Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(2): 151 – 168 (2022)
www.ejabf.journals.ekb.eg

Bacterial pathogens causing the blue crab (*Callinectes sapidus*) mortality at Suez Canal (El-Temsah Lake) in Ismailia Governorate

Soad S. Abd El-Halim Salama[1], Mohamed E. AbouElatta[2], El Sayed K. Abd El Rahman[2], Ahmed H. Sherif [3*]

1. Fish Diseases Research Department, Animal Health Research Institute, AHRI, Dokki, Agriculture Research Centre ARC, 12619 Egypt.
2. Fish Health and Management Department, Central Laboratory for Aquaculture Research (El-Abbassa), Agriculture Research Center, Egypt.
3. Fish Diseases Department, Animal Health Research Institute AHRI, Agriculture Research Centre ARC, Kafr- Elsheikh, 12619, Egypt

[*]**Corresponding Author: ahsherif77@yahoo.com**

ARTICLE INFO

Article History:
Received: Feb. 26, 2022
Accepted: March 3, 2022
Online: March 21, 2022

Keywords:
El-Temsah Lake,
Blue Crab
Callinectes sapidus,
Enterococcus gallinarum,
Ammonia

ABSTRACT

El-Temsah Lake is considered the largest water body in Ismailia governorate, with a total area of 14 km^2. A total number of 120 moribund crabs *(Callinectes sapidus)* were collected from El-Temsah Lake in front of the youth house, Ismailia Governorate, in September 2021. The analyses of water parameters revealed high concentrations of un-ionized ammonia and nitrite. Clinically, moribund *C. sapidus* showed loss of escape reflex, lethargy, sluggishness, easily catching, and shell lesions on the dorsal cuticle, swimming legs, and walking legs. Several affected *C. sapidus* showed softening of the cuticle. Internally, the intestine is devoid of food, gills show blackening, and a straw yellow fluid is accumulated around the hepatopancreas. *Enterococcus gallinarum* was isolated from hemolymph, gills, and shell then identified by traditional bacterial methods, Vitek 2 system, and DNA sequence. *C. sapidus* was infected with *E. gallinarum*, withrates of 90% and 83.33%, respectively, in sites 1 (El-Taween club) and 2 (Beach club). *E. gallinarum* was sensitive for Ciprofloxacin and Rifampicin; whereas, it showed resistance to Vancomycin, Erythromycin, Chloramphenicol, and Tetracycline. It was obvious that the high mortality rate of *C. sapidus* was associated with deleterious water quality and *E. gallinarum* infection.

INTRODUCTION

El-Temsah Lake is a concavity evolved along a fault trough covered with Nile Delta sediments. It is a saline and shallow lake; the largest water body is near Ismailia City at a point of 80 km to the south of Port Said. It lies approximately mid-way between the south of Suez City and the north city of Port Said between latitude of 30^{o}35′46.55‘‘N and longitude of 32^{o}19′30.54"E **(El-Serehy *et al.*, 2018)**. The entire depth of El-Temsah Lake ranges between 4 and 10m. In the western lagoon and southwestern part of the lake,

the water depth is shallower, ranging from 2.5 to 4m. It is a land-engulfed embayment with a total area of 14km^2 **(El-Serehy & Sleigh, 1992)** and containing about 80×10^6 m^3 of water. For water salinity of the lake, saline was converted to fresh water due to different water resources supplying the lake with different kinds of water (freshwater from Ismailia sweet freshwater canal, partially treated waste-water through several agricultural, industrial, domestic sewage drains, such as drains of Al-Mahsama, Al-Bahtimi, Al-Dabiaia, Al-Wadi and Al-forsan) along with the saline water from the Suez Canal **(EEAA, 2010, 2011)**. Unfortunately, the upsurge activities of the temporary and permanent residents have participated in forming high quantities of waste, including raw liquid and solid municipal sewage, agricultural drainages and industrial wastewater, all of which are discharged in El-Temsah Lake. The lake under study plays an important role in most of the activities in Ismailia City (Ismailia Governorate) such as tourism and navigation; it is and considered a fishery source for the local fishermen **(Kaiser *et al*., 2009)**.

Fish can be infected with various microbial agents, among which serious bacterial infections are considered. Those infections go beyond infecting fish. Their nature is either communicable or zoonotic, viz. *Aeromonas*, *Vibrio*, *Mycobacteria* and *Streptococcus* **(Russo, *et al*., 2012)**. Streptococcosis (Strep disease) or pop eye disease is a septicemic disease affecting both fresh and marine water fishes, in both farmed and feral populations; it results in high economic losses accounting for hundred million dollars annually **(Karsidani, *et al*., 2010)**. Streptococosis was misdiagnosed with other closely related bacteria, viz. *Lactococcus*, *Enterococcus*, and *Vagococcus*, which cause clinical signs similar to those of streptococcal infection **(Yanong & Francis-Floydm, 2013)**. Numerous other fish species of edible and ornamental character are susceptible to this infection, including tilapia, mullet, eel, sturgeon, rosey barbs, striped bass, rainbow sharks, red-tailed black, danios and sharks; infection was observed in 2 forms: acute and chronic diseases **(Yanong & Francis-Floyd, 2013)**. Stress is usually one of the predisposing factors resulting in bacterial outbreaks such as poor environmental conditions: rise of the environmental temperature, harvesting, handling, transportation and poor water quality (high ammonia and nitrite levels) **(Yanong & Francis-Floyd, 2013; Sherif *et al*., 2020a, b; Sherif *et al*., 2021a)**.

Crab *(Callinectes sapidus)* is decapod crustacean of the infra order Brachyura. Decapod crustaceans broadly categorize shrimps, lobsters, and *C. sapidus* that gain public interest due to their importance as a global food source accounting over 24 billion dollars **(Bondad-Reantaso, *et al*., 2012**). *C. sapidus* is rich in high quality protein, sodium, potassium and phosphorus. Moreover, it contains good amount of minerals; namely, Fe, Zn, Cu and Mg in addition to vitamins A, C, B6, thiamin and riboflavin **(Nishidha, 2006)**. *C. sapidus* is bottom feeders i.e. feeding on algae, mollusks, worms, other crustaceans, fungi, bacteria, plants and animal matter **(Kennish, 2019)**. Vibrio sp. (*V.*

harveyi, *V. alginolyticus*, *V. anguillarum*), *Aeromonas hydrophila*, *Pseudomonas fluopescens*, *Tenacibaculum maritimum*, *Leucathrix mucor*, *E. coli*, *Providencia stuari*, and *Proteus mirabils* were isolated from shell, gills, hemolymph, hepatopancreas and muscles of shrimp, Mantis shrimp and *C. sapidus* **(Salama *et al.*, 2019)**. Microbial groups most commonly isolated from crab meat were: *Vibrio*, *Aeromonas*, *Enterobacteraceae*, *Moroxella*, *Acintobacter*, *Pseudomonas*, *Shewanella*, *Micrococcus*, *Bacillus lolitha* and *Thampuran*. **Mahmoud (2015)** and **Osman *et al.* (2017)** isolated *E. gallinarum* from *O. niloticus* in aquaculture and wild sites in Egypt. Additionally, **Khafagy *et al.* (2009)** isolated *Enterococcus faecalis* from tilapia in El-Temsah Lake in Ismailia governorate.

The current study aimed to shed some light on bacterial pathogens causing crab (*Callinectes sapidus*) mortalities in El-Temsah Lake in an attempt to solve this problem.

MATERIALS AND METHODS

2.1. Sampling and study site

A total of 120 moribund crabs (*Callinectes sapidus*) were collected from El-Temsah Lake, which was suffering from mass mortality at the governorate of Ismailia in September 2021. Two collection sites were selected; namely, the El-Taween and Beach clubs. The weights of the specimens ranged from 25–110 and 15–100g, respectively. The geographical site of El-Temsah Lake is illustrated in Table (1) and Fig. (1) **(El-Serehy *et al.*, 2018)**. *C. sapidus* samples were transported alive in aerated water tanks and euthanized by immersion in tricaine methane sulfonate (MS222) solution at a concentration of 250 mg/l (25–30°C), following the recommendations of **AVMA (2007)**. During this study, all applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Table 1. Characteristics of the El-Temsah Lake

Parameter	El-Temsah Lake
Location	Ismailia City on the Suez Canal
Origin	Natural
Latitude	30°35′46.55‘‘N
Longitude	32°19′30.54"E
Surface area (km^2)	14
Water volume (m^3)	80 ×10^6
Average depth (m)	2–28

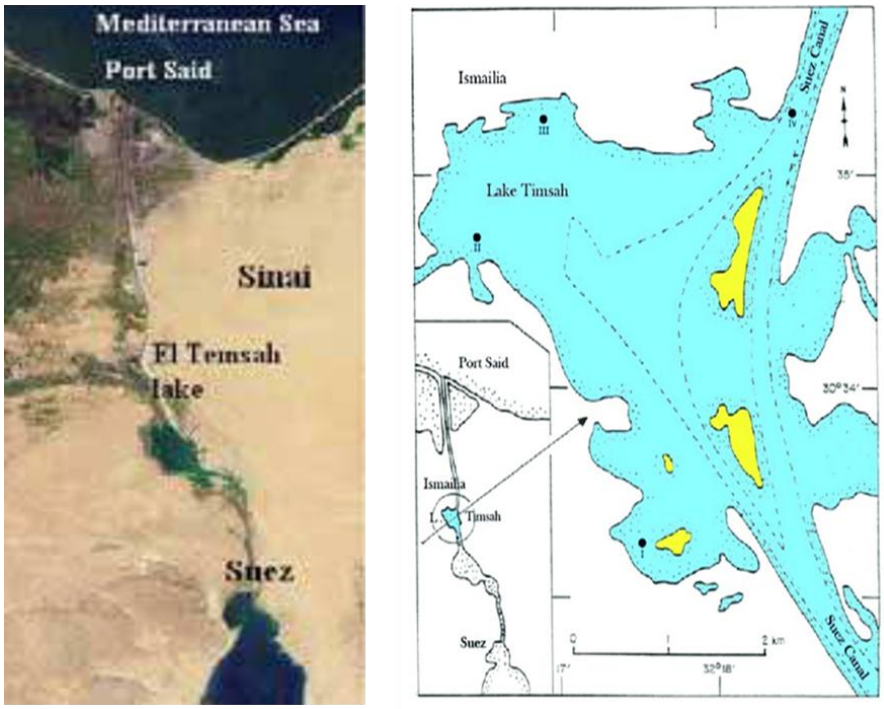

Fig. 1. Map of El-Temsah Lake

2. 2. Analyses of the water parameters

Water temperature was measured by using a simple pocket thermometer. Two water samples were collected from a depth of 30cm using bottles, then stored in acid-washed polyethylene bottles for analysis of pH, dissolved oxygen, salinity, un-ionized ammonia, and nitrite (NO_2) according to the procedures of **APHA (1992)**.

2.3. Clinical and post mortem investigations

C. sapidus samples under investigation were grossly examined for swimming ability, any discoloration or lesions in the shell from dorsal aspect or ventral aspect or in the appendages, eyestalks and texture of carapace (hard or soft). The specimens were externally examined according to the study of **Noga (2010)**. The samples were dissected for detection of any abnormality in gills, hepatopancreas, hemolymph, foregut and hindgut. The samples were examined by a compression method. The muscles, gills, and internal organs were pressed between two glass plates and searched for metacercariae under a stereomicroscope **(Noga, 2010),** the examination did not reveal any harmful parasites that cause fish diseases.

2.4. Bacteriological examination and identification

The swabs for bacteriological analyses from the *C. sapidus* were collected (exocuticle, muscle, hemolymph, gills and hepatopancreas) under aseptic condition and immediately streaked onto tryptic soy broth with or without 3% NaCl and blood agar, then incubated at 10°C and 24°C for 24-48h. **(Tonguthai *et al.*, 1999)**.

2.4.1. Primary identification

Pure colonies were streaked onto TSA, aeromonas agar, pseudomonas agar and TCBS agar plates (Oxoid Ltd.,USA). Suspected colonies were picked, purified, and subjected to the phenotypic characterization of the bacterial isolates and confirmed according to the studies of **Bergey (1994)**, **Elmer *et al.* (1997)** and **Madigan and Martinko (2005)**. In addition to the Gram-positive identification (GPI), VITEK® 2 compact cards (BioMérieux) **(Lusiastuti, *et al.*, 2013)** was used following the manufacturer's instructions.

2.4.2. Polymerase chain reaction and bacterial sequence

2.4.2.1. DNA extraction

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, a volume of 200µl of the sample suspension was incubated with 10µl of proteinase K and 200µl of lysis buffer at 56ºC for 10min. After incubation, a volume of 200µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

2.4.2.2. Oligonucleotide primers

Primers (Table 2) were supplied from **Metabion (Germany)** and utilized in a 25-µl reaction containing 12.5µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer (20 pmol), 4.5µl of water, and 6µl of DNA template. The reaction was performed in an applied biosystems 2720 thermal cycler. The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1× TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15µl of the PCR products were loaded in each gel slot. A gene ruler of 100bp DNA ladder (Fermentas, Thermo Scientific, Germany) and Gelpilot 100bp plus ladder (Qiagen, Germany, Gmbh) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra), and the data was analyzed through computer software.

2.4.2.3. DNA sequences

PCR products were purified using QIAquick PCR Product extraction kit. (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) was used for the sequence reaction and then it was purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems3130 genetic analyzer (HITACHI, Japan), a BLAST® analysis (Basic Local Alignment Search Tool) **(Altschul*et al.*, 1990)** was initially performed to establish sequence identity to GenBank accessions. The phylogenetic tree was created by the MegAlign module of Lasergene DNAStar version 12.1 of **Thompson *et al.* (1994**). While, the Phylogenetic analyses were done using maximum likelihood, neighbor joining, and maximum parsimony in MEGA6 **(Tamura *et al.*, 2013)**.

Table 2. Primers sequences of the target genes

Gene name	Primers sequences 5'-3'	Amplified segment (bp)	Annealing temperature	Reference
Vibrio 16S rRNA	F:CGGTGAAATGCGTAGAGAT	663	56˚C	**Tarr *et al.*, 2007**
	R:TTACTAGCGATTCCGAGTTC			
Aeromonas 16S rRNA	F:CTACTTTTGCCGGCGAGCGG	953	50˚C	**Gordon *et al.*, 2007**
	R:TGATTCCCGAAGGCACTCCC			
S. aureus 23S rRNA	F:ACGGAGTTACAAAGGACGAC	1250	55˚C	**Bhati *et al.*, 2016**
	R:AGCTCAGCCTTAACGAGTAC			
Bacterial 16S rRNA	F:AGAGTTTGATCMTGGCTCAG	1485	56˚C	**Lagacé *et al.*, 2004**
	R:TACGGYTACCTTGTTACGACTT			

2.5. Antibiogram sensitivity discs

Sensitivity was determined by the agar diffusion method (**Quinn *et al.*, 2002**) using 6mm diameter commercial discs (Oxoid) including the following antibiotics: Streptomycin 10µg (S10), Ciprofloxacin 5µg (CIP 5), Ampicillin 10µg (AMP10), Rifampicin 5µg (RP5), Chloramphenicol 30µg (C30), Penicillin 10µg (P10), Nitrofurantoin 300mcg(F300), Tetracycline 30µg (T 30), Erythromycin 15µg (E15), Vancomycin 30µg (VA30) and Gentamycin 10µg (GN 10). Antibiotic sensitivity was tested on Mueller-Hinton agar, and inhibition zone diameters were interpreted as sensitive, intermediate, and resistant according to **CLSI (2010)**.

2.6. Biosafety measures

In the experimental work, the authors followed the biosafety measures regarding pathogen safety data sheets: Infectious substances—*E. galliniurm*, Pathogen Regulation Directorate, **EU (2010)**.

RESULTS

3.1. Clinical and post mortem investigations and prevalence of bacterial infection

The *C. sapidus* were examined macroscopically for swimming ability (loss of first pair of legs), discoloration black spot on cuticles and legs, softshell, exophthalmia or eye loss, the blackness of gills. Post mortem mortalities were recorded in all life stages with empty intestines and offensive smell (Figs. 2 & 3).

After culturing the bacterial isolates on a set of specific bacterial media and bacterial sequencing, the infection rates of *E. gallinarum* were as follows: 81 out of 90 (90 %) and 25 out 30 (83.33 %) in sites 1 and 2, respectively.

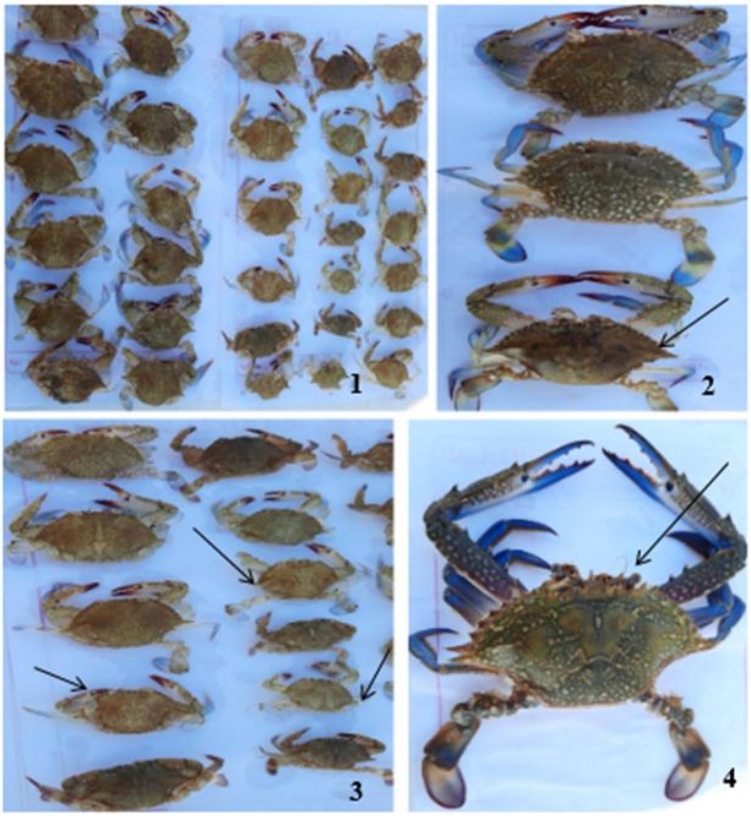

Fig. 2. Photo 1 showing a high mortality rate in all growing stages. **Photo 2** showing brown to black discoloration on dorsal aspect of diseased *C. sapidus* (black arrow). **Photo 3** showing destructed of walking and swimming legs in diseased *C. sapidus* (black arrow). **Photo 4** showing exophthalmia of eyes in diseased *C. sapidus* (black arrow)

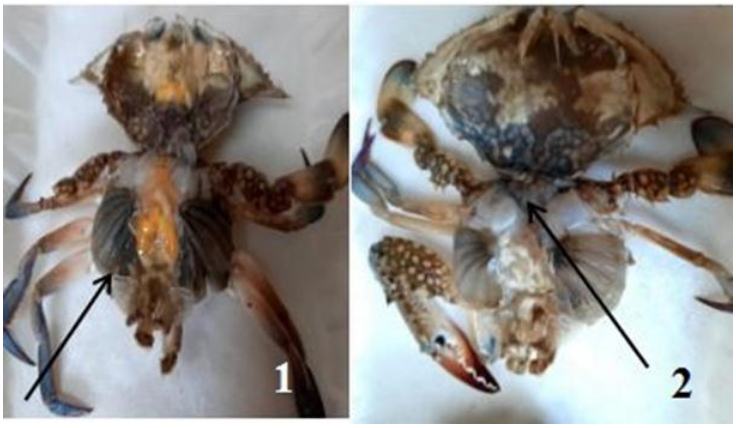

Fig. 3. Photo 1: Naturally diseased *C. sapidus* showing empty intestines (black arrow). **Photo 2:** Naturally diseased *C. sapidus* showing blackness of gills (black arrow).

3.2. Water parameters

The physiochemical parameters of water are recorded in Table (3), showing that physicochemical parameters were in suitable ranges for normal *C. sapidus* culture. While, unionized ammonia 0.023 mg/l and nitrite 0.35 mg/l were higher than the permissible level recommended for *C. sapidus*.

Table 3. Water quality of the samples from El-Temsah Lake

Water parameter	Results		Permissible limits
	Sample 1	Sample 2	
Temperature °C	22±3	22±3	--
pH	7.97±0.2	8.18±0.4	7.5 - 8.5
Dissolved oxygen mg/l	5.5±0.5	5.4±0.9	5 – 6 mg/l
Salinity g/l	31.5±0..4	32.3±1.2	28 – 35 ppt for marine farm
Un ionized ammonia mg/l	0.0125±0.00	0.023±0.001	0.0 – 0.0125 mg/l
Nitrite (NO_2) mg/l	0.24±0.04	0.35±0.1	0.0 - 0.3 mg/l

3.3. Traditional bacterial identification

The microbiological cultures on TSA from the samples that were taken from the internal organs revealed the presence of dewdrop colonies in pure culture and non-β hemolytic on blood agar that can grow at 10ºC after 48h (very faint), but good after 72 h. The isolated bacteria were identified as *E. gallinarum* which is Gram–positive bacteria, catalase –ve. *E. gallinarum* appears as short–chain cocci (3-4) under microscope. Table (4) shows the biochemical characterization of *E. gallinarum* by using the VITEK® 2 system.

3.4. The identification of *E. gallinarum* using 16S rRNA gene sequence

The assembled of *Enterococcus* sp. was submitted to the database of GenBank under the accession number of OL823003. Depending on the comparative sequence analysis, the identity of the current isolate is confirmed to be *E. gallinarum* (Fig. 4). The BLAST analysis of the 16S rRNA gene sequence from *E. gallinarum* in this study exhibited 99.81 - 99.52% identity, with *E. gallinarum* (MN208191.1– CP046307.1- MF682953.1- MH111599.1- MH111595.1). Remarkably, the phylogenetic tree (Fig. 4) of sequenced 16S rRNA of *E. gallinarum* was grouped with known sequences of *E. gallinarum* and separated from other sequences belonging to *E. gallinarum, E. pseudoavium, E. avium, E. faecalis, F. faecium* and *E. mundtii.*

Table 4. *E. gallinarum* identification using VITEK® 2 system and hemolytic pattern

Biochemical test	Results	Biochemical test	Results
D-Amygdalin (AMY)	+	Polymixin B resistance(POLYB)	+
Phosphatidylinositol-Phospholipase C	_	D-Galactose (dGAL)	+
Arginine Dihydrolase 1- 0.111mg	_	D-Ribose (dRIB)	+
Beta-Galactosidase (BGAL)	+	L-Lactatealkalinization (ILATK)	_
Alpha –Glucisidase (AGLU)	_	N-Acetyl-D-glucosamine (NAG)	+
Ala-Phe-ProArylamidase (APPA)	_	D-Maltose (dMAL)	+
Cyclodextrin (CDEX)	+	Bacitracin resistance(BACI)	_
L-Aspartate Arylamidase(AspA)	+	Novobiocinn resistance(NOVO)	_
Beta Galactopyranosidase (BGAR)	+	Growth in 6.5% NaCL (NC6.5)	_
Alpha –Mannosidase (AMAN)	_	D-Mannitol (dMAN)	_
Phosphatase (PHOS)	_	D-Mannose (dMNE)	+
Leucine ARYLAMIDASE (LeuA)	_	Methyl-B-DGlucopyranoside(MBdG)	+
L-Proline Arylamidase (ProA)	_	Pullulan (PUL)	_
Beta Glucuronidase(BGURr)0.0018 mg	_	D-Raffinose (dRAF)	+
Alpha–Galactosidase (AGAL)	_	O/129 Resistance (comp.vibrio.)	_
L-Pyrrolydonyl-Arylamidase-(PyrA)	+	Salicin (SAL)	+
Beta-Glucuronidase (BGUR) 0.0378mg	_	Saccharose/Sucrose (SAC)	+
Alanine Arylamidase (AlaA)	+	D-Trehalose (dTRE)	+
Tyrosine Arylamidase (TyrA)	_	ArginineDihydrolase2(ADH2s)0.27mg	_
D-Sorbitol (dSOR)	_	Optochin Resistance(OPTO)	_
Urease (URE)	_	Catalase test	_

3.5. Antibiogram of *E. gallinarum*

The results of sensitivity to antibiotic revealed that *E. gallinarum* was sensitive to Ciprofloxacin and Rifampicin (100%), while resistant to Chloramphenicol, Erythromycin, and Vancomycin (100%) (Table 5).

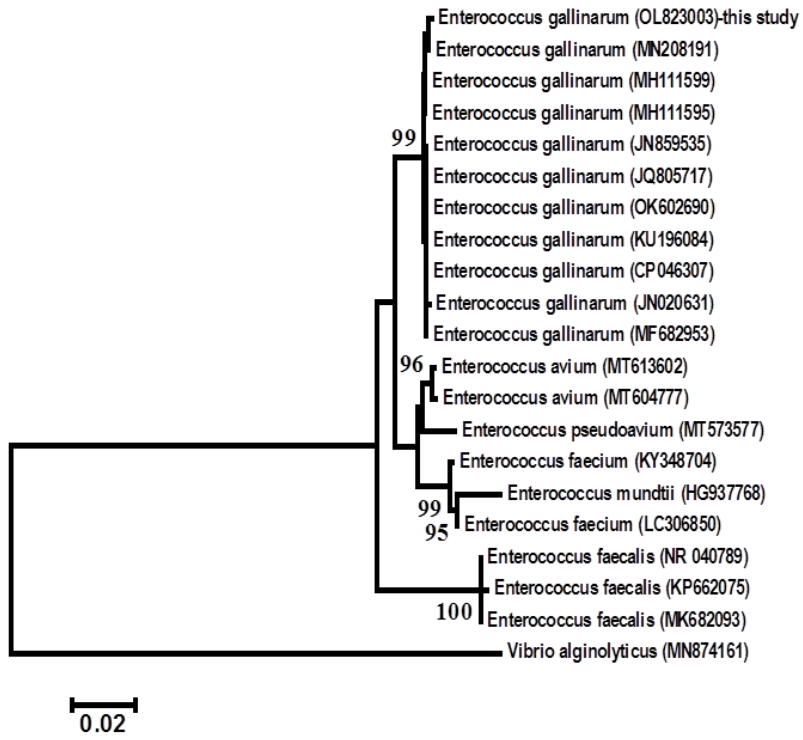

Fig. 4. The phylogenetic tree showing the comparative sequence 16S rRNA analysis of the current *E. gallinarum* against 20 different accession numbers recorded from *E. gallinarum* (10), *E. pseudoavium* (1), *E. avium* (2), *E. faecalis* (3), *F. faecium* (2), *E. mundtii* (1) and *V. alginolyticus* (1).

Table 5. Results of antibiotic sensitivity test of *E. gallinarum*

Antibiotic disc	Antibiotic sensitivity
Streptomycin 10 µg (S10)	I
Ampicillin 10 µg (AMP10)	I
Vancomycin 30 µg (VA30)	R
Chloramphenicol 30 µg (C30)	R
Penicillin 10 µg (P10)	I
Rifampicin 5 µg (RP5)	S
Nitrofurantoin 300mcg(F300)	I
Ciprofloxacin 5 µg (CIP 5)	S
Erythromycin 15 µg (E15)	R
Gentamycin 10 µg (GN 10)	I
Tetracycline 30 µg (T 30)	R

S= Sensitive, R= Resistance, and I=Intermediate

DISCUSSION

Enterococcus spp is occurred and persisted in the aquatic environment (both fresh and marine_water) and was regularly isolated from drinking water and wastewater treatment plants; it was isolated from pets, food, and wildlife animals such as birds and fish **(Roberts *et al.*, 2009;Getachew *et al.*, 2013; Oravcova *et a*l., 2013; Lozano *et al.*, 2015)**. *Oreochromis niloticus* was infected with *E. gallinarum* and was isolated from the liver and kidney at a rate of 1.3% **(Osman *et al.*, 2017)**. In addition, *E. gallinarum* was isolated from various disease-infected fishes in Bangladesh **(Iqbal *et al.*, 1996; Chowdhury *et al.*, 1998)** as well as from the aquatic environment (water and sand) along the recreational beaches of the West Coast in the USA, mainly from one site at the beach with a large population of pelicans **(Roberts *et al.*, 2009)**.

In this study, the infection rates of *E. gallinarum* ranged from 83.33% to 90 % in *C. sapidus*. The clinical signs and post mortem observed in the collected *C. sapidus* lost the swimming ability and black coloration and empty intestine were detected. Similar results are found in the study of **Osman *et al.* (2017)** on the diseased *Oreochromis niloticus* with various clinical signs, viz. pop-eye with a purulent and hemorrhagic fluid surrounding the eyes and ventral petechial hemorrhages. While, post mortem examination revealed hemorrhages in the abdominal fat, pericarditis, hepatomegaly, splenomegaly and inflamed kidney. Similarly, **Carson *et al.* (1993)** found that *Enterococcus* strain caused a high mortality rate, reaching up to 60% in rainbow trout, (*Oncorhynchus mykiss*) farmed in Australia and South Africa; the infected fish developped septicemia and characteristic exophthalmos. Furthermore **Nieto *et al.* (1995)** reported that, *Enterococcus* strain caused similar lesions of in turbot, *Scophthalmus maximus* (L.), cultured in several farms in the the Northwest of Spain, where enterococcal septicemia occurredshowing extensive hemorrhages, ulcerations, and purulent inflammation on the skin, while desquamative enteropathy and necrosis were observed in the tissues of spleen and kidney.

By using the comparative sequence 16S rRNA, assembled of the isolated *E. gallinarum* was submitted to the database of GenBank under the accession number OL823003. Using the same technique, ten virulent strains of *E. faecalis* were isolated and identified from naturally infected catfish and tilapia farmed in Bangladesh **(Rahman *et al.*, 2017)** and *Edwardsiella tarda* infected *Oreochromis niloticus* **(Sherif *et al.*, 2021b)**. Multiplex PCR was used to identify four common species; namely, *E. faecalis, E. faecium, E. casseliflavus* and *E. gallinarum* **(Kariyama *et al.*, 2000)**. A number of 48 enterococcal isolates were identified using the multiplex PCR, and the majority of the isolates were *E. faecalis* (32.2%, 31/96), *E. faecium* (7.2%, 7/96), *E. casseliflavus* (7.2%, 7/96) and *E. gallinarum* (3.1%, 3/96) **(Hammad *et al.*, 2014)**. Meanwhile, **Osman *et al.* (2017)** analysis of the 16S rRNA gene confirmed that the studied 17 tilapia isolates were

as follows: 6/17 *E. faecalis*, 2/17 *E. gallinarum*, 3/17 *Streptococcus pluranimalium* 2/17 *Aerococcus viridans*, 1/17.

The results of sensitivity to antibiotic revealed that *E. gallinarum* was sensitive to Ciprofloxacin and Rifampicin (100%), while resistance to Chloramphenicol, Erythromycin, and Vancomycin (100%) was determined. These results agree with those of **Osman *et al.* (2017)** who claimed that, the *E. gallinarum* isolates were sensitive to Ciprofloxacin and Rifampicin (100%), whereas they were resistant to Chloramphenicol, Vancomycin and Erythromycin (100%). Moreover, **Oluwole *et al.* (2013)** recorded that *E. gallinarum* was resistant to Erythromycin, Ampicillin and Chloramphenicol. Contrarily, **Oladipo *et al.* (2014)** found that the isolates of *E. gallinarum* were sensitive to all tested antibiotics except for Vancomycin. These variations in results revealed the sensitivity and susceptibility of any microorganism from one area to another according to the usage of the antibiotics **(Sherif *et al.*, 2021c)**.

The physical characteristics of the bacterial media affect the growth of the cultured pathogens and the production of bacterial toxins (**Weinberg 1985**; **Arp 1988)**. Identically, **Ramesh *et al.* (1989)** found that environmental parameters including temperature, pH, salinity, and ammonia compounds impact the growth of the bacteria. In this study, the water salinity of the El-Temsah Lake was 30 g/l and pH 7.8, while un-ionized ammonia and nitrite were 0.023 mg/l and 0.25 mg/l, respectively, creating a favorable condition for the occurrence of the bacterial infection. Similarly, the intensity of the *Enterococcus* infection in *M. rosenbergii* is upsurge influenced by different environmental parameters such as temperature (33–34 °C), and pH (8·8–9·5) affecting their survival rate, while it was downregulated with low salinity (5–10 ppt) **(Cheng & Chen, 1998)**. On the other hand, **Cheng and Chen (2002)** claimed that ammonia decreases the virulence of *Enterococcus* pathogen and increases the susceptibility to *Enterococcus* by reducing the immune ability of Giant freshwater prawn *Macrobrachium rosenbergii*.

CONCLUSION

From the obtained investigation, the deleterious water quality proved to greatly impact the health of crabs *(C. sapidus)* in the El-Temsah Lake and subsequently species under study became vulnerable to bacterial infection. *E. gallinarum* prevailed in the collected samples with a rate of 83.33% to 90%. Thus, it is recommended to minimize the municipal wastewater discharged into the lake. In addition, water parameters and *C. sapidus* quality should be regularly monitored.

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