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A FIRST RECORD OF *FACKLAMIA HOMINIS* ISOLATED FROM COMMON CARP (*CYPRINUS CARPIO*) CULTIVATED IN FLOATING CAGES AT AL-HILLA RIVER, BABYLON PROVINCE: ANTIBIOTIC RESISTANT AND SUSCEPTIBILITY OF PLANT SPECIES

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Abstract

This study was carried out to investigate the occurrence of potentially pathogenic species of Gram-positive bacterium in common carp cultivated in floating cages at Al-Hilla river. A total of 144 fishes were collected from fish farms during the period December 2017- November 2018. Bacteria were identified using the VITEK 2 system and selected biochemical tests. Here we report the first fish's case of gills due to *F. hominis*, found in common carp cultivated in floating cages at Al-Hilla river. Antibiotic susceptibility (17 antimicrobial) was using the VITEK 2 system. Significant sensitive was produced only by Ciprofloxacin, Ertapenem and Levofloxacin, when tested *in vitro*. The plant species susceptibility test was conducted using disc diffusion method on blood agar. Procedure was based on the standardized disc agar diffusion method for antimicrobial susceptibility tests. After incubation, the diameter of the zone of inhibition was measured to determine the sensitivity of the six plant species extracted with alcohol in four different concentrations (10, 15, 20 and 25%). The potential of plant extracts to inhibit the infection caused by *F. hominis*, all plants inhibited the growth of *F. hominis* (pd" 0.01) in the highest concentration (25%), ginseng (*P. ginseng*) appeared to be the most effective, whereas olive leaves (*O. europaea*) was the least effective. The farm owners should be concerned about the presence of these pathogenic bacteria which also contributes to human health risk and should adopt best management practices for responsible aquaculture to ensure the quality of fish.

Key words: *Facklamia hominis*., common carp, Babylon Province

Introduction

Iraq have many water resources with (area of 1074000 hectare), such as Euphrates and Dijlah Rivers with their tributaries, lakes, swamps, ponds and all these resources can be used for fish culture in different ways (Mhaisen, 1987). Fish diseases are one of the major problems in fish farms (Al-Niaeem, 2006; Al-Salim *et al.,* 2009a; Al-Salim *et al.,* 2009b; Mhaisen *et al.,* 2010; Mhaisen *et al.,* 2012; Al-Niaeem *et al.,* 2015; Al-Jubouri *et al.,* 2017 and AlYahya *et al.,* 2018). The Gram-positive, catalase-negative many agents which are pathogenic to man and other animals (Facklam and Elliot, 1995). The economic losses due to bacterial diseases are significant in aquaculture. Intensive stock conditions and seasonal changes in water quality result in stress that allows frequent infection by opportunist pathogens in fish ponds (Austin and Austin, 2007).

The genus *Facklamia* was first described in 1997 using 16S rRNA sequencing and has since been identified from both a wide range of animal sources and infrequently as a human pathogen (Collins *et al.,* 1997 and Takamatsu *et al.,* 2006). Bacteremia has been associated with endocarditis, necrotizing gangrene, chorioamnionitis, and central nervous system disease (Pérez Alonso *et al.,* 2012 and McCann *et al.,* 2014). Although *Facklamia* spp. have been implicated in invasive infections, the virulence of this organism has not been fully determined, because co-occurrence with other bacteria (LaClaire, 2000).

This study aims to isolate and identify *F. hominis* bacteria from common carp cultivated in floating cages at Al-Hilla river, Babylon Province and the susceptibility pattern of bacterial isolates to 17 antimicrobial drugs and

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susceptibility of six plant species.

Materials and Methods

Al-Hilla river is a part of Euphrates that irrigate very large distances of agricultural areas and used also for human and industrial uses. The long of this river is 101 km (in Babylon Province) with unstable water level. This study involved the diagnosis of pathogenic species of Gram-positive bacterium in common carp cultivated in four farms of floating cages at Al-Hilla river during the period December 2017- November 2018. The first and second stations, before the city center and the third and fourth stations, after the city center.

The fishes were collected from four farms. The live fishes were transported in oxygenated pond water filled plastic bags and put into another plastic bag filled with ice flakes. Samples were transported to the Lab. in College of Veterinary Medicine, University of Al-Qasim Green and processed immediately for examination. Bacteria were identified using the VITEK 2 system and selected biochemical tests (Table 1).

Antibiotic susceptibility (17 antimicrobial) was using the VITEK 2 system. The plant species susceptibility test was conducted using disc diffusion method on blood agar at 37°C for 24 h. Procedure was based on the standardized disc agar diffusion method of the National Committee for Clinical Laboratory Standards for antimicrobial susceptibility tests (Finegold and Martin, 1982). After incubation, the diameter of the zone of inhibition was measured to determine the sensitivity of the six plant species extracted with alcohol in four different concentrations (10, 15, 20 and 25%).

The plant species were from collected from Iraqi local markets, powdered and extracted with ultrapure alcohol, Olive leaves (*Olea europaea*), Cinnamon (*Cinnamomum zeilanicum*), Clove (*Eugenia caryophyllata*), Turmeric (*Curcuma longa*), Ginseng (*Panax ginseng*) and Thyme leaves (*Thymus vulgaris*).

The statistical calculations of the results were completed using SPSS version 20, one way (ANOVA) to determine the difference.

Results and Discussion

F. hominis is a gram-positive facultative an aerobic coccus that was initially isolated from urine, blood and abscesses in humans (Collins *et al.,* 1997). Here we report the first fish's case of gills due to *F. hominis*, found in common carp cultivated in floating cages at Al-Hilla river in third station (after the city center, increase in household waste).

The study was conducted during the December 2017-November 2018 in which there is a fluctuation in water quality parameters in the aquaculture throughout the studied stations (Table 2). 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 relatively from 6.4 to 8.1.

The fish 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 fish to become susceptible to disease infection (Rijnsdorp *et al.,* 2009 and Albert and Ransangan, 2013).

The growth of bacteria in water is increased by high levels of organic matters, high salinity, high water temperature 25 °C to 32°C and pH 5-9 (Kiriratnikom *et al.,* 2000). These favorable conditions for bacteria were also observed in the present study.

Infected cases 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), and this is may be related to that,

Table 1: Biochemical profile of *F. hominis.*

Biochemical Details								
-	dXYL	5	-	PIPLC	4	+	AMY	2
-	AGLU	11	+	BGAL	9	+	ADH1	8
-	AspA	15	-	CDEX	14	+	APPA	13
-	PHOS	19	-	AMAN	17	-	BGAR	16
-	BGURr	24	+	ProA	23	-	LeuA	20
-	BGUR	27	+	PyrA	26	-	AGAL	25
-	dSOR	30	-	TyrA	29	-	AlaA	28
-	dGAL	37	-	POLYB	32	-	URE	31
-	LAC	42	-	lLATk	39	-	DRlB	38
-	BACl	46	-	dAML	45	-	NAG	44
+	dMAN	52	-	NC6.5	50	-	NOVO	47
-	PUL	56	-	MBdG	54	-	Dmne	53
-	SAL	59	-	O129R	58	-	Draf	57
-	ADH2$_s$	63	-	Dtre	62	-	SAC	60
						-	OPTO	64

Positive, -: Negative

Table 2: The water parameters of studied stations.

Station	Temp (°C)	Salinity (ppt)	pH
	Range (mean)		
1st station	11.9 - 32.7 (22.66)	480 - 560 (517.5)	6.8 - 8.1 (7.36)
2nd station	10.7 - 32.9 (22.9)	460 - 570 (516.66)	6.5 - 8.1 (7.33)
3rd station	13 - 32.6 (23.8)	480 - 580 (530.8)	6.4 - 7.6 (7.09)
4th station	12.5 - 31.2 (22.86)	460 - 560 (513.33)	7.1 - 7.9 (7.35)

the extensive use of antibiotics can cause the development of antibiotic-resistant pathogens which can infect both cultured animals (DiMasi *et al.,* 2016; Bingyun and Thomas, 2018).

On the basis of the 17 antibiotics tested, it can be stated that the majority is entirely ineffective against *F. hominis*. The scale of antibiotic sensitivity is arbitrary, since the smallest inhibition zone was taken into consideration (Table 3). According to that scale, significant inhibition was produced only by Cefazolin, Trimethoprim/ Sulfamethoxazole and Ampicillin/ Sulbactan. The 17 antibiotics examined are not suitable for therapeutic treatment (particularly as there are cheaper and more effective agents available) (Woo and Bruno, 1999).

An increase in bacterial resistance to antibiotics and the lack of new antibiotics introduced intothe market resulted in a need to find alternative strategies so as to cope with infections resulting from drug-resistant bacteria (Bajera *et al.,* 2016). Several bioassays such as well diffusion, disk-diffusion, and broth or agar dilution are well known and commonly used methods (Balouiri *et al.,* 2016).

The potential of plant extracts to inhibit the infection caused by *F. hominis*, all plants inhibited the growth of *F. hominis* ($p \leq 0.01$) in the highest concentration (25%), ginseng (*P. ginseng*) appeared to be the most effective, whereas olive leaves (*O. europaea*) was the least effective (Fig. 1). This study suggests that widely available and inexpensive plant extracts might be effective natural agents to prevent the *F. hominis* infection in fish. More research is needed in order to ascertain the efficiency of the plant extracts in vivo as well as to find a proper practice used in aquaculture.

Antibacterial and properties of different plants have been used in Chinese medicine for a long time. Recently, many studies have been published in order to study the actual functional mechanisms as well as the exact chemical composition of the plants. Herbal compounds, such as phenolics, polyphenols, alkaloids, quinones, terpenoids, lectines and polypeptides, have been shown to be effective alternatives to synthetic compounds against pathogens (Citarasu, 2010).

A variety of bacterial species have been inhibited by the essential oil constituents extracted from the *Cymbopogan citratus* and *Satureja montana*. The most appropriate method for determining the bactericidal effect as well as a strong tool for obtaining information about the dynamic interaction between the anti-microbial agent and the microbial strain is the time-kill test, also a time-dependent or a concentration-dependent antimicrobial effect is revealed by the time-kill test (Costa *et al.,* 2015 and Sonam *et al.,* 2017).

Factors determining the activity of essential oils are composition, functional groups present in active components, and their synergistic interactions (Dorman *et al.,* 2000). The antimicrobial mechanism of action varies with the type strain of the microorganism used. It is well known that in comparison to Gram-negative bacteria, Gram-positive bacteria are more susceptible to eosinophils (Azhdarzadeh and Hojjati, 2016).

Herbal extracts and animal originated product have a potential application as an immunostimulant in fish culture, primarily because they can be easily obtained, are not expensive and act against a broad spectrum of pathogens. However, the effect is dose-dependent, and

Table 3: Susceptibility (zone of inhibition in mm) of *Facklamia hominis* antibiotics.

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin/ Sulbactan	>=32	R	Meropenem	8*	R
Piperacillin/ Tazobactan	8	S	Amikacin	<=2	S
Cefazolin	>=64	R	Gentamicin	<=1	S
Ceftazidime	<=1	S	Tobramycin	<=1	S
Cefriaxone	<=1	S	Ciprofloxacin	<=0.25	S
Cefepime	<=1	S	Levofloxacin	0.5	S
Aztreonam	<=1	S	Tigecycline	1	S
Ertapenem	<=0.5	S	Trimethoprim/Sulfamethoxazole	40	S
Imipenem	2	I			

MIC*: Minimum Inhibitory Concentration* (ig/ml), S*: Sensitive, R: Resistant, I: Intermediate.* Ciprofloxacin, Ertapenem and Levofloxacin appear sensitive when tested *in vitro*.

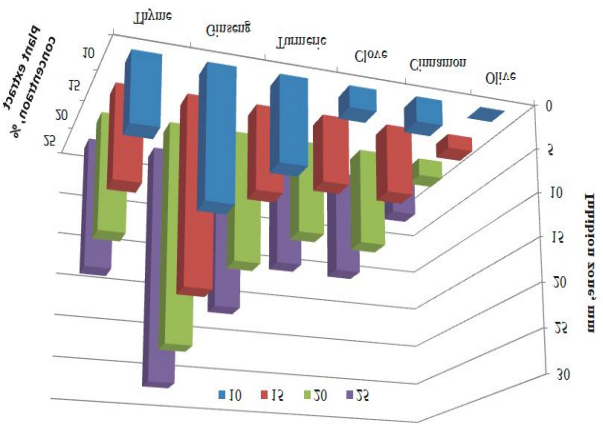

Fig. 1: Susceptibility (zone of inhibition in mm) of the six plant species extracted with alcohol in four different concentrations on the growth of *Facklamia hominis*.

there is always a potential for overdosing consequently, dosage optimization is strongly recommended. The use of such plant products as immunostimulants in fish culture systems may also be of environmental value because of their biodegradability. Due to their beneficiary attributes, we conclude that herbal extracts and animal originated product can be used in fish culture as alternatives to vaccines, antibiotics or chemotherapeutic agents.

Conclusion

Keeping the health of fish depends on the relationship between fish, the environment and pathogens. The results of the present study demonstrated the presence of pathogenic *F. hominis* nearly in all fish farms. So the farm owners should be concerned about the presence of these pathogenic bacteria which also contributes to human health risk and should adopt best management practices for responsible aquaculture to ensure the quality of fish.

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