

Isolation and identification of bacteria from *Coptodon zillii* (Gervais, 1848) and study of some virulence factors in Al-Diwaniya River/middle of Iraq

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ABSTRACT

This study was conceded to examining the occurrence of possible pathogenic species of bacterium in *C. zillii* in the Al-Diwaniya River. A total of 100 fishes of *C. zillii*, during the period (April) 2021 till (March) 2022. Bacteria were recognized using the VITEK 2 system and designated biochemical tests. Species were recognized with different percentages in *C. zillii*, *P. luteola*, *S. thalophilum*, *S. lentus*, and *A. sobria*. Antimicrobial susceptibility test of 16 antimicrobials, which are (Ampicillin, piperacillin/tazobactam, amikacin, cefazolin, Ceftriaxone, Ertapenem, Gentamycin, ceftazidime, ciprofloxacin, imipenem, ceftazidime, levofloxacin, cefepime, tigecyclin, Nitrofurantoin, and trimethoprim-sulphamethoxazole) was recognized and studied for antimicrobial susceptibility test using the Vitek II system. The best antimicrobial susceptibility were ciprofloxacin ($\leq 0.11 \mu\text{g/ml}$), Imipenem ($\leq 0.25 \mu\text{g/ml}$), when tested in vitro on *P. luteola*, *S. thalophilum*, *S. lentus*, *A. sobria*, and Levofloxacin (≤ 0.22 , ≤ 0.20 , ≤ 0.25 , $\leq 0.21 \mu\text{g/ml}$), when examined in vitro on *P. luteola*, *S. thalophilum*, *S. lentus*, *A. sobria* while, it was resistant to cefazolin (≥ 70 , ≥ 70 , ≥ 76 , $\geq 68 \mu\text{g/ml}$), when examined in vitro on *P. luteola*, *S. thalophilum*, *S. lentus*, *A. sobria*.

Keywords: *Coptodon. zillii* bacteria Antibiotics, Iraq.

INTRODUCTION

Tilapia fish is a main commodity in aquaculture marketed worldwide¹. Tilapias is a typical name given to a group of fish inside the Cichlidae². Tilapias have 1524 species³; in any case, Fishbase recorded 1699 species as per⁴. spread *C. zillii* in southern Morocco, Turkey, Egyptian, Jordan, the United States, America and the Philippines. The *C. zillii* (Gervais, 1848) is boundless in the tropics and sub-tropical locales and is broadly in Africa and Russia. In Africa, its spreading ranges from Morocco and Egypt in the North, Côte d'Ivoire and Nigeria in the West, to the Democratic Republic of Congo in central Africa⁵. Strange *Coptodon zillii* fish have been kept in Iraqi waters in the Musayyib regions on the Euphrates⁶. Fish diseases are a major limiting factor in fish production and cause high mortality. Researchers and aquaculture farmers are looking for ways to maximize yield

per unit volume of water to reduce costs in aquaculture farms⁷. Economic losses resulting from bacterial illnesses are impressive in aquaculture. The seasonal variations in water quality and severe stock conditions result in tension, which permits recurrent infection at opportunist pathogens in communal carp cultured⁸. Bacteria persist well in a river stream independently of their hosts. Additionally, bacterial illnesses have become principal barriers to aquaculture, especially when the water temperature is warm.

⁹⁻¹⁰ It has been reported that bacterial species having a place with something like 13 genera are pathogenic to aquatic animals, including gram-negative microbes, and gram-positive microbes and species reported to be associated with bacterial diseases are responsible for many diseases and heavy mortalities in farmed fish. The bacteria are sent by fish that have connected with other infected fish. Bacterial fish illness and diseases are incredibly high and are one of the most challenging medical conditions to deal with. Bacteria in the fish body through the gills or skin, or they can remain on the outer layer of the body¹¹.

MATERIALS AND METHODS

AL-Diwaniya River is an expansion of the Al-Hilla River, a part of the Euphrates River, in the center of Iraq. It is 123 km long, 25-30 m wide and depth 3-5 m. The samples were selected from two locations in the river between the Daghhara barrier through Sinniyah region to the AL-Diwaniya city ($44^{\circ}54' - 57^{\circ}55' E$ $59^{\circ}86' 14' 31'' N$) (Fig. 1). A total of 100 fishes of *C. zillii*, Fishing sampled was done by using seine net, gill nets, cast net. The sampling was completed during the period from April 2021 to March 2022. The length of the fish ranged between 16-30 cm, and the weight was 70-470 g. The living fishes were sent to oxygenated pool water before moving to the lab in the Veterinary Medicine College, Al-Qasim Green University. The bacteria were collected aseptically from analyzed fishes' skin, gills and intestine using a sterile loop. For bacterial isolation, MacConkey agar medium was utilized. Incubation of the inoculated plate was done at 37°C for 24 h. Bacteria were recognized, and antimicrobial susceptibility (Ampicillin, Piperacillin- Tazobactam, Cefazoline, Cefoxitine, Ciftazidime Ciftriaxone, Cifepime, Ertapeneme imipeneme, Amikacine, Gentamycin Nitrofurantoin, Ciprofloxacin, Livofloxacin, Tigecycline, and Trimethoprim- Sulphamethoxazole) by using the Vitek 2 system. The bacteria were re-cultured on MacConkye agar, placed in the incubator for 24 h. and transported for investigation in the Vitek 2 (Tables 1,2,3 and 4).



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

Biochemical Details		Reaction	Biochemical Details		Reaction
2	APPA	+	3	ADO	-
4	PyrA	+	5	IARL	-
7	dCEL	-	9	BGAL	+
10	H ₂ S	+	11	BNAG	-
12	AGL Tp	+	13	dGLU	+
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	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	BLUR	-
58	O129R	-	59	GGAA	-
61	IMLTa	-	62	ELLM	-
64	ILATa	-			

Table 1. Biochemical Details of Isolated *Pseudomonas luteola* .Positive (+), Negative (-)

Biochemical Details		Reaction	Biochemical Details		Reaction
2	AMY	+	5	dXYL	-
4	PIPLC	-	8	ADHI	+
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	ILATk	-	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	BLUR	-	37	dGAL	-
45	dMAL	+	54	MBdG	+
46	BACI	-	56	PAUL	+
62	dTRE	+	63	ADH2s	-
64	OPTO	+			

Table 2. Biochemical Details of isolated *Staphylococcus lentus* Positive (+), Negative (-)

Biochemical Details		Reaction	Biochemical Details		Reaction
2	AMY	-	5	dXYL	-
4	PIPLC	-	8	ADHI	+
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	ILATk	+	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	BLUR	-	37	dGAL	+
45	dMAL	+	54	MBdG	+
46	BACI	+	56	PAUL	-
62	dTRE	+	63	ADH2s	-
64	OPTO	+			

Table 3. Biochemical Details of isolated *Streptococcus thoraltensis*. Positive (+), Negative (-)

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	-
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	BLUR	-
58	O129R	-	59	GGAA	+
61	IMLTa	+	62	ELLM	+
64	ILATa	-			

Table 4. Biochemical Details of Isolated *Aeromonas sobria*. Positive (+), Negative, (-)

RESULTS

Results of the current study showed species *P. luteola*, *S. thalpopphilum*, *S. lentus* and *A. sobria* were collected from the skin, gills, fins and intestine of *C. zillii* (Table 5). In the Al-Diwaniya River, they are hazardous and might be undesirable for general health when consumed species. The emergence of diseases in the fish farms may be linked to the stress factors experienced by the fish or a possibility of bacteria transition either from water to fish or the opposite or by handling. 13 described 181 bacterial isolates, primarily *A. hydrophila/caviar*, *S. agalactiae*, *P. shigelloides*, *A. sobria*, and *V. cholera*, all of which exhibited susceptibility to chloramphenicol and ceftriaxone.

Bacteria	skin	Gills	fins	Intestines
<i>Pseudomonas luteola</i>	+	-	+	-
<i>Sphingobacterium thalpopphilum</i>	-	+	-	-
<i>Staphylococcus lentus</i>	+	-	+	-
<i>Aeromonas sobria</i>	-	-	-	+

Table 5. isolated bacteria from the skin, gills, fins and intestines of *C. zillii* fish. (+): found, (-): not found

The monthly changes in the average water temperature values varied from the lowest value of 11.2 °C during February to the highest value of 33.8 °C during August. Dissolved oxygen values varied from 4.5.0 mg/L in August to 9.0 mg/L cm in February. Values of salinity fluctuated from 0.50 in April to 0.69 in October. A narrow range in pH was detected during the study period, with the maximum value on average being 8 in March and the minimum being 6.1 in August in Al-Diwaniyah River (Fig. 2).

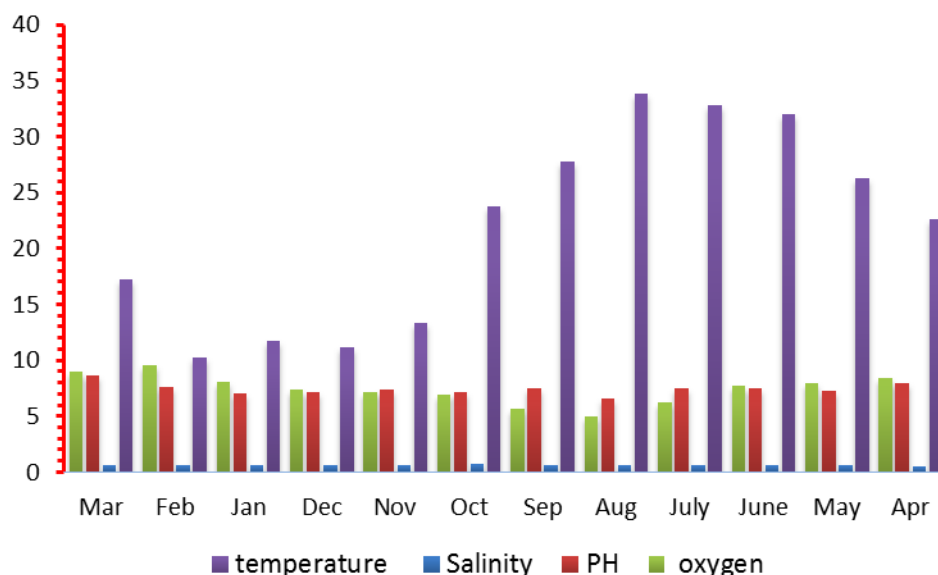


Figure 2. Monthly variations in water temperature, oxygen, salinity and pH in Al-Diwaniya River.

Antimicrobials have been viewed as the most used synthetic compounds for the greater part of the century worldwide. Antimicrobials are a group of natural or synthetic mixtures that kill or inhibit the development of microorganisms and are moreover used as a development promoter and illness treatment and/ or prevention¹⁸

Ciprofloxacin ($\leq 0.11 \mu\text{g/ml}$), Imipenem ($\leq 0.25 \mu\text{g/ml}$), were the best antimicrobial susceptibility when tested in vitro on *P. luteola*, *S. thalpopphilum*, *S.*

lentus, *A. sobria*, and Livofloxacin (≤ 0.22 , ≤ 0.20 , ≤ 0.25 , ≤ 0.21 , $\mu\text{g/ml}$) when tested in vitro on *P. luteola*, *S. thalpophilum*, *S. lentus*, *A. sobria* while, it was resistant to Cefazolin (≥ 70 , ≥ 70 , ≥ 76 , ≥ 68 , $\mu\text{g/ml}$), when tested in vitro on *P. luteola*, *S. thalpophilum*, *S. lentus*, *A. sobria* (Table 6).

P. luteola					
Antimicrobial	MIC*	Interpretation	Antimicrobial	MIC*	Interpretation
Ampicillin	≥ 28	R	Imipenem	≤ 0.25	R
Piperacillin/ Tazobactan	≤ 4	S	Amikacin	≤ 4	S
Cefazolin	≥ 70	R	Gentamicin	≤ 5	S
Cefoxitin	≤ 4	S	Nitrofurantoin	44	R
Ceftazidime	≤ 15	S	Ciprofloxacin	≤ 0.11	S
Ceftriaxone	≤ 8	S	Levofloxacin	≤ 0.22	S
Cefepime	≤ 8	S	Tigecycline	3	R
Ertapenem	≤ 0.62	S	Trime- thoprim/Sulfameth oxazole	≤ 25	S
S. thalpophilum					
Antimicrobial	MIC*	Interpretation	Antimicrobial	MIC*	Interpretation
Ampicillin	≥ 22	R	Imipenem	≤ 0.25	R
Piperacillin/ Tazobactan	≤ 4	S	Amikacin	≤ 4	S
Cefazolin	≥ 76	R	Gentamicin	≤ 5	S
Cefoxitin	≤ 4	S	Nitrofurantoin	44	R
Ceftazidime	≤ 15	S	Ciprofloxacin	≤ 0.11	S
Ceftriaxone	≤ 8	S	Levofloxacin	≤ 0.20	S
Cefepime	≤ 8	S	Tigecycline	3	R
Ertapenem	≤ 0.62	S	Trime- thoprim/Sulfameth oxazole	≤ 25	S
S. lentus					
Antimicrobial	MIC*	Interpretation	Antimicrobial	MIC*	Interpretation
Ampicillin	≥ 40	R	Imipenem	≤ 0.25	R
Piperacillin/ Tazobactan	≤ 4	S	Amikacin	≤ 4	S
Cefazolin	≥ 70	R	Gentamicin	≤ 5	S
Cefoxitin	≤ 4	S	Nitrofurantoin	44	R
Ceftazidime	≤ 15	S	Ciprofloxacin	≤ 0.11	S
Ceftriaxone	≤ 8	S	Levofloxacin	≤ 0.25	S
Cefepime	≤ 8	S	Tigecycline	3	R
Ertapenem	≤ 0.62	S	Trime- thoprim/Sulfameth oxazole	≤ 25	S

A. sobria.					
Antimicrobial	MIC*	Interpretation	Antimicrobial	MIC*	Interpretation
Ampicillin	≥ 33	R	Imipenem	≤ 0.25	R
Piperacillin/ Tazobactan	≤ 4	S	Amikacin	≤ 4	S
Cefazolin	≥ 68	R	Gentamicin	≤ 2	S
Cefoxitin	≤ 2	S	Nitrofurantoin	44	R
Ceftazidime	≤ 3	S	Ciprofloxacin	≤ 0.11	S
Ceftriaxone	≤ 8	S	Levofloxacin	≤ 0.21	S
Cefepime	≤ 1	S	Tigecycline	≤ 1	R
Ertapenem	≤ 0.60	S	Trime- thoprim/Sulfame thoxazole	≤ 25	S

Table 6. Antibiotic susceptibility *P. luteola*, *S. thalophilum*, *S. lentus*, *A. sobria*.*MIC: Minimum Inhibitory Concentration ($\mu\text{g/ml}$), S: Sensitive, R: Resistant. Several antimicrobials were tested on *S. agalactiae* - a microbes collected from the soil of a tilapia farm in Pampanga, Philippines. A group of three antimicrobial concentrations was examined in the antimicrobial assay: a) 10 μg of (ampicillin, gentamycin, and penicillin); b) 20 μg of (nalidixic acid and amoxicillin) 30 μg of (tetracycline, vancomycin and chloramphenicol). Their results revealed that four antimicrobials (such as chloramphenicol, gentamycin, nalidixic acid and tetracycline) inhibit the development of *S. agalactiae*. Therefore, these antimicrobials are suggested to treat *S. agalactiae* infection in tilapia. Furthermore, other antimicrobials were discovered such as amoxicillin, ampicillin, penicillin and vancomycin, which led to intermediate susceptible²³.

DISCUSSION

^{14,15} state that a fish farming system always exposed to various stresses becomes more sensitive to disease contagion. Due to higher water temperature levels from 25°C to 32°C, organic matter, salinity and pH 5-9, the number of bacteria in the water increased increased^{16,17}.

^{19,20} reported that the highest susceptibility of drug assessment in several microorganisms is a practicable method that can reduce antimicrobial dosage; in contrast, there are particular hazards for using antimicrobials; initially, the medication can enter the biological barrier and tissues; for instance, it can enter through the blood vessels in the brain, moreover, using antimicrobials for a long period resulting in the resistance of bacteria, accumulation of the drug in fish tissues and environmental problems. Isolates of *Aeromonas* spp. were susceptible to enrofloxacin and bacteria *A. hydrophila* is sensitive to oxytetracycline, ofloxacin, azithromycin, norfloxacin, doxycycline and chlortetracycline, while it showed resistance to amoxicillin, ampicillin, cefuroxime, flumequine and erythromycin^{21,22}

CONCLUSION

The presence of *P. luteola*, *S. thalophilum*, *S. lentus*, and *A. sobria* in *Coptodon zillii* is considered a public health hazard to consumers. It harbors virulence factors responsible for its pathogenesis. Therefore, hygienic measures must be applied to control microbial contamination in the aquatic environment and during fish transport until it reaches consumers. In the current study, the bokashi bio-fertilizer and the bio-fungi *P. cyclopium*, *T. harzianum*, demonstrated their inhibitory activity in decreasing the incidence and severity of root rot disease and a significant increase in the growth parameters of the plant.

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Author's Contribution

All authors actively contributed to the performance of the assignment, document lettering and review. All authors accepted the last kind of the document.

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