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A First Record of *Proteus hauseri* and *Pseudomonas putida* Isolated from Common Carp (*Cyprinus carpio*) Cultivated in Floating Cages at Al-Hilla River, Babylon Province, Iraq: Antibiotic Resistance

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Abstract: This study was carried out to investigate the occurrence of potentially pathogenic species of Gram-nagative bacteria in common carp (*Cyprinus carpio*) cultivated in floating cages at Al-Hilla river. A total of 144 fishes was collected from fish farms during the period from December 2017 till November 2018. Bacteria were identified using the VITEK 2 system. Here, we reported the first record in Iraq of *Proteus hauseri* and *Pseudomonas putida*, which were isolated from skin, gills and intestine of common carp. *P. hauseri* and *P. putida* were identified with a different percentage in common carp as the following: *P. hauseri* (0.69-10.09%) and *P. putida* (0.69-2.88%). Antibiotic susceptibility (Ampicillin, Piperacillin/ Tazobactan, Cefazolin, Cefoxitin, Ceftazidime Ceftriaxone, Cefepime, Ertapenem, Imipenem, Amikacin, Gentamicin, Nitrofurantion, Ciprofloxacin, Levofloxacin, Tigecycline and Trimethoprim/ Sulfamethoxazole) was by using the VITEK 2 system. The results indicated that the bacteria were sensitive to minimum inhibitory concentration (MIC) of levofloxacin (0.25 µg/ ml) to *P. hauseri* and Imipenem and Ciprofloxacin (<= 0. 25) to *P. putida*, but resistant to cefazolin (>= 64 µg/ ml).

Keywords: *Cyprinus carpio*, *Proteus hauseri*, *Pseudomonas putida*, Antibiotics, Iraq.

Introduction

Fish diseases are one of the major problems in fish farms (Al-Niaeem, 2006; Mhaisen et al., 2010, 2012; Al-Niaeem et al., 2015; Al-Jubouri et al., 2017; AlYahya et al., 2018). A variety of environmental and biological factors, husbandry and management practices may influence the abundance and impacts of diseases in farmed fish (Bagum et al., 2013). Information on impacts of the economic losses may help to develop management strategies against diseases in fish farms and to reduce the magnitude of the parasitism (Tavares-Dias & Martins, 2017).

Proteus bacteria inhabit the environment and the *Proteus* species are widely distributed in the natural environment. They can be found in polluted waters and they are also present in the intestine of humans and animals as opportunistic pathogens (Mordi & Momoh, 2009; Ahmed, 2015). These microorganisms under favorable conditions cause a number of infections. *Proteus* opportunistic pathogens express virulence factors associated with adherence, motility, immune avoidance, nutrient acquisition, host damage and endotoxicity (Drzewiecka, 2016). *Proteus* is Gram-negative, facultative anaerobic and rod shaped bacteria. Its swarming motility and urease activity do not usually ferment lactose (Mordi & Momoh, 2009). Since it belongs to the family Enterobacteriaceae, general behaviors are applied on this genus: It is actively motile, non-spore forming, non-capsulated oxidase-negative, but catalase and nitrate positive (Elsherief et al., 2014; Drzewiecka, 2016).

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Pseudomonas putida is a rod-shaped, flagellated and Gram-negative bacterium that is found in most soil and water habitats where there is oxygen. It grows optimally at 25-30° C and can be easily isolated (Lombardi et al., 2002). *P. putida* is significant to the environment due to its complex metabolism and ability to control pollution. There is a high versatility of bacterial communities towards contaminations which are further increased by certain catabolic sequences on the TOL plasmids in the cell (Haffaressas et al., 2018). Even the plasmids are important in sensing the environmental stress, some of the environmental stresses are caused by benzene, xylene, and toluene, the main components of gasoline and are major sources of water contamination. *P. putida* can degrade the hydrocarbons of these organic solvents through oxidative reactions therefore placing *P. putida* as one of the most important microbes in bioremediation (Otenio et al., 2005).

Due to the little information pertains to *P. hauseri* and *P. putida* infection in the fish farms particularly in Iraq, the aim of the present study is to identify the prevalence and antibiotic resistance in common carp, *C. carpio* cultivated in floating cages in Al-Hilla river and the susceptibility pattern of bacteria to 16 antimicrobial drugs.

Materials and Methods

Al-Hilla river is a part of Euphrates that irrigate very large extent of agricultural areas and used also for human and industrial uses. The length of this river is 101 km (in Babylon province) with unstable water level. A total of 144 fishes of common carp from four farms of floating cages at Al-Hilla were collected. The first farm, St 1 (44˚16' 32.16" E 32˚42' 42.61" N) and second farm, St 2 (44˚24' 9.55" E 32˚33' 43.32" N) are situated before the city center (44˚27' 47.75" E 32˚26' 62.59" N), while the third farm, St 3 and the fourth farm, St 4 (44˚40' 50.4" E 32˚23' 2.84" N) are situated after the city center (Figure 1) where an increase in household waste occurred. The sampling was done during the period from December 2017 till November 2018. The fish length ranged between 25-40 cm and the weight was 303-1006 g. The live fishes were transported to oxygenated pond water before their transferring to the laboratory in the College of Veterinary Medicine, University of Al-Qasim Green.

The collected fishes were dissected and bacteria were taken aseptically, by using a sterile loop, from skin, gills 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 (Ampicillin, Piperacillin/ Tazobactan, Cefazolin, Cefoxitin, Ceftazidime Ceftriaxone, Cefepime, Ertapenem Imipenem, Amikacin, Gentamicin Nitrofurantion, Ciprofloxacin, Levofloxacin, Tigecycline, and Trimethoprim/ Sulfamethoxazole) by using the Vitek 2 system. The bacteria were re-cultured on MacConkye agar, placed in the incubator for 24 h. and sent for examination in the Vitek 2 (Tables 1 & 2).

Table 1: Biochemical features of isolated *P. hauseri*.

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	-			

Table 2: Biochemical features of isolated *P. putida*.

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	-			

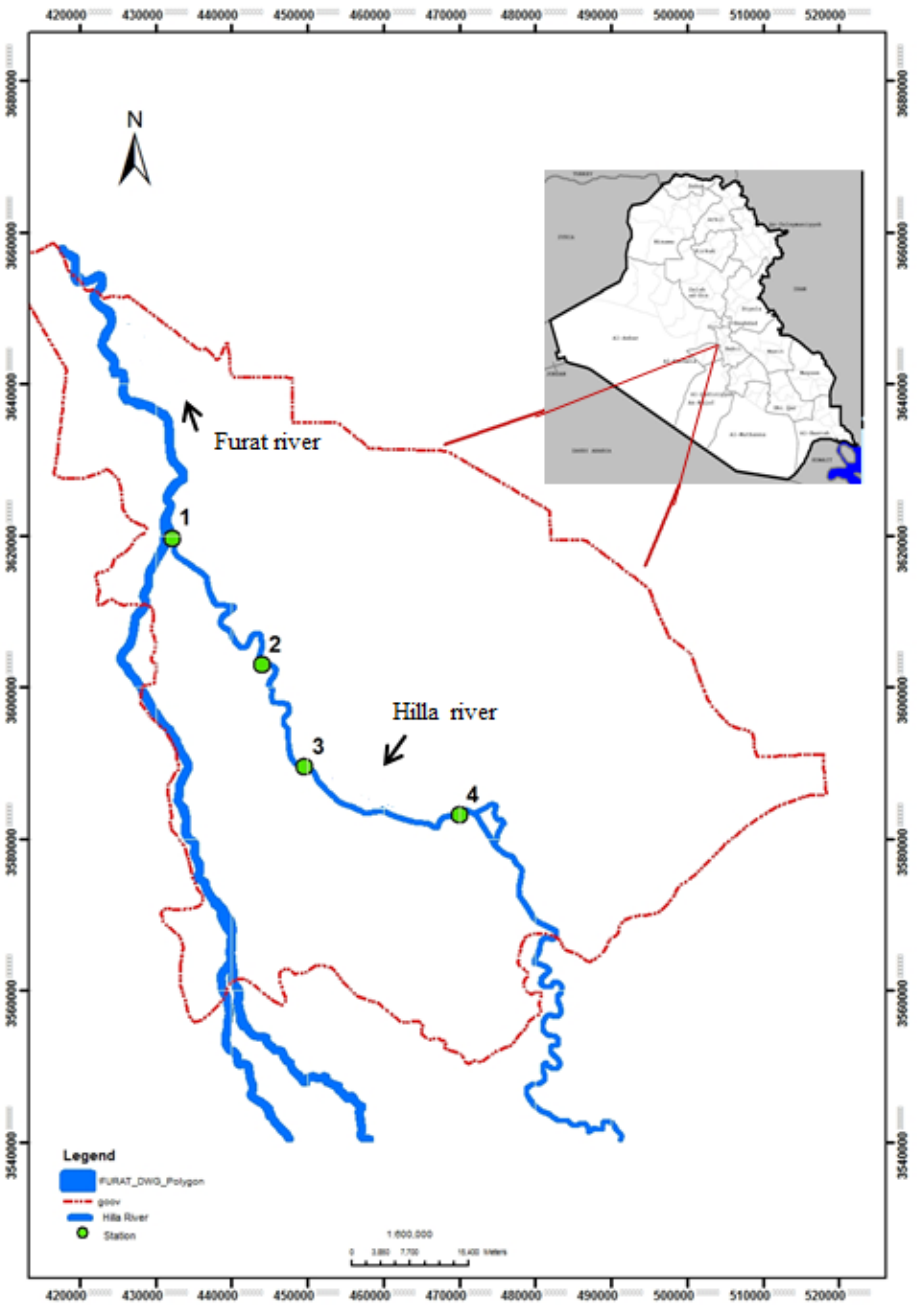

Figure 1: Map of field sampling stations in Al-Hilla river.

Results and Discussion

The study was conducted during December 2017-November 2018 in which there is a fluctuation in water quality parameters in the aquaculture throughout the sampling sites (Table 3). The mean of temperature fluctuated from 10.7 °C to 32.9 °C. Meanwhile, the mean of salinity was recorded to range from 460 to 580 ppt. The pH was fluctuated relatively from 6.4 to 8.1.

Fishes in a culture system always exposed to a variety of stressors which including high stocking density, handling, transportation and poor water quality. On the other hand, fish immunity is reduced during a stressful event which causes the fishes to become susceptible to disease infection (Rijnsdorp et al., 2009; Albert & Ransangan, 2013).

The growth of bacteria in water is increased by high levels of organic matters, high salinity, high water temperature of 25 °C to 32°C and pH of 5-9. Disposal of sewage wastes into a

large volume of water could increase the biological oxygen demands to such a high level that all the available oxygen may be removed (Kiriratnikom et al., 2000). These favorable conditions for bacteria were also observed in the present study.

Table 3: The water parameters of studied stations.

Stations (St)	Temperature (°C)	Salinity (ppt)	Ammonia (mg/ l)	pH
	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)

P. hauseri and *P. putida* were isolated from the skin, gills and intestine of common carp in all stations, except the first station. The clinical signs of affected fishes are haemorrhages on the body along with red patches over the operculum and lower abdomen. These bacteria were identified with a different percentage in common carp (Figures 3 & 4). The species of bacteria were different between farms and this may be related to pollution of the aquatic environment due to the heavy release of agricultural and sewage wastes (Koshy & Nayar, 1999).

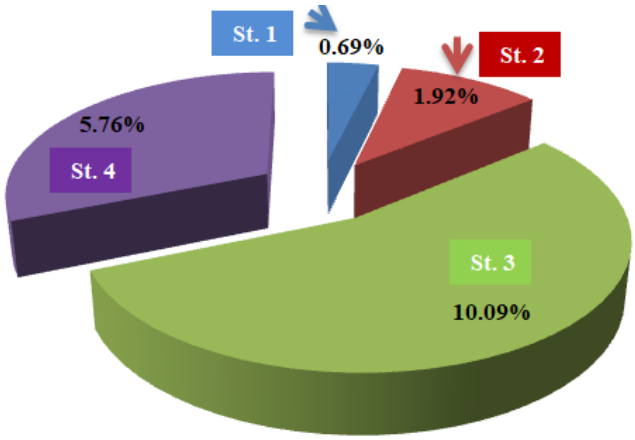

Figure 2: Percentage of isolated *Proteus hauseri* from the skin, gills and intestine of *C. carpio*.

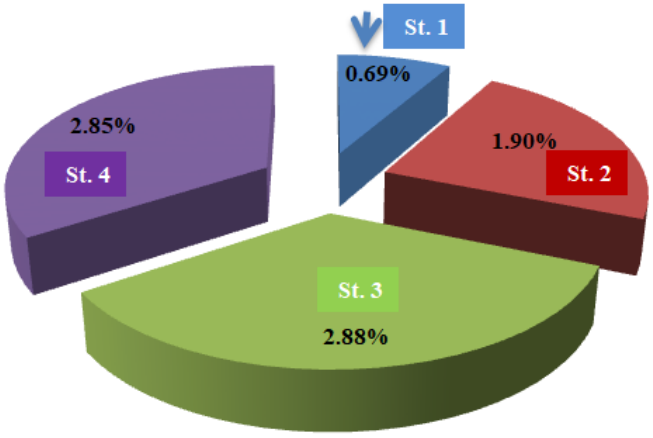

Figure 3: Percentage of isolated *Pseudomonas putida* from the skin, gills and intestine of *C. carpio*.

P. hauseri recorded a higher proportion of other bacteria in all stations. This shows their widespread in the aquatic environment. These bacteria are widely distributed in freshwater environments with organic load (Kumar et al., 2015; Zhang et al., 2016). *P. hauseri* and *P. putida* in the present study have been detected among common carp, although the managers of the farms stated that they use Oxytetracycline (1%) in fish farm. Oxytetracycline is widely used to treat bacterial infections in aquaculture farms (Reed et al., 2006). High level of resistance of bacteria to Oxytetracycline as seen in this report, may be linked to the abuse of this antibiotic in aquaculture. This pattern has been reported in India (Singh et al., 2009) and Chile (Claudio & Raul, 2002). As much as possible, abuse of antibiotics and non-adherence to withdrawal periods should be discouraged as these practices have public health and economic implications.

Antibiotics are normally used to prevent or treat disease outbreaks in fish farming. However, extensive use of antibiotics in fish farming can cause the development of antibiotic-resistant pathogens which can infect both cultured animals as well as humans (DiMasi et al., 2016; Bingyun & Thomas, 2018). High allergy of drug examination in various organisms is a feasible technique that can clearly decrease antibiotic dosage.

At present, the most widely used method of controlling *P. hauseri* and *P. putida* in cultured fishes is the use of antimicrobial drugs. The widespread antibiotic use is associated with an increased antibiotic resistance in aquatic bacteria. Thus, antimicrobial susceptibility tests are important for an effective treatment (Skwor et al., 2014). Screening drugs with a high sensitivity in multiple organisms is a practical method that can sharply reduce antibiotic doses.

In this study, we found that the *P. hauseri* was intensely susceptible to Levofloxacin (0.25 μg/ ml) and *P. putida* was intensely susceptible to Imipenem and Ciprofloxacin (<= 0.25) as indicated in Tables 4 & 6. So, it was suggested that the two drugs may be suitable to prevent *P. hauseri* and *P. putida*. However, the consistent use of antibiotics is associated with a certain risk. On the one hand, the drugs hardly cross the biological barriers to kill bacterial pathogens in tissues, especially the blood-brain barrier (El-Bouhy et al., 2011; Li et al., 2019). On the other hand, the long-term use of antibiotics causes bacterial resistance, drug residues in fishes and environmental safety problems.

Table 4: Antibiotic susceptibility *Proteus hauseri.*

Antimicrobial	MIC*	Interpretation
Ampicillin	>= 32	R
Piperacillin/ Tazobactan	<= 4	S
Cefazolin	>= 64	R
Cefoxitin	<= 4	S
Ceftazidime	<= 1	S
Ceftriaxone	<= 1	S
Cefepime	<= 1	S
Ertapenem	<= 0. 5	S
Imipenem	4	R
Amikacin	<= 2	S
Gentamicin	<= 1	S
Nitrofurantion	128	R
Ciprofloxacin	<= 0. 25	S
Levofloxacin	0. 25	S
Tigecycline	4	R
Trimethoprim/Sulfamethoxazole	<= 20	S

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

Table 5: Antibiotic susceptibility *Pseudomonas putida*.

Antimicrobial	MIC*	Interpretation
Ampicillin	-	-
Piperacillin/ Tazobactan	8	S
Cefazolin	>= 64	R
Cefoxitin	-	-
Ceftazidime	4	S
Ceftriaxone	8	S
Cefepime	2	S
Ertapenem	-	-
Imipenem	<= 0. 25	S
Amikacin	<= 2	S
Gentamicin	<= 1	S
Nitrofurantion	-	-
Ciprofloxacin	<= 0. 25	S
Levofloxacin	1	S
Tigecycline	2	S
Trimethoprim/Sulfamethoxazole	80	R

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

Conclusions

Keeping the health of fishes depends on the relationship between fishes, the environment and pathogens. The results of the present study demonstrated the presence of pathogenic *P. hauseri* and *P. putida* in floating cages at Al-Hilla river. So, the farm owners should be concerned about the presence of these pathogenic bacteria which also contribute to human health risk and should adopt best management practices for responsible aquaculture to ensure the quality of fishes. On the other hand, the long-term use of antibiotics causes bacterial resistance, drug residues in fishes and environmental safety problems.

Collecting and analyzing data on fish disease outbreaks remains a major tool in implementing measures to be put in place to avoid epizootics that have socio-economic consequences. Knowledge of prevalence of specific bacterial disease conditions in a region within the country is very important to quarantine officers as this will guide them to take measures when planning to export live aquatic animals from such regions.

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