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Isolation of Bacteria *Sphingobacterium Thalpophilum* and *Streptococcus Thoraltensis* from *Oreeochromis Aureus* (Steindachner, 1864)) and Antibiotic Susceptibility in Al-Diwaniyah River / Middle Iraq

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Abstract: This study was carried out to investigate the occurrence of potentially pathogenic species of bacterium in *O. aureus* in Al-Diwaniya River. A total of 100 fishes of *O. aureus*, Fish during the period April 2021 till March 2022. Bacteria were identified using the VITEK 2 system and selected biochemical tests. species were identified in a *O. aureus* which are S. thoraltensis, S. thalpophilum. Antibiotic susceptibility test of 16 antibiotics, which are (Ampicillin, piperacillin/ tazobactan, amikacin, cefazolin, Ceftriaxone, Ertapenem, Gentamicin, cefoxitin, ciprofloxacin, Imipenem, ceftazidime, levofloxacin, cefepime, tigecycline, Nitrofurantion, and trimethoprim/ sulfamethoxazoleby) were identified and examined for antibiotic susceptibility using the Vitek II system. The results indicate that the bacteria sensitive to Minimum Inhibitory antibiotics susceptibility were Ciprofloxacin(<= 0. 23, <= 0. 25 µg/ ml), on S. *thoraltensis*, and S. *thalpophilum*. Imipenem and Levofloxacin (<= 0. 20, <= 0. 25 µg/ ml), when tested in vitro on *S. thoraltensis, S. thalpophilum*, while it was resistant to Cefoxitin(<= 66, <= 70 µg/ ml) on *S. thoraltensis*, and *S. thalpophilum*.

Keywords: *Oreeochromis aureus*, Fish bacteria, Antibiotic susceptibility, Iraq.

Introduction

Aquaculture, especially fish, is one of the fastest growing projects in the world. Global fish production has witnessed a significant increase during the last five decades, and aquaculture is an increasingly important option in the production of animal protein (FAO, 2018). *O. aureus* (Steindachner, 1864) belong to the Cichlidae family of 1524 species (Eli, 2004). spread in southern Morocco, Turkey, Egypt, Jordan, America and the Philippines, (Khalifa., *et al* 2020).the O. aureus was recorded in Iraqi waters for the first time in the southern part of the general estuary at the city of Basra (Mutlak and Al-Faisal, 2009).

One of the main risks to the sustainability of fish aquaculture globally is the proper control and maintenance of infectious diseases (Rodger, 2016; Terzi, 2018).

Despite being somewhat hardy Tilapia fish (Romana-Eguia et al., 2020), tilapias are still susceptible to diseases such as bacterial, parasitic, fungal, and viral diseases (Amal & Zamri-Saad, 2011; Romana-Eguia et al., 2020). Even with antibacterial properties (Sajorne & Mabuhay-Omar, 2020). The stressors are poor water quality on the farm environment, increased intensification, and insufficient proper sanitary maintenance (Romana-Eguia et al., 2020).

Important bacterial diseases that have been globally reported and that have a negative impact on tilapia aquaculture are streptococcus, transmissible septicemia, vibriobiosis, staphylococcus, pseudomonas, franciselle, spine disease, Edward's disease, onychomycosis, and others. Pathogens have been identified in tilapia as S. iniae, S. agalactiae, Edwardsiella

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tarda and Pseudomonas spp. and Francisella noatunensis, Nocardia seriolae, and Aeromonas spp. and Flavobacterium spp. (Rodger, 2016; Romana-Eguia et al., 2020; Kayansamruaj et al., 2020). Bacteria are the main responsible for diseases in farmed fish and cause great economic losses, and that the real role of bacteria in causing such diseases may vary or vary from a primary cause to a secondary cause, as some of these bacteria live in a throw-away, but when the fish become weak, they cause injury and cause a condition certain satisfactory (Aravenaromán *et al*., 2014). Water, especially water containing a high percentage of organic matter, is a good medium for the growth of many types of bacteria, and many researchers consider the bacteria that naturally occur on fish to be a reflection of the bacteria present in the water in general (Aravenaromán *et al*., 2014; Hassan *et al* 2017 and Jauneikaite *et al*., 2018).

Materials and Methods

A total of 100 live O. aureus fish was collected from the AL-Diwaniya River between Daghghara barrier through Sinniyah district to the AL-Diwaniya city, The fish length ranged between 10-25 cm and the weight was 40-450 g. It is AL-Diwaniya River 123 km long, 25-30m wide and 3-5m depth (Fig. 1). Fish sampling was using seine net , gill nets , cast net. The sampling was done during the period from April 2021 till March 2022. 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).

Figure. 1. Map of field sampling sites in Al-Diwaniya River

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Table 1: Biochemical Details of isolated *Streptococcus thoraltensis.*

Biochemical Details		Reaction	Biochemical Details		Reaction
2	AMY	–	5	dXYL	–
4	PIPLC	–	8	ADHl	+
13	APPA	–	15	AspA	–
14	CDEX	–	16	BGAR	–
20	LeuA	+	24	BGURr	–
23	ProA	–	25	AGAL	+
28	AlaA	+	30	dSOR	–
29	TyrA	+	31	URE	–
38	dRIB	+	42	LAC	–
39	lLATk	+	44	NAG	+
47	NOVO	+	52	dMAN	+
50	NC6.5	–	53	dMNE	+
57	dRAF	+	59	SAL	–
58	O129R	–	60	SAC	+
9	BGAL	+	17	AMAN	–
11	AGLU	+	19	PHOS	–
26	PyrA	–	32	POLYB	–
27	BGUR	–	37	dGAL	+
45	dMAL	+	54	MBdG	+
46	BACl	+	56	PUL	–
62	dTRE	+	63	ADH2s	–
64	OPTO	+			

Positive, + Negative, _

Table 2: Biochemical Details of isolated *Sphingobacterium thalpophilum.*

Biochemical Details		Reaction	Biochemical Details		Reaction
2	APPA	+	3	ADO	_
4	PyrA	_	5	IARL	_
7	dCEL	_	9	BGAL	+
10	H2S	+	11	BNAG	_
12	AGL Tp	_	13	dGLU	+
14	GGT	_	15	OFF	_
17	BGLU	+	18	dMAL	_
19	dMAN	_	20	dMNE	_
21	BXYL	_	22	BAlap	_

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23	ProA	+	26	LIP	+
27	PLE	-	29	TyrA	+
31	URE	+	32	dSOR	_
33	SAC	_	34	dTAG	_
35	dTRE	_	36	CIT	_
37	MNT	_	39	5KG	_
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	O129R	_	59	GGAA	_
61	IMLTa	_	62	ELLM	_
64	ILATa	_			

Positive, + Negative, _

Results Discussion

The study was conducted during the period from April 2021 till March 2022, there is a fluctuation in water collected from in Al-Diwaniya River (Fig. 2). The mean of temperature varied from the lowest value 11.2oC during February to the highest value 33.8oC during August. Dissolved oxygen values fluctuated from 4.5.0mg/l in August to 9.0mg/l cm in February Salinity values ranged from 0.50‰ in April to 0.69‰ in October. Narrow fluctuation of pH was observed during the study period, with the highest average value was 8 in March and a lowest of 6.1 in August, in AL-Diwaniya River.

Bacterial diseases in fish generally do not develop as a result of exposure to an infectious agent, but in most cases, disease occurs as a result of complex interactions between pathogens, fish and environmental stresses, affecting fish susceptibility to disease (Wedekind *et al*., 2010; Siri *et al*., 2011). Most of the bacteria that cause fish diseases are Gram-negative Bacillus, but there are some pathogens of bacteria that are Gram-positive (Öztürk and Altinck, 2014; Arifin *et al.*, 2013). The reason for this is that most of the fresh surface water, including Al-Diwaniyah River, is polluted with sewage water, and it is a source of bacterial contamination, especially near residential communities as a result of the wastewater being discharged and not treated. Therefore, fresh water is a carrier of most types of pathogenic bacteria(Amal *et al*., 2015).

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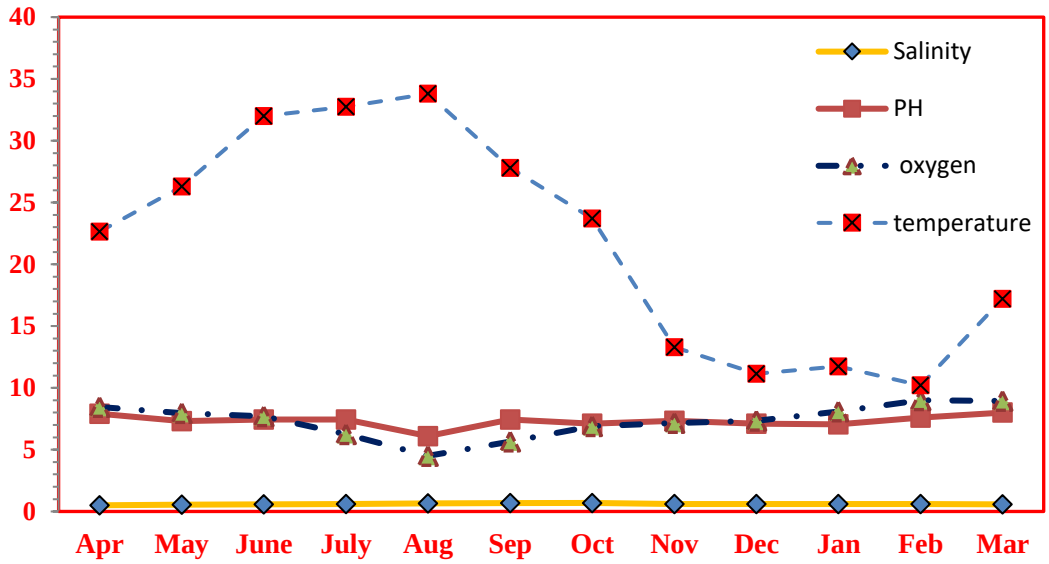

Figure 2. Monthly variations in water temperature, oxygen, salinity and pH in Al-Diwaniya River.

The results of present study showed that S. thoraltensis, S. thalpophilum.isolated and identified from O. aureus fish the in Al-Diwaniya River are a dangerous and may be unhealthy for public health when consumed (Table 3). Abdel-Latif and Seddik (2017) isolated several types of Enterobacteriaceae in *Oreochromis niloticus* experiencing cutaneous hemorrhages and ulcers with marked focal hemorrhages and areas of necrosis in the liver and congestion in the gills and spleen of Beheira Republic Egypt, these species are *K. aerogenes, E. cloacae, C. freundii, E. coli, Klebsiella sp, Proteus mirabili* and *P. vulgaris.*

Isolates by Marathe *et al*. (2016) and Thongkao and Sudjaroen (2017) Many species of human infecting bacteria from the gut of tilapia fish such as *Enterobacter spp, Serratia marcescens, E. coli, Klebsiella pneumoniae,* and showed that the presence of these bacteria in the gut of tilapia fish is of concern, and indicates that these fish are considered as a potential reservoir for some human pathogens in the environment.

Marcel *et al.* (2013) in a study on the presence of pathogens in red tilapia fish cultured in cages in two water bodies in Malaysia by isolate *Micrococcus spp*, the fish showed no clinical signs but the somatic lesions included swelling or congestion in the kidneys with pallor of the liver.

(Table 3): isolated bacteria from the skin, gills, fins and intestines of *O. aureus* fish.

Bacteria	skin	Gills	fins	Intestines
Streptococcus thoraltensis	+	-	-	-
Sphingobacterium thalpophilum	-	+	+	+

(+): found**, (-):** not found

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Antibiotics have been considered the most utilized chemicals for more than half-century worldwide. Antibiotics are a group of organic or chemical compounds that kill or inhibit the growth of pathogens and are also used as a growth promoter and disease treatment or prevention (Lulijwa *et al*., 2020).

The best antibiotics susceptibility were Ciprofloxacin (<= 0. 11µg/ ml), Imipenem and Levofloxacin (<= 0. 25, <= 0. 22 µg/ ml), when tested in vitro on P. luteola, A. sobria and S. thalpophilum while, it was resistant to Cefazolin (>= 70 µg/ ml) on P. luteola, A. sobria and S. thalpophilum (Table 3). In vitro, The best antibiotics susceptibility were Ciprofloxacin (<= 0. 23, <= 0. 25 µg/ ml), on *S. thoraltensis*, and *S. thalpophilum*. Imipenem and Levofloxacin (<= 0. 20, <= 0. 25 µg/ ml), when tested in vitro on *S. thoraltensis, S. thalpophilum,* while it was resistant to Cefoxitin(<= 66, <= 70 µg/ ml) on *S. thoraltensis,* and *S. thalpophilum* (Table 4). Kumar *et al*. (2015) that P. hauseri bacteria were sensitive to ampicillin, cephalexin and ciprofloxacin and resistant to gentamycin, oxytetracycline and chlorphenicol. While these bacteria were resistant to ampicillin and cefazolin, while they were highly sensitive to levofloxacin and ciprofloxacin in the current study. Wamala *et al*. (2018) Between that In a study to determine the sensitivity of some bacterial species isolated from Oreochromis niloticus and African Silurus fish in Uganda, A. hydrophila and A. sobria, Proteus spp. and Citrobacter spp. and Klebsiella spp. High levels of resistance to penicillin, oxycycline and ampicillin. Some bacteria that have a high sensitivity against some drugs can be used to significantly reduce the dose of the antibiotic(DiMasi *et al*., 2016).

(Table 4): *Antibiotic susceptibility S. thoraltensis* and *S. thalpophilum.*

S. thoraltensis					
Antimicrobial	**MIC***	**Interpretation**	**Antimicrobial**	**MIC***	**Interpretation**
Ampicillin	5	R	Imipenem	<= 0. 20	R
Piperacillin/ Tazobactan	<= 4	S	Amikacin	<= 4	S
Cefazolin	>= 41	R	Gentamicin	<= 5	S
Cefoxitin	<= 66	S	Nitrofurantion	67	R
Ceftazidime	<= 15	S	Ciprofloxacin	<= 0. 23	S
Ceftriaxone	<= 8	S	Levofloxacin	<= 0. 25	S
Cefepime	<= 8	S	Tigecycline	3	R
Ertapenem	<= 0. 62	S	Trimethoprim/Sulfamethoxazole	<= 25	S
S. thalpophilum					
Antimicrobial	**MIC***	**Interpretation**	**Antimicrobial**	**MIC***	**Interpretation**
Ampicillin	2	R	Imipenem	<= 0. 25	R
Piperacillin/ Tazobactan	<= 4	S	Amikacin	<= 2	S
Cefazolin	>= 33	R	Gentamicin	<= 1	S
Cefoxitin	<= 70	S	Nitrofurantion	128	R

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Ceftazidime	<= 1	S	Ciprofloxacin	<= 0. 25	S
Ceftriaxone	<= 1	S	Levofloxacin	0. 25	S
Cefepime	<= 1	S	Tigecycline	2	R
Ertapenem	<= 0. 5	S	Trimethoprim/Sulfamethoxazole	<= 20	S

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

Conclusion

Extensive and uncontrolled use of antimicrobial drugs may result in multiple resistance to Antimicrobials. So the antimicrobial sensitivity test in the genus Citrobacter infection and others. There is an effect of environmental factors on the spread of bacteria in fish, especially temperature, ammonia, nitrite, the biological requirement for oxygen and salinity in fish culture water.

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