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Vector Potential of Cymothoid Isopods in The Transmission of *Vibrio alginolytcus* in *Tilapia zilli* and *Solea aegyptiaca* in Egypt

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ABSTRACT

Cymothoid isopods are known to have harmful impacts on fish. Hence, their host-parasite relationship must receive much attention. This study investigated the phenotypic and genotypic characterization of Vibrio alginolyticus recovered from both fish and isopod which may correlate isopod to the transmission of V. alginolyticus. Thirty-two V. alginolyticus strains from both freshly dead moribund fishes and their isopods were studied; 18 strains from Tilapia zilli in Manzla Lake and 14 strains from Solea aegyptiaca in Qaroun Lake. In addition,, 3 strains were isolated from the water of both lakes. Biochemical, antibiogram, species-specific PCR and phylogenetic analysis of 16S rRNA gene were performed. Moreover, water physicochemical factors and isopod identification were done. The physiochemical analysis of water samples from both lakes showed bad water quality of high temperature, low dissolved oxygen levels and high values of almost all chemical parameters. The results revealed the isolation of Nerocila orbignyi isopod in both Manzla and Qaroun Lakes, while Levonica redmanii isopod was only found in Qaroun Lake. V. alginolyticus strains from fish, isopods and water showed the exact antimicrobial susceptibility profile. They were susceptible to erythromycin, imipenem and trimethoprim/ sulphamethoxazole, while they were resistant to ampicillin, lincomycin, colistin and penicillin. In the species-specific PCR using the collagenase gene, all tested isolates showed the characteristic V. alginolyticus amplicon of 737 bp. The phylogenetic analysis of partial 16S rRNA gene sequence revealed that V. alginolyticus isolates from fish and isopod showed 100% similarity with each other and 99.23% similarity with water strain, suggesting that the isopod was the likely source of infections. In conclusion, we demonstrated that the deterioration of water quality strongly related to the high prevalence of isopods could be a potential vector in the transmission of *V.alginolyticus* in *T.zilli* and *S. aegyptiaca* fish.

INTRODUCTION

Although mariculture is a significant investment for the Egyptian fishermen, diseases and high feeding cost are the main challenges hindering the sustainability and economic success of this field **(Abdelaziz *et al*., 2017)**.

Disease poses a serious threat to aquaculture environments owing to fish living in poor conditions besides pathogens **(Addo *et al.*, 2017)**. Inadequate disease surveillance and climate change are additional variables that raise the risk of disease. Climate change has a potential impact on parasites, directly or indirectly via changes to host characteristics, such as distribution, physiology, behavior and mortality **(Callaway *et al*., 2012; Lõhmus & Björklund, 2015)**.

Aquaculture offers an ideal habitat for parasitic infections because of high stocking densities and hosts' susceptibility **(Barber & Poulin, 2002)**. Isopods are marine parasites that prefer to live in warmer waters **(Rameshkumar & Ravichandran, 2014)**, causing serious economic losses to fishermen by killing, stunting or injuring fishes **(Ravichandran *et al*., 2016)**. Mechanical damage to fish epithelial tissues, occurring as a result of parasite invasion and movement, generates entry points for pathogens **(Xu *et al.*, 2012)**. In addition, the parasite itself may act as vector of other pathogenic organisms **(Horton & Okamura, 2001)**. Rising in temperature might enhance parasite fitness and speed up the spread of the disease in a single outbreak **(Lõhmus & Björklund, 2015)**.

Vibrio alginolyticus is one of the most dangerous pathogens in marine aquaculture, causing serious losses among many fish and shellfish species **(Austin *et al.*, 2012; Kang *et al*., 2016)**. Skin darkening, scale loss, hemorrhages on numerous regions of the body surface, including the mouth, base of fins, abdomen, opercula and around the anal opening, eroded fins and ascites were all observed in *V. alginolyticus* infected fish **(Elsayed *et al*., 2021)**. Vibriosis is linked to high salinity (30-35ppt), high temperature, parasite infestation and mechanical damage **(Haenen *et al*., 2014; El-Bouhy *et al.*, 2016; Abdelaziz *et al.*, 2017)**.

Infestation with *Nerocila phaiopleura* isopod induce vibriosis in *Stolephorus commersonii* **(Rajkumar *et al*., 2007)**. In this context, **(Younes *et al*., 2016b)** discussed a correlation between *N. orbigny* isopod and bacterial infections (*V. alginolyticus, A. sobria* and *S. aureus*), causing sever mortalities among *Tilapia zilli* and *Solea vulgaris*. Though our previous study illustrated concurrent isopod infestation and vibriosis among *T. zilli* and *S. aegyptiaca* (**El-kabany *et al.*, 2023)**, little is known about the host-parasite relationship in inducing the disease. Therefore, the present study was designed to investigate the phenotypic and genotypic characterization of *V. alginolyticus* recovered from both fish and isopod that may correlate isopod infestation to the transmission of *V. alginolyticus* in *T. zilli* and *S. aegyptiaca* fish in Egypt's marine environment.

MATERIALS AND METHODS

1. **Fish sampling**

During the summer of 2019, 120 dead moribund fish (44 *T. zilli* and 76 *Solea aegyptiaca*) were gathered from the marine environment of the Deeba triangle, Manzla lake and Qarun Lake in the Fayoum province. After being properly identified, samples were taken to the lab for additional analysis.

2. **Chemical analysis of water**

Water samples were collected**;** temperature, dissolved oxygen, pH and salinity were measured *in situ* using digital thermometer (HANNA®, range 0–100°C), Do meter (HI 9147- Europe, Romania), digital pH meter (Jenway® Model: 550) and salinometer (Portable optical TDS salinometer/ refractometer®). Total hardness, chlorides (CL), ammonia (NH3), nitrate (NO3) and nitrite (NO2), Sulfates (SO4) and phosphorus(PO4) were determined according to the methods of **APHA (2005)**. Iron was also determined using specific kit (HI-3834 Iron test kit). Results were compared to recommended permissible limits of **USEPA (2006)**.

3. Bacterial isolation

3.1. Fish

32 *vibrio* strains were isolated from freshly dead moribund infested fishes; 18 strains from *T. zilli* in deeba triangle, Manzla Lake and 14 strains from *S. aegyptiaca* from Qaroun Lake **(El-kabany *et al.*, 2023)**.

3.2. Isopods

Isopods were removed from the fish body surface, gills and branchial region using fine-tipped sterile forceps. After identification of samples, collected isopods were sent to the laboratory for parasitological identification and bacterial isolation. For bacterial isolation, isopods were washed three times using phosphate buffer saline and surface-sterilized with 70% Ethanol. After blotting their bodies dry, they were pooled, homogenized and serially diluted in a sterile physiological saline solution **(Delhoumi *et al*., 2020)**. All prepared samples were then inoculated into brain heart infusion broth (BHIB; HIMEDIA, India) and incubated at 28°C for 24h. A loopful of the obtained broth culture was then streaked on the *Vibrio* selective media Thiosulfate-citrate-bile salt-Sucrose (TCBS; HIMEDIA, India) supplemented with (2% NaCl) at 28°C for 24h.

3.3. Water

Bacterial isolation from water samples was performed according to **(APHA, 2005*)***. Briefly: Ten-fold serial dilutions were made aseptically then streaked on TCBS media at 28°C for 24h. Pure colonies were stored in BHI + 15% (vol/ vol) glycerol at- 20 °C for further identification.

4. Bacterial Identification

4.1. Morphological and biochemical examination

Pure cultures isolated from fish, isopod and water were identified morphologically, based on colony, Gram staining and motility, and biochemically according to the study of **(Brenner *et al*., 2005)**.

4.2. Antimicrobial susceptibility test

Isolates of *V. alginolyticus* were tested for antimicrobial susceptibility by Disk diffusion method **(Bauer *et al*., 1966**) on Mueller–Hinton agar (Oxoid, UK) with 2%

(w/v) NaCl. Result of each antimicrobial was classified as susceptible (S), resistant (R), or intermediately resistant (I) according to the guidelines developed by **CLSI (2020)**. The following antimicrobial discs (HIMEDIA) were used: ampicillin (AMP; 10µg), ciprofloxacin (CIP; 5µg), erythromycin ((E; 15µg), linomycin (L; 2µg), oxytetracycline (OTC; 30 µg), Imipenem (IPM; 10 µg), trimethoprim-sulfamethoxazole (SXT; 25 µg), gentamicin (GEN; 10 µg), rifampicin (RIF; 5 µg), colistin (CL; 10 µg) & penicillin (P; 10 µg).

4.3. Genotypic identification

DNA was extracted using boiling technique **(Devi *et al.*, 2009)**. *V. alginolyticus* was identified by species-specific PCR using collagenase gene according to **Di Pinto *et al*. (2005)**. Genetic relatedness was performed by the amplification of partial *Vibrio* 16S rRNA according to **Montieri *et al.* (2010)**. The 16S rRNA PCR products of 3 strains from fish and its isopod and from water in Deeba triangle were sequenced using Genetic Analyzer 3500 (Applied Biosystems) and blasted at the NCBI Blast home page (https://blast.ncbi.nlm.nih.gov/Blast/). Multiple alignments were conducted using the BioEdit Clustal W program (version 7.0.1.4). A neighbour-joining phylogenetic tree was built using MEGA X software version 7.0 **(Kumar *et al.*, 2018)**.

RESULTS

1. Water analysis

The physiochemical analysis of 3 water samples from both Deeba triangle and Qaroun Lake showed bad water quality of high temperature, low levels of dissolved oxygen and high values of almost all chemical parameters (Table 1).

2. Postmortem examination

The postmortem examination of isopoda infested fish revealed opened mouth, skin darkness, extensive external hemorrhages all over the body, around the head and at the base of the fins, abdominal distention, turbid eye, abrasions, body emaciation, detached scales, external excessive mucous secretion, presence of one or more than one isopod in gill chamber (unilateral or bilateral) or on the body surface attached to skin and slight protrusion of gill cover (operculum) (Fig. 1).

Fig. 1. Infested *T. zilli* showing open mouth, turbid eye, external excessive mucous secretion, hemorrhage on slightly protruded gill cover and abdomen and slight abdominal distention

Table 1. Physicochemical analysis of water samples in Manzla and Qaroun Lakes

	Manzla Lake	Qaroun Lake	Permissible limits (USEPA, 2006)
Temp.(°C)	31.5	29.9	8 – 28[a]
pH	9	8.7	6.5–8.5
DO(mg/L)	4.2	4.7	> 5[a]
Salinity	34.13	36.6	Varies according to geographical region
Hardness (mg/L)	737	650	>300 mg/l considered Hard water
Chloride (mg/L)	769.6	405	250 mg/l
Ammonia (mg/L)	2.3	1.5	0.05–0.5 mg/l
Nitrite (mg/L)	3.3	1.5	0.01–0.03 mg/l
Nitrate (mg/L)	7.5	6.2	2-5 mg /l
Sulphate (mg/L)	146.7	214	200 mg/l
Phosphate (mg/L)	2.7	2.4	0.5–0.7 mg/l
Iron	2.7	2	1

[a] (CCME, 2007)

The necropsy findings showed accumulation of serous fluids in the abdominal cavity, congested spleen, kidney and liver, while in other advanced cases, the liver appeared pale.

3. Isopod Identification

Two species of cymothoids (*Crustacea,:Cymothoidae*) were detected; *Nerocila orbignyi* in Manzla Lake (Fig. 2), while in Qaroun Lake, *N. orbignyi* and *Levonica redmanii* were found (Fig. 3).

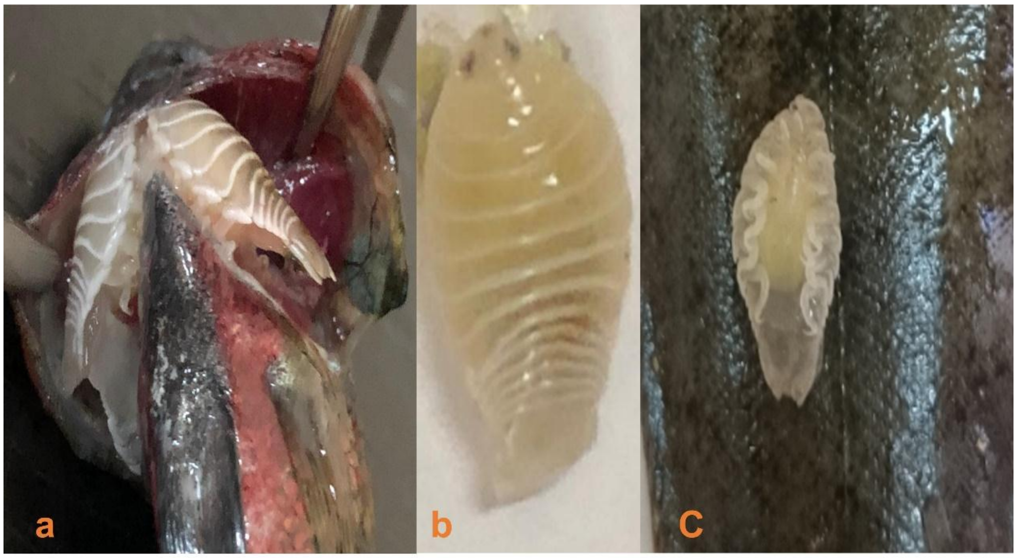

Fig. 2. (a) *N. orbignyi* isopod infesting both gills of *T. zilli* in Deeba triangle, Manzla Lake; **(b)** Dorsal view of *N. orbignyi*; **(c)** Ventral view of *N. orbignyi*

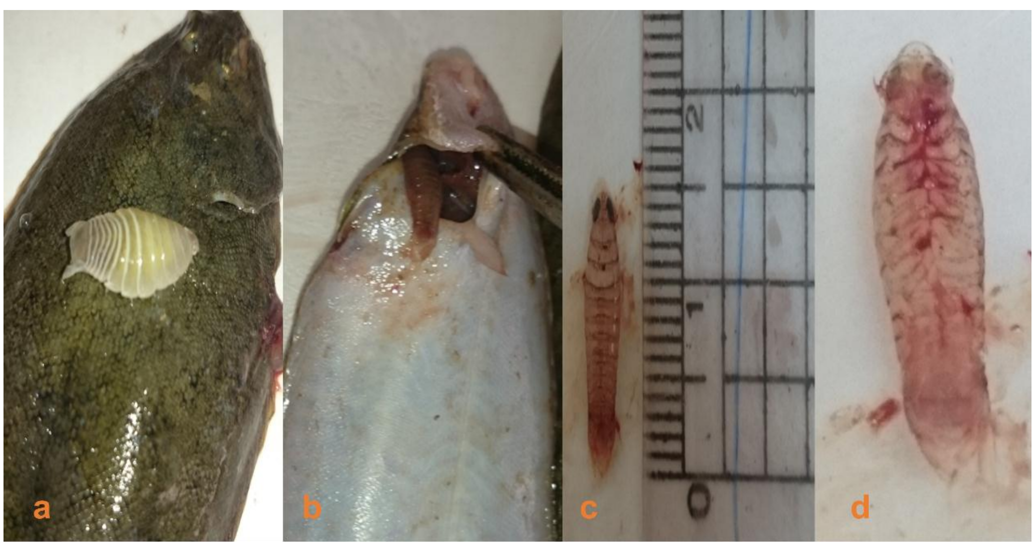

Fig. 3. (a) *N. orbignyi* isopod attached to *S. aegyptiaca* in Qaroun Lake; **(b)** *Levonica redmanii* infesting gills of *S. aegyptiaca* in Qaroun Lake; **(c)** Dorsal view of *L. redmanii*; **(d)**Ventral view of *L. redmanii*

4. Phenotypic characterization

From Manzla Lake, 18 isolates were retrieved from both *T.zilli* and their isopods. While from Qaroun lake, 14 isolates were purified from both *S. aeqyptiaca* and their isopods. From water samples, 3 strains were isolated from both Manzla and Qaroun Lakes. On TCBS agar plates, large greenish yellow colonies of *V. alginolyticus* were observed. Under microscope, Gram-negative short comma- shaped curved rods and motile bacteria were viewed. Isolates from fish and their isopod and from water samples showed the same biochemical characteristics. All isolates were positive in oxidase, catalase, indole, Vogas Proskauer, methyl red production, fermentation of glucose and sucrose, gelatin hydrolysis and nitrate reduction. Isolates were all negative in urease and H_2S production.

Table (2) shows antimicrobial sensitivity of *V. alginolyticus* strains isolated from Manzla Lake. All isolates from fish and their isopod showed 100% susceptibility profile agreement for all tested antibiotics. Water isolates showed almost the same profile that was reported in fish and isopod isolates. All strains were sensitive to erythromycin, imipenem and Trimethoprim /sulphamethoxazole (SXT). Oppositely, they were resistant to ampicillin, oxytetracycline, lincomycin, rifampicin, colistin and penicillin. Intermediate resistance was observed for ciprofloxacin and gentamycin.

Table 2. Antimicrobial sensitivity of *V. alginolyticus* isolates fish (n = 18), isopod (n = 18), water (n = 3) in Deeba triangle, Manzla Lake

	Fish sample			**Isopod samples**			**Water samples**		
	S	I	R	S	I	R	S	I	R
Amp10		2 11.1%	16 88.9%		2 11.1%	16 88.9%		1 33.3%	2 66.7%
CIP5	10 55.6%	8 44.4%		10 55.6%	8 44.4%		1 33.3%	2 66.7%	
E15	18 100%			18 100%			3 100%		
L2			18 100%			18 100%			3 100%
OTC30		2 11.1%	16 88.9%		2 11.1%	16 88.9%		1 33.3%	2 66.7%
IPM 10	18 100%			18 100%			3 100%		
SXT 25	18 100%			18 100%			3 100%		
GEN 10	11 61.1%	7 38.9%		11 61.1%	7 38.9%		1 33.3 %	2 66.7%	
RIF 5			18 100%			18 100%			3 100%
CL 10			18 100%			18 100%			3 100%
P 10			18 100%			18 100%			3 100%

Table (3) displays antimicrobial sensitivity of *V. alginolyticus* strains isolated from Qaroun Lake. All isolated strains from water, fish and their isopod showed 100% susceptibility profile agreement for all tested antibiotics. All isolates were sensitive to ciprofloxacin, erythromycin, oxytetracycline, imipenem, SXT, gentamycin and rifampicin. While, they were resistant to ampicillin, lincomycin, colisitin and penicillin.

Table 3. Antimicrobial sensitivity of *V. alginolyticus* isolates for fish (n = 14), isopod (n = 14), water (n = 1) in Qaroun Lake

	Fish samples			Isopod samples			Water samples		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Amp10			14 100%			14 100%			1 100%
CIP5	14 100%			14 100%			1 100%		
E15	14 100%			14 100%			1 100%		
L2			14 100%			14 100%			1 100%
OTC30	14 100%			14 100%			1 100%		
IPM 10	14 100%			14 100%			1 100%		
SXT 25	14 100%			14 100%			1 100%		
GEN 10	14 100%			14 100%			1 100%		
RIF 5	14 100%			14 100%			1 100%		
CL 10			14 100%			14 100%			1 100%
P 10			14 100%			14 100%			1 100%

5. Genotypic characterization

V. alginolyticus was confirmed by species-specific PCR using collagenase gene. All tested isolates showed the characteristic *V. alginolyticus* amplicon size of 737 bp.

The partial sequences for *V. alginolyticus 16S rRNA* gene from Deeba triangle, Manzla Lake were submitted with accession numbers (MZ413925), (MZ423872), (MZ497102) for fish, isopod and water, respectively, in the Gene Bank https://blast.ncbi.nlm.nih.gov/Blast. The derived sequences were clustered with their relevant sequences using neighbor-joining phylogenetic tree. The phylogenetic analysis showed two major clades. The first clade contains our *V. alginolyticus* isolates which are embedded among other *V. alginolyticus* isolates with >96-97% strong bootstrap value, as demonstrated in Fig. (4). *V. alginolyticus* isolates from fish and isopod showed 100% similarity with each other and 99.23% similarity with water strain. *V. alginolyticus* strains, derived from fish, isopod and water showed identity of 99.85%, 99.84% and 99.56% respectively, with *V. alginolyticus* (MK102582-KR347340) from china, *V. alginolyticus* LC456723 from Indonesia, *V. alginolyticus* MG438511 from Thailand and *V. alginolyticus* KT005561 from India.

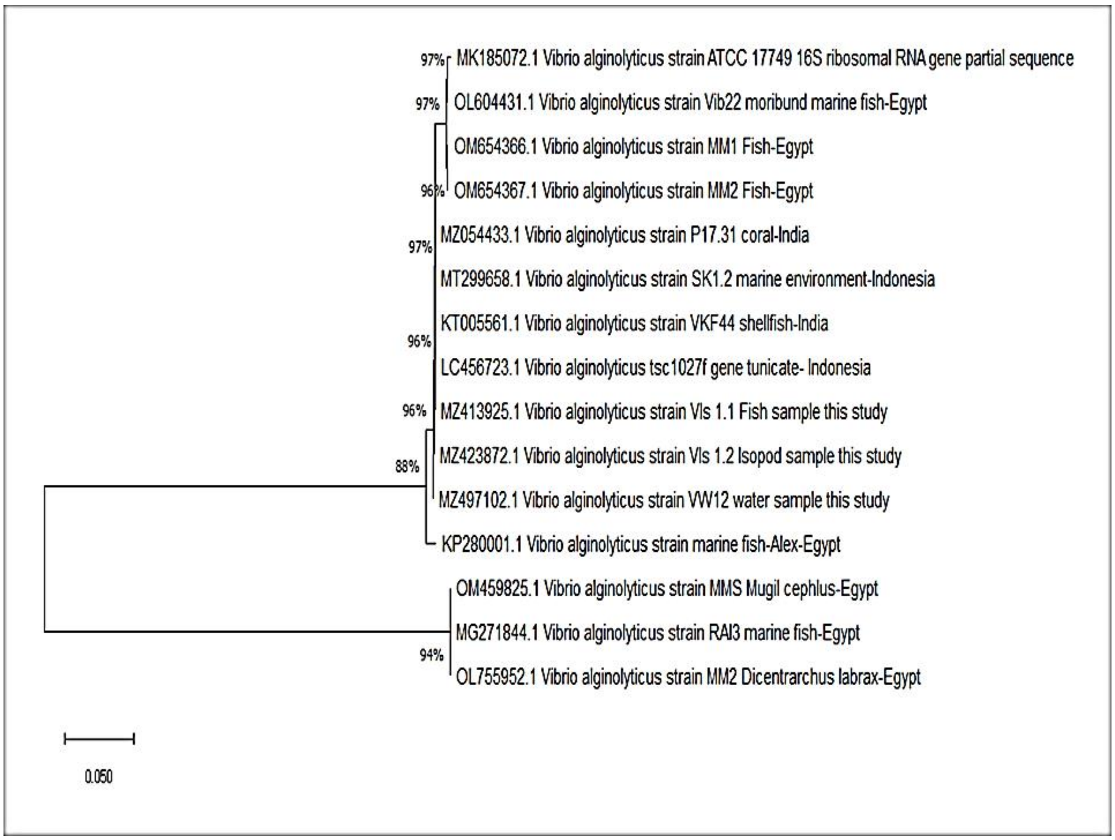

Fig. 4. Neighbor-joining phylogenetic tree showing sequences of *V. alginolyticus* partial *16S rRNA* gene with accession numbers (MZ413925), (MZ423872), (MZ497102) for fish, isopod and water, respectively, and the most closest other sequences.

DISCUSSION

Cymothoid isopods, the most frequent category of fish crustacean parasites are known to have harmful impacts on fish. Hence, their host-parasite relationship must receive a lot of attention **(Mariasingarayan *et al.*, 2020)**. This study investigated the phenotypic and genotypic characterization of *V. alginolyticus* recovered from both fish and isopod that may correlate isopod infestation to the transmission of *V. alginolyticus* in *T. zilli* and *S. aegyptiaca.*

Unproper water physicochemical factors have a strong impact on fish health and their resistance against diseases **(Younes *et al*., 2016a**; **Abou-Okada *et al*., 2017)**. In addition, increased water temperature contributes to higher prevalence of parasitic infestations and bacterial invasions of fish **(Karvonen *et al.*, 2010)**. In our study, high water temperature was recorded in both Manzla and Qaroun; (31.5℃) and (29.9℃), respectively. This is the optimum temperature for *V. alginolyticus* **(Austin *et al.*, 2012)**. Moreover, *V. alginolyticus* adhesion on intestinal mucus, a temperature-related virulence factor, occurs at 30℃ **(Yan *et al.*, 2007)**. Water temperature also had a significant positive correlation with the isopod infestation rate **(Mahmoud *et al*., 2019)**. In this study, pH level of 9.0 and 8.7 was recorded in Deeba triangle and Qaroun Lake, respectively. This pH is slightly different from that recorded in the study of **Marcogliese and Cone (1996)** who stated that, alkaline pH has slight effect on the parasite abundance. For salinity, we observed 34.13 ppt and 36.6 ppt in Deeba triangle and Qaroun Lake, respectively. High water salinity has been strongly correlated with *Vibrio* infections in fish **(Abdelaziz *et al.*, 2017)**. In addition, The synthesis of peritrichous flagella by *V. alginoliticus*, which are responsible for a swarming movement on solid surfaces, depends mainly on temperature and salt concentration **(Ulitzur, 1975)**. The present study found high ammonia levels in both Manzla and Qaroun; (2.3 mg/L), (1.5 mg/L), respectively. This result agrees with the findings of **Ahmad *et al.*** (**2016**) and **Mahmoud *et al*. (2019)** who found a positive relation between ammonia and isopod infestation rate. Iron level (2.7 mg/l), (2mg/l) in Deeba triangle and Qaroun Lake, respectively, was higher than the permissible limits. Dissolved oxygen; (4.2 mg/L), (4.7 mg/L) was lower than normal acceptable range. **(Chen *et al*., 2011)** explained that the sharp decline in dissolved oxygen accompanied by high levels of ammonia and iron are most likely factors that trigger infection development and impair fish immune system.

Isopods infest many species of fish worldwide, resulting in major economic losses **(Ravichandran *et al*., 2011)**. At present, little is known about the role of isopods as probable vector for bacterial diseases. During the current study, two cymothoid isopods were found: *L. redmanii* identified according to **Brusca (1981)** and *N. orbignyi* identified according to **Al-zubaidy and Mhaisen (2013)**. *N. orbignyi* was isolated from gill chambers of *T. zilli* in Deeba triangle. This result coincides with those of **Abdel-latif (2016)** and **Younes *et al.* (2016b)**. For *S. aegyptiaca, N. orbignyi* was isolated from the skin. This agrees with the studies of **Samn *et al*. (2014)** and **Mahmoud *et al.* (2016)***.* *L. redmanii* was collected in the present work from the gills of *S. aegyptiaca*, which is the same site of infestation reported in the study of **Khalaf-allah and Yousef (2019)**. Infested fish showed slight protrusion of gill cover (operculum), atrophy and hemorrhage at attachment site; this may be due to feeding activity, attachment, fixation and locomotion of isopod, resulting in a decrease in the respired oxygen levels of damaged gill epithelium. These results are in agreement with those of **Ali and Aboyadak (2018)** and **Khalaf-allah and Yousef (2019)**. Other common recorded clinical signs for *T.zilli* and *S. aegyptiaca* were darkened external body surfaces, hemorrhage on several parts of the body surface, mouth, base of fins, abdomen, opercula and around the anal opening, swollen abdomen, excessive mucus secretion, turbid eyes, ulcers and detached scales. This result is supported by previous studies **(El-Bouhy *et al*., 2016; Abdelaziz *et al*., 2017; Khafagy *et al.* 2021)**. While, the necropsy findings are nearly similar to those of **Abdelaziz *et al*. (2017)** and **El-Sayed *et al*. (2019)**.

Regarding *V. alginolticus* characterization, all fish, isopods and water isolates exhibited the same phenotypic characters. Our results concur with those of **Khafagy *et al*. (2021)**. In the current study, *V. alginolyticus* strains from fish, isopod and water in Manzla and Qaroun Lakes showed the exact antimicrobial susceptibility profile. They were susceptibile to erythromycin (100%), imipenem (100%) and trimethoprim/sulphamethoxazole (SXT) (100%) while resistant to ampicillin; (66 - 100%), lincomycin (100%), colistin (100%) and penicillins (100%). These results are in consent with those of **Abd El Tawab *et al*. (2018)** and **Khafagy *et al.* (2018)**. Isolates showed high resistance to oxytetracyline and rifampcin in Deeba triangle, whereas they were highly sensitive to them in Qaroun Lake. On the other hand, isolates showed intermediate susceptibility to ciprofloxacin and gentamycin in Deeba triangle, this was in complete accordance with **(Aly *et al.* 2019),** while they were highly sensitive to ciprofloxacin and gentamycin in Qaroun Lake; this finding in consistent with those recorded in previous studies **(El Sayed *et al*., 2019; Sadat *et al*., 2021)**. Variations in the results of antibiotic sensitivity tests between Manzla Lake and Qaroun Lake may be due to environmental pressure of repeated usage of antibiotics in previous outbreaks leading to the dramatic growth of antimicrobial resistance **(Abdel-Aziz *et al*., 2013)**, making it urgent to make antibiogram susceptibility test before application of antimicrobial agents in aquaculture farms to overcome problems of antimicrobial resistance.

In this study, phenotypic, biochemical and PCR identification of our *V. alginolyticus* isolates were confirmed by 16S rRNA sequence analysis. Phylogenetic tree exhibited that *V. alginolyticus* isolates from fish and isopod showed 100% similarity with each other and 99.23% similarity with water strain, confirming that fish and isopod strains are of the same origin. This result strongly suggests the transmission of *V. alginolyticus*, either from fish to isopod or from isopod to fish. Even though the isopod has got *V. alginolyticus* from the hosted fish, it could be able to detach and attack another fish, with the possibility of transmitting the infection to the new host. Our results are supported by recent findings of **Goffredi *et al.* (2023)** who found that, *Vibrio* (100% prevalence) dominated the microbiomes of marine obligate blood-feeders (OBF), including isopods (*Nerocila*), and that these microbiomes have been low in range as compared to host fish skin surfaces and seawater. Fluorescence microscopy evaluation revealed that *Vibrio* cells were limited to the digestive lumen inside and across the blood meal for all examined OBF species.

In conclusion, the deterioration of water quality by sewage and agriculture discharges strongly related to high prevalence of isopod could be a potential vector in the transmission of *V.alginolyticus* in *T.zilli* and *S. aegyptiaca* fish inhabiting Manzla and Qaroun Lakes.

REFERENCES

Abd El Tawab, A. A.; Ibrahim, A. M. and Sittien, A. E. S. (2018). Phenotypic nd Genotypic characterization of *Vibrio* species isolated from marine fishes. Benha Veterinary Medical Journal, 34(1): 79–93.

Abdelaziz, M.; Ibrahem, M. D.; Ibrahim, M. A.; Abu-Elala, N. M. and Abdel-moneam, D. A. (2017). Monitoring of different *vibrio* species affecting marine fishes in Lake Qarun and Gulf of Suez : Phenotypic and molecular characterization. The Egyptian Journal of Aquatic Research. 43(2): 141–146. doi: 10.1016/j.ejar.2017.06.002.

Abdel-Aziz, M.; Eissa, A. E.; Hanna, M. and Abou-Okada, M. (2013). Identifying some pathogenic *Vibrio*/ *Photobacterium* species during mass mortalities of cultured Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) from some Egyptian coastal provinces. International Journal of Veterinary Science and Medicine, 1(2): 87–95. doi: 10.1016/j.ijvsm.2013.10.004.

Abdel-latif, H. M. R. (2016). Cymothoid Parasite, *Nerocila Orbigni* Inflicts Great Losses on *Tilapia Zilli* in Lake Qarun at El-Fayoum Province. International Journal of Innovative Studies in Aquatic Biology and Fisheries, 2(3): 1–9. doi: 10.20431/2454-7670.0203001.

Abou-Okada, M.; Abdel-Aziz, M. and Hanna, M. (2017). Characterization and Pathogenicity of *Vibrio Alginolyticus* Isolated From Diseased Cultured Gilthead Seabream, Journal of the Egyptian Veterinary Medical Association, 77(4): 865 – 877.

Addo, S., Carrias, A. A., Williams, M. A., Liles, M. R., Terhune, J. S., & Davis, D. A. (2017). Effects of *Bacillus subtilis* strains and the prebiotic Previda® on growth, immune parameters, and susceptibility to *Aeromonas hydrophila* infection in Nile tilapia, *Oreochromis niloticus*. Aquaculture Research, 48(9): 4798-4810.

Ahmad, I.; Afshan, K.; Ramzan, M.; Hayat , S.; Rizvi, S. S. R. and Qayyum, M. (2016). Effect of water quality parameters on isopod parasite *alitropus typus* (aegidae) of ectotherms in chashma lake, Pakistan. Pakistan Journal of Zoology, 48(3): 769–779.

Ali, N. G. and Aboyadak, I. M. (2018). Histopathological alterations and condition factor deterioration accompanied by isopod infestation in *Tilapia zilli* , *Mugil capito* and *Solea aegyptiaca* from Lake Qaroun. The Egyptian Journal of Aquatic Research, 44(1): 57–63. doi: 10.1016/j.ejar.2018.03.001.

Aly, S. M.; Eisa, A. A. and Elbanna, N. (2019). Characterization of *vibrio Alginolyticus* Infection in gilthead seabream (*Sparus auratus*) cultured in Egypt. Suez Canal Veterinary Medical Journal (SCVMJ), 24(1): 553–571. doi: 10.21608/EJABF.2020.76562.

Al-Zubaidy, A. B. and Mhaisen, F. T. (2013). The First Record of Three Cymothoid Isopods from Red Sea Fishes, Yemeni Coastal Waters, International Journal of Marine Science, 3(21): 166-172. doi: 10.5376/ijms.2013.03.0021.

APHA (American Public Health Association) (2005). Standard Methods for the Examination of Water and Wastewater. 21st ed. Washington, DC, USA.

Austin, B.; Austin, D.A. and Munn, C.B. (2012). Bacterial Fish Pathogens; Springer: Berlin/Heidelberg, Germany,; ISBN 9400748841.

Barber, I., & Poulin, R. (2002). Interactions between fish, parasites and disease. Handbook of fish biology and fisheries. Volume 1: Fish biology, 359-389.

Bauer, A. W.; Kirby, W. M.; Sherris, J. C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology, 45: 493-496.

Brenner, D. J.; Krieg, N. R.; Staley J. T. and Garrity, G. M. (2005). Bergey's Manual of Systematic Bacteriology, 2nd Edition, Vol. 2 (The Proteobacteria), part C (The Alpha-, Beta-, Delta-, and Epsilonproteobacteria), Springer, New York.

Brusca, R. C. (1981). A monograph on the Isopoda *Cymothoidae* (Crustacea) of the eastern Pacific. Zoological Journal of the Linnean Society, 73(2): 117–199. doi: 10.1111/j.1096-3642.1981.tb01592.x.

Callaway, R.; Shinn, A. P.; Grenfell, S. E.; Bron, J. E.; Burnell, G.; Cook, E. J.; ... & Shields, R. J. (2012). Review of climate change impacts on marine aquaculture in the UK and Ireland. Aquatic Conservation: Marine and Freshwater Ecosystems, 22(3): 389-421. doi: 10.1002/aqc.2247.

CCME (Canadian Council of Ministers of the Environment) (2007). For the protection of aquatic life. In: Canadian environmental quality guidelines, 1999. Canadian Council of Ministers of the Environment, 1999, Winnipeg.

Chen, M. X.; Li, H. Y.; Li, G. and Zheng, T. L. (2011). Distribution of *vibrio alginolyticus*-like species in Shenzhen coastal waters, China. Brazilian Journal of Microbiology,42(3): 884–896. doi: 10.1590/S1517-83822011000300007.

CLSI (Clinical and Laboratory Standards Institute) (2020). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA

Delhoumi, M.; Catania, V.; Zaabar, W.; Tolone, M.; Quatrini, P. and Achouri et al. (2020). The gut microbiota structure of the terrestrial isopod *Porcellionides pruinosus* (Isopoda: *Oniscidea*). European Zoological Journal, 87(1): 357–368. doi: 10.1080/24750263.2020.1781269.

Devi, R.; Surendran, P. K. and Chakraborty, K. (2009). Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from shrimp farms along the Southwest coast of India. World Journal of Microbiology and Biotechnology, 25(11): 2005–2012. doi: 10.1007/s11274-009-0101-8.

Di Pinto, A.; Ciccarese, G.; Tantillo, G.; Catalano, D. and Forte, V. T.(2005). A Collagenase-Targeted Multiplex PCR Assay for Identification of *Vibrio alginolyticus* , *Vibrio cholerae* , and *Vibrio parahaemolyticus*. Food Protection, 68(1): 150–153.

El-Bouhy, Z.; El-Nobi, G.; El-Murr, A. and Abd El-Hakim, S. (2016). Study On Vibriosis In *Mugil Capito* In El-Dakahlia And Damitta Governorates, Egypt. Abbassa Int. J. Aquat., 9(1): 19–35.

El-kabany, N. M.; Badawy, M. F.; Laban, S. E. and Ismail, T. F. (2023). Natural Parasitic and Bacterial Coinfection in Some Fish Species in Egypt. Egyptian Journal of Aquatic Biology and Fisheries, *27*(1): 319-334.

EL-Sayed, M. E.; Algammal, A., M.; Abouel-atta, M. E.; Mabrok, M. and Emam, A. M. (2019). Pathogenicity, genetic typing and antibiotic sensitivity of *Vibrio alginolyticus* isolated from *Oreochromis niloticus* and *Tilapia zillii*. Revue de Médecine Vétérinaire, 170(4-6): 80-86.

ELSayed, M. R.; Osman, A. E.; Emam, A. M.; Abd el-galil, M. A. A. and Sayed, H. H. (2021). Studies On *Vibrio Alginolyticus* Infection Among Some Red Sea Fishes At Hurghada Mohamed.Assiut Veterinary Medical Journal, 67(170): 37–50.

Goffredi, S. K., Appy, R. G., Hildreth, R., & deRogatis, J. (2023). Marine vampires: Persistent, internal associations between bacteria and blood-feeding marine annelids and crustaceans. Frontiers in Microbiology, 13.

Haenen, O. L. M.; Zanten, E. V.; Jansen, R.; Roozenburg, I.; Engelsma, M. Y.; Dijkstra, A.; Boers, S. A.; Voorbergen-Laarman, M. and Möller, A. V. M.(2014). *Vibrio vulnificus* outbreaks in Dutch eel farms since 1996: Strain diversity and impact. Diseases of Aquatic Organisms, 108(3): 201–209. doi: 10.3354/dao02703.

Horton, T. and Okamura, B. (2001). Cymothoid isopod parasites in aquaculture: a review and case study of a Turkish sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus auratus*) farm. Diseases of Aquatic Orgaisms, 46: 181-188.

Kang, C. H.; Shin, Y.; Jang, S.; Jung, Y. and So, J. (2016). Antimicrobial susceptibility of *Vibrio alginolyticus* isolated from oyster in Korea. Environmental Science and Pollution Research, 23(20): 21106–21112. doi: 10.1007/s11356-016-7426-2.

Karvonen, A.; Rintamäki, P.; Jokela, J. and Valtonen, E. T. (2010). Increasing water temperature and disease risks in aquatic systems: Climate change increases the risk of some, but not all, diseases. International Journal for Parasitology. Australian Society for Parasitology Inc., 40(13): 1483–1488. doi: 10.1016/j.ijpara.2010.04.015.

Khafagy A. R.; Essawy, A. E.; Noura, H. A.E. and Ayoub, H. F. (2021). Prevalence and Antimicrobial susceptibility of Pathogenic Bacteria in *Tilapia zilli* and *Mugil capito*. Suez Canal Veterinary Medical Journal. SCVMJ, 26(1): 155–169. doi: 10.21608/scvmj.2021.78822.1042.

Khafagy, A. A. R.; Farag, A. A.; Ibrahim, M. S. and Ibrahim,R. A.(2018). Isolation, identification and antibiotic resistance of *Vibrio alginolyticus* isolated from *Mugil seheli* - Suez Governorate, Egypt. Egyptian Journal for Aquaculture, 8(2): 1–16. doi: 10.21608/eja.2018.36229.

Khalaf-allah, H. M. M. and Yousef, O. E. A. (2019). Infestation Study of *Livoneca Redmanii* (Isopoda, Cymothoidae) on *Solea Solea* in Lake Qarun, Egypt. Journal of the Egyptian Society of Parasitology, 49(1): 105–114. doi: 10.21608/jesp.2019.68292.

Kumar, S.; Stecher, G.; Li, M.; Knyaz, C. and Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35(6): 1547–1549. doi: 10.1093/molbev/msy096.

Lõhmus, M. and Björklund, M. (2015). Climate change: What will it do to fish-parasite interactions. Biological Journal of the Linnean Society, 116(2): 397–411. doi: 10.1111/bij.12584.

Mahmoud, N. E.; Fahmy, M. M.; Abuowarda, M. and Khattab, M. S. (2016). Parasitic cymothoid isopods and their impacts in commercially important fishes from Lake Qarun, Egypt. International Journal of Chem. Tech. Research, 9(12): 221–229.

Mahmoud, N. E.; Fahmy, M. M.; Abuowarda, M. M.; Zaki, M. M.; Ismael, E.and Ismail E. M.(2019). Influence of water Quality Parameters on the Prevalence of *Livoneca redmanii* (Isopoda; Cymothoidae) Infestation on Mediterranean Sea Fishes, Egypt. International Journal of Veterinary Science, 8(3): 174–181.

Marcogliese, D. J. and Cone, D. K. (1996). On the distribution and abundance of eel parasites in Nova Scotia: Influence of pH. Journal of Parasitology, 82(3): 389–399. doi: 10.2307/3284074.

Mariasingarayan, Y.; Danaraj, J.; Perumal, A. & Narayanasamy, R. (2020). Two new host records of the cymothoid isopods from Rameswaram Island, southeast coast of India. Journal of Parasitic Diseases, 44: 486-490.

Montieri, S.; Suffredini, E.; Ciccozzi, M. and Croci, L. (2010). Phylogenetic and evolutionary analysis of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* isolates based on toxR gene sequence. New Microbiologica, 33(4): 359-372.

Rajkumar, M.; Thavasi, R.; Perumal, P. and Trilles, J. (2007). Parasite induced vibriosis in *Stolephorus commersonii*. Research Journal of Microbiology, 2(12): 972–977. doi: 10.3923/jm.2007.972.977.

Rameshkumar, G. and Ravichandran, S. (2014). Problems caused by isopod parasites in commercial fishes. Journal of Parasitic Diseases, 38(1): 138–141. doi: 10.1007/s12639-012-0210-4.

Ravichandran, S.; Rameshkumar, G. and Trilles, J. P. (2011). New records of two parasitic cymothoids from Indian fishes. Journal of Parasitic Diseases, 35(2): 232–234. doi: 10.1007/s12639-011-0046-3.

Ravichandran, S.; Sivasubramanian, K.; Parasuraman, P.; Rajan, D. K. and Kumar, J. R. (2016). Isopod Parasite Induced Secondary Microbial Infection in Marine Food Fishes. Journal of fish pathology, 29(1): 1–5. doi: 10.7847/jfp.2016.29.1.001.

Sadat, A.; El-Sherbiny, H.; Zakaria, A.; Ramadan, H., and Awad, A. (2021). Prevalence, antibiogram and virulence characterization of *Vibrio* isolates from fish and shellfish in Egypt: a possible zoonotic hazard to humans. Journal of Applied Microbiology, 131(1): 485–498. doi: 10.1111/jam.14929.

Samn, A. A. A. M. ; Metwally, K. M. ; Zeina, A. F. and Allah, H. M. M. K. (2014). First occurrence of *Nerocila bivittata*: parasitic isopods (skin shedders) on *Lithognathus mormyrus* (*Osteichthyes, Sparidae*) from Abu Qir Bay, Alexandria, Egypt.The journal of American Science, 10(7):171-179.

Ulitzur, S. (1975). Effect of temperature, salts, pH and other factors on the development of peritrichous flagella in *Vibrio alginolyticus*. Archives of Microbiology, 104: 285-288.

USEPA (United States Environmental Protection Agency) (2006). risk-based concentration table. www.epa.gov/reg3hwmd/risk/human/rbc/rbc1006.pdf

Xu, D.; Shoemaker, C. A. and Klesius, P. H. (2012). *Ichthyophthirius multifiliis* as a potential vector of *Edwardsiella ictaluri* in channel catfish. FEMS Microbiol Lett., 329(2): 160-167. doi:10.1111/j.1574-6968.2012.02518.x

Yan, Q.; Chen, Q.; Ma, S.; Zhuang, Z.;Wang, X. (2007). Characteristics of adherence of pathogenic *Vibrio alginolyticus* to the intestinal mucus of large yellow croaker (*Pseudosciaena crocea*). Aquaculture, 269: 21–30.

Younes, A. M.; Fares, M. O.; Gaafar, A. Y. and Mohamed, L. A. (2016a). Isolation of *Vibrio alginolyticus* and *Vibrio vulnificus* Strains from Cultured *Oreochromis niloticus* Around Qarun Lake , Egypt. Global Veterinaria,16(1): 1–5. doi: 10.5829/idosi.gv.2016.16.01.10214

Younes, A. M.; Noor Eldin, A. I. and Abd Ellatif, M. A. (2016b). A contribution of crustacean isopodoa, bacterial infections and physicochemical parameters in mass mortalities among fishes in Lake Qarun. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7(2): 1906–1911.