila with probability (98%) and Staphylococcus lentus (90%), were implicated in the seasonal autumn mortalities of farm-raised Cyprinus carpio, Basrah Governorates, Iraq. Infected fishes were collected, have increased in the mortality rate and suffering from skin necrosis, hemorrhage and acute infections during the autumn season of the year 2019, the swabs were taken from the skin, gills and intestine. Bacterial strains were identified using the VITEK ii system. The sensitive antibiotic test was applied using Muller Hintom agar manufactured India, five antibiotics were used: Ampicillin 20mg (Amp), Erythromycin 15 mg (Eritrea), Florfenicol 10 mg (Flo), Enofloxacin 10mg (Eno) and Neomycin 30mg (Neo). Therapeutic course treatment was used 2000 aquaculture fish, therapeutic course treatment was used in 100 x 10 meters with depth 2 meters' pond using 10% concentration of enrofloxacin therapy, the results indicated that a remarkable decrease in morbidity and mortality, and contributing to important progresses in the health of the fish population leading to a dramatic reduction in morbidity and mortality, and contributing to significant advances in the health of the general population also results of the study suggest that the enrofloxacin therapy could be potential therapeutic options for treating septic wounds caused by above mentioned bacterial. . The results of the study suggest that the both bacterial species are very influential pathogens in aquaculture operations, cause high mortality in cultured ponds. The requires making with correct treatments in a short time, use of antibiotics after allergy testing can lead to very fast solutions and treatments can control these diseases in commitment right help. The uses of enrofloxacin therapy treatment is the first attempts in the world to treat fish diseases, where it is intended for poultry diseases. Antibiotics should not use as an easy alternative by farmers in person without adequate diagnosis of medical condition to fish farming practices. Health organizations in relevant institutions must follow strict programs to using antibiotics to residual products environment of antimicrobials in aquaculture production. Control programs must undergo treatment exchange views to surveillance under the grant or authorization in using antibiotic at the national level is the latest legislation and standards based on sound science.

Keywords. Isolation, treatments, Staphylococcus lentus, Aeromonas hydrophila, autumn mortalities Cyprinus carpio, Iraq.

1. Introduction

Chemotherapy has been applied to bacteria in aquaculture for more than 60 years, changing the antibiotic treatment of infectious diseases, which led to a significant reduction in morbidity and mortality, which was reflected and contributed significantly in progress public health and reduce disease in general [1]. Pharmaceutical research in the field of health and aquaculture focused mainly on some antibiotics widely used in aquaculture [2], which has made consumer protection organizations concerned about excessive use of these types of treatments, especially when misuse or overuse use, which affect directly or indirectly on environmental and public health regulations, and this requires stand firm through the process of legalization of use and bring it under strict control. Antibacterial are among the most-used drugs in veterinary medicine, the uses of antibiotics in fish farms are a lot and often without control by a science specialists control in this field reflected the effectiveness of many antibiotics and lower actual effects. Therefore, the current study was designed to suggest the suitable scientific treatment system through allergy screening for disease or bacterial factor and to the appropriate treatment and the appropriate dose for treatment and long enough to control the disease.

2. Materials and Methods

2.1. fish farm study

The samples were collected from the common carp farms north of Basrah city. A total of 30 live infected fishes was collected, during the autumn season of the year 2019, samples were placed in ice box and

transferred to the laboratory of department marine vertebrate marine science center and to Al Noor vet. lab. Within 3hrs. Fish weights and lengths from l50 -350 gm, and 15-35cm respectively.

2.2. Bacterial Isolation

Bacterial samples were taken from the skin, gills and intestines using a sterile scalpel, samples were homogenized in 9 ml of sterile normal saline solution; one milliliter aliquots of the homogeneous solutions were serially diluted (10-1 to 10-7), aliquots of 0.1 ml of the serial dilutions were inoculated on tryptic soya agar (TSA) (Hi media- India). Nutrient agar (Hi media- India) in duplicate using the spread plate method and the plates were incubated at 37°C for 24-48 h. Then after, Bacteria were identified using the VITEK II system.

2.3. Sensitivity Tests

The bacterial sensitivity testing used following [3] Bauer method, through the use of a sterile swab of vaccination on a predefined inoculate a plate of Mueller Hinton agar. Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 35 C for 24 h (temperatures over 35 C). After overnight incubation, using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts. Five antibiotics used in the study were: ampicillin 20mg (Amp), Erythromycin 15 mg (Eritrea), Florfenicol 10 mg (Flo), Enofloxacin 10mg (Eno), Neomycin 30mg (Neo).

2.4. Therapeutic Course Treatment

The amount of processed fish for therapeutic course treatment was 2000 fish which kept in a clay bottom pond with size of 100x 10 m, with depth 2 m. The treatments were done by adding 10% concentration of Enrofloxacin in fish feed directly with ratio with a percentage of 15 mg/kg of cultures fish for five days

3. Results

3.1. Bacterial isolation

Through the results of the current study, conducted during the autumn season of the year 2019. The result showed Fluctuation in quality standards in aquaculture throughout the sampling sites and an accompanied the sudden change of temperature, and also a decline in the level of scientific knowledge for diagnosis by educators which contributed significantly in the activity and proliferation of pathogens during this time of year. The main change is ammonia construction, and temperature fluctuated from 20.5- 35°C, pH was relatively from 6.5 - 7.5.

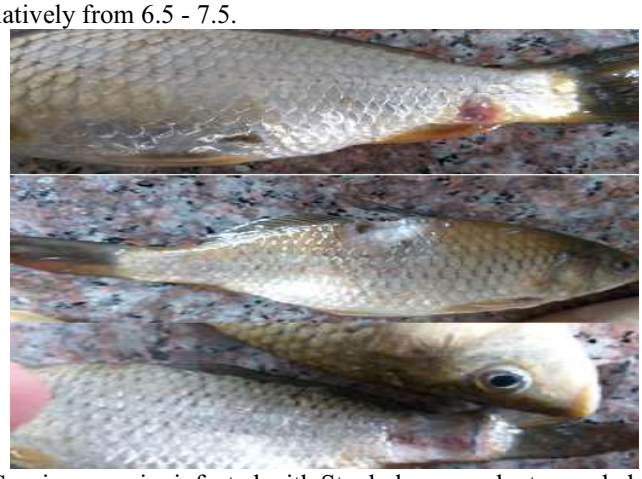

Figure 1. Cyprinus carpio, infected with Staphylococcus lentus probability (90%), Aeromonas hydrophila (98%). Suffering from skin necrosis.

Table 1. Biochemical Details Identification of isolates bacterial species by Vitek II system. report of Aeromonas hydrophila /caviae with probability (98%)

	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 2. Biochemical Details Identification of isolates bacterial species by Vitek II system. report Staphylococcus lentus caviae with probability (90%).

	AMY	-	***4***	**PIPLC**	-	***5***	**dxyL**	-	***8***	**ADH1**	-	***9***	**BGAL**	-	***11***	**AGLU**	+
13	APPA	-	*14*	CDEX	-	15	AspA	-	*16*	BGAR	-	*17*	AMAN	-	*19*	PHOS	-
20	LeuA	-	*23*	ProA	-	24	BGURr	-	*25*	AGAL	-	*26*	PyrA	+	*27*	BGUR	_
28	AIaA	-	*29*	TyrA	-	30	Dsor	-	*31*	URE	-	*32*	POLYB	+	*37*	Dgal	-
38	Drib	+	*39*	ILATk	-	*42*	LAS	-	*44*	NAG	+	*45*	Dmal	+	*46*	BACI	+
47	NOVO	-	*50*	NC6.5	+	*52*	Dman	-	*53*	Dmne	(+)	*54*	MBdG	-	*56*	PUI	_
57	Draf	-	*58*	O129R	+	*59*	SAL	-	*60*	SAC	+	*62*	dTRE	+	*63*	ADH2s	_
64	OPTO	+															_

According to the fish data collection sample that infected, fishes suffering increased in the mortality rate and they were suffering from skin necrosis and hemorrhage and acute infections, skin discoloration, red patches around the base of the fins and mouth and necrotic intestine Fig (1). The bacterial strain dominant are Gram-negative *Staphylococcus lentus* with probability (90%), *Aeromonas hydrophila* with probability (98%). Bacterial strains were identified using the VITEK II system. (table 2, 3).

3.2. Antibiotic Sensitivity of Pathogenic Bacteria.

After overnight incubation. The endpoint of inhibition is where growth starts. Five antibiotics used in the study were: ampicillin 20mg (Amp), Erythromycin 15 mg (Eritrea), Florfenicol 10 mg (Flo), Enofloxacin 10mg (Eno), Neomycin 30mg (Neo). We found that the Enofloxacin it was more effective in inhibiting the growth of both types of bacteria table 1. which encouraged the treatment tests on infected fish.

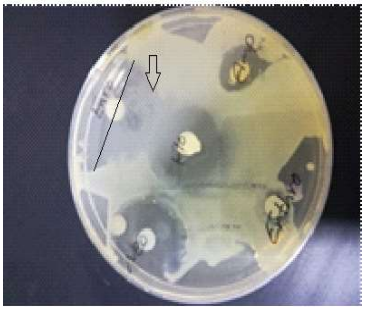

Figure 2. sensitive test of antibiotic ampicillin 20mg (Amp), Erythromycin 15mg (Eritrea), Florfenicol 10 mg (Flo), Enofloxacin 10mg (Eno), Neomycin 30mg (Neo) with Muller Hinton agar.

3.3. Therapeutic Course Treatment

New therapeutic course was used treatment of fish farms, the treatments were done by adding 10% concentration of Enrofloxacin in fish feed, with a percentage of 15 mg/kg of cultures fish for five days 'treatments, after calculating the approximate total weight of fish to determine the amount of treatment used. Through therapeutic course treatment, the first day observed a significant decrease in the mortality rate and amount of the fish losses, whereas it was prior to treatment ranging from 50-70 fish daily from the ranch. On the second day of treatment observed increase in the mortality rate due to a high concentration of ammonia, we stop the feeding and continue treatment with the same concentration and quantity. A sharp decline was observed in the mortality rate of fish farmed. By the fifth day the losses were 2 fish only. We stopped the treatment and began the feeding naturally and without any significant problems. The results indicated that a remarkable decrease in morbidity and mortality, and contributing to important progresses in the health of the fish population leading to a dramatic reduction in morbidity and mortality, and contributing to significant advances in the health of the general population. The results of the study suggest that the enrofloxacin therapy could be potential therapeutic options for treating septic wounds caused by above mentioned bacterial. The uses of enrofloxacin therapy treatment are the first attempts in the world to treat fish diseases, where it is intended for poultry diseases.

Table 3. Antibiotics sensitive test of (A) Aeromonas hydrophila and (S) Staphylococcus lentus to antimicrobial agents. type of resistance. inhibition zone(mm).

Type of antibiotics	Concentration (mg/g) (A)	Type of resistance (A)	Diameter of inhibition zone(A)	Concentration (mg/g) (S)	Type of resistance (S)	Diameter of inhibition zone (S)
Ampicillin	10	R	12 mm	1	s	14mm
Erythromycin	15	R	14mm	15	R	14mm
Florfenicol	10	S	13mm	10	S	18mm
Enrofloxacin	10	S	20 mm	10	S	21mm
Neomycin	30	R	13mm	30	R	13mm

The results also showed the presence of disease-causing species in almost all fish farms in the area and with the same symptoms. We are considering that the study is the first in an extensive about diagnosis, allergy testing and application of new types and description of treatments and track results directly with cultured for best results. The study also recommends that the fish farmers must be worried about the existence of these diseases causing bacteria which could also contribute to public health risk and should therefore be to adopt best management practices for aquaculture is responsible for ensuring the quality of the fish and also biosecurity.

4. Discussions

Different types of bacteria were isolated, these bacteria known to cause serious disease in fish and those of interest as diverse as fish pathogens. Aeromonas hydrophila is common pathogens known to cause serious mortalities in fish among the symptoms observed a skin ulcer, protruded, bleeding, ascites and rotting fins very sick from eating all fish skin bleeding especially carp of farmed fish as explained [4].

The Enrofloxacin, Florfenicol and ceftiofur have been widely used to treat a broad array of infectious diseases, it has a capability to change the proportions among lymphocyte subset in lymphatic organs and thus might have an impact on the immune response to bacterial endotoxins in chicks two [5] , This has been proven through testing, particularly sensitive and therefore the limited concerns of global use have been tested for the first time in Iraq as an antibiotic for use in fish farms and gave full healing rate and reduce the incidence of mortality to zero within a very short period of treatment. Increasing concentrations of 0.5 to 8 mg/ml of enrofloxacin associated with increased activity, in vivo, this researcher explained on infected poultry of salmonids were effective treatment for one day, much less effective treatments for 2 to 5 days, Enrofloxacin added to drinking water each day for 5 to 10 days-day

treatments showed similar efficacy to those used in treatment for 5 days for this group of antibiotics [6]. Despite of the widespread use of bacterial antibiotics in the world and that is what many researchers referred to specific types and quantities of antibiotics used [7] [8], As well as, most of them it is probable that the majority of antibacterial is not associated disease agent's factors of the target bacterium [9], also it is necessary that there is also a need for assurance that the usage will not harm animals or humans [10].

Level of virulent of gram negative annexing land that rely on fish species, doses of infections, exposure time and age of host species and pathogens of bacterial strains [11] [12] [13].

Aeromonas hydrophila and Staphylococcus lentus are a widespread species in the aquatic environment, isolate from different aquatic environments, and it is a responsible for bacterial disease of Hemorrhagic Septicemia in freshwater fish, in addition to other species, Furthermore the main reason for its spread is not following proper farming systems and food quality and lack of oxygen and intensive farming play a key in the spread of this type of bacterial

The presence of thesis bacteria as a real indicator of severe water contamination and this underlines the presence of high numbers of fish mortalities domain as accompanies this shortage pollution biomarkers of fish including dissolved oxygen and this has been confirmed by many international studies which the most important"[15] [16]. The results of the study suggest that the both bacterial species are very influential pathogens in aquaculture operations, cause high mortality in cultured ponds. The requires making with correct treatments in a short time, use of antibiotics after allergy testing can lead to very fast solutions and treatments can control these diseases in commitment right help.

5. Conclusion

1. Antibiotics should not use as an easy alternative by farmers in person without adequate diagnosis of medical condition to fish farming practices.
2. Health organizations in relevant institutions must follow strict programs to using antibiotics to residual products environment of antimicrobials in aquaculture production.
3. Control programs must undergo treatment exchange views to surveillance under the grant or authorization in using antibiotic at the national level is the latest legislation and standards based on sound science.

6. Acknowledgements

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