



Vibriosis triggered mass kills in Pacific white leg shrimp (*Litopenaeus vannamei*) reared at some Egyptian earthen pond-based aquaculture facilities

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ABSTRACT

Vibriosis is a serious bacterial disease causing significant mortalities among farmed marine shrimp. The current study aimed to identify and characterize *Vibrio* spp. incriminated in the mass kills of the *Litopenaeus vannamei* shrimp from earthen ponds located in the vicinity of Lake Manzala in Shatta City, Damietta, Egypt. Moribund shrimp exhibited lethargic swimming behavior and redness/congestion of almost all body appendages and telson. Ponds' water parameters remarkably deteriorated. A high surge in water temperature (32°C), elevated levels of unionized ammonia (0.9 mg/L), and a remarkable decrease in dissolved oxygen (DO) (3mg/L) were all recorded during the period of disease outbreak and mortality episode from July through September 2021. *Vibrio parahaemolyticus* (30 isolates) and *V. alginolyticus* (23 isolates) were exclusively isolated from clinically affected shrimps collected during the mass kills episode. The identity of the retrieved isolates was confirmed through gene sequence and phylogenetic analysis. Histopathological examination revealed different degrees of degeneration, sloughing, and necrotic changes through hepatopancreatic tissues together with marked inflammatory haemocytic infiltrations within the muscular tissues of the affected shrimps. A combined therapeutic strategy was adopted to stop mortalities and enhance shrimp health within the affected shrimp ponds. Permanent baths of hydrogen peroxide 40 % solution (in water) alternated with *Yucca schidigera* extract solution for 1 week,

together with Florfenicol powder at a dosage of 25 mg / Kg shrimp biomass in diet were very effective in reducing mortalities, improving swimming behavior, normalizing feeding intake, and enhancing the general health of the affected shrimps.

INTRODUCTION

Shrimp farming makes a significant contribution to global food security and economy (**Bardera *et al.*, 2021**). Shrimp aquaculture started in Egypt in 1985 (**Sadek *et al.*, 2002**). The industry is steadily growing with the existence of numerous megaprojects to establish a competent shrimp farming sector (**Elgendy *et al.*, 2015**). The total production in 2019 approached 8305 MT, with a value of 103 million US dollars. The aquaculture sector contributes with 2 % of the total shrimp production in Egypt, about 123 MT (**FAO, 2018**). *Penaeus semisulcatus*, *P. japonicas*, and *Metapenaeus stebingi* are among the most common species in Egypt (**Sadek *et al.*, 2002**).

The white leg shrimp, *Litopenaeus vannamei*, is one of the most economically valuable species in the shrimp aquaculture, accounting for more than 70% of the industry's total world output (**FAO, 2018**). The suitability of *L. vannamei* for farming over a wide variety of environmental conditions (e.g., salinity, temperature), its rapid growth rates, and its capacity to withstand high stocking densities are all positive attributes of this shrimp species (**Bondad-Reantaso *et al.*, 2012**). Extensive, semi-intensive, and intensive production systems may be used in commercial shrimp cultivation tactics (**Sadek *et al.*, 2002**). However, to satisfy the increasing demand, the intensification of shrimp production in commercial ponds is growing rapidly (**Elgendy *et al.*, 2015**). Several production outputs, such as growth performance, health, and survivability of shrimp, are compromised as a consequence of overstocking (**Bondad-Reantaso *et al.*, 2012**).

The increasing incidence of infectious diseases in farmed shrimp is commonly coupled with a combination of negative factors, including water quality degradation, accumulation of organic wastes, bad management practices, and the occurrence of abnormal behaviors such as cannibalism (**Elgendy *et al.*, 2015**). All those factors collectively compromise the immune defense mechanisms, making shrimp susceptible to invading pathogens (**Elgendy *et al.*, 2015**).

Vibrios are important pathogens in the global shrimp farming industry. *Vibrio* spp. are ubiquitous in the aquatic environment, and they initiate destructive infections upon environmental deterioration (**Elgendy *et al.*, 2015**). *Vibrio parahaemolyticus*, the causative agent of acute hepatopancreatic necrosis, causes fatal infections, especially at the early life stages of shrimp, with massive mortality exceeding 70% and colossal economic losses. In addition, other *Vibrio* spp., such as *V. splendidus*, *V. harveyi*, *V. alginolyticus*, and *V. mimicus* can cause infections in penaeid shrimp (**Elgendy *et al.*, 2015**; **Kongrueng *et al.*, 2015**; **Soto-Rodríguez *et al.*, 2015**).

The present study aimed to unlock the puzzling etiologies of shrimp mortalities in earthen pond-based facilities at Damietta. Phenotypic, genotypic, and molecular characterization of retrieved isolates, together with the histopathology of moribund shrimps, was an essential asset to determine the definite etiology behind the mass kills. Ultimately, a field-based treatment trial to stop the uprising mortalities and control the disease outbreak was strictly applied through the affected earthen pond facilities.

MATERIALS AND METHODS

Case history and sampling

Five earthen ponds stocked with Pacific white leg (*L. vannamei*) shrimp located in the vicinity of Lake Manzala at Shatta City, Damietta, Egypt, have faced mass mortalities (60% mortalities) during the period from July through September 2021. The shrimp stocking densities were 100.000 /acre through the affected farms. The average body weight of shrimp was 16.5 g. Shrimps were fed a 40 % protein pelleted diet from Skretting® in Egypt. The daily water exchange rate in shrimp ponds was 25%.

Moribund shrimp exhibited lethargic swimming behavior and redness/congestion of almost all body appendages and telson. Pond's water parameters recorded during the disease outbreak and mortality episode were noticeably poor. A high surge in water temperature (32°), elevated levels of unionized ammonia (0.9 mg/L), and a remarkable decrease in dissolved oxygen (DO) (3mg/L) were reported shortly before and during the disease outbreak.

A total of 100 moribund shrimp weighing 16.5 g on average were collected from five earthen ponds through the outbreak area suffering from mass kills (20 samples/farm). Shrimp were transported alive in fiberglass tanks with pond's water supplied with constant oxygen supply to the Aquatic Animal Medicine and Management Laboratory (AAMML), Faculty of Veterinary Medicine, Cairo University Egypt, for clinical, microbiological, molecular and histopathological investigations.

Bacteriological examination

Hepatopancreas from moribund shrimps were aseptically removed and homogenized in microfuge tubes containing 400µl of sterile saline solution (2.5% NaCl). The homogenates (100 µl) were spread onto thiosulphate citrate bile sucrose (TCBS) (Oxoid™) and marine agar plates (Difco™) and then incubated at 30°C for 24h. Random colonies representative of the different colony types obtained on the agar plates were re-streaked onto TCBS and phenotypically identified using Gram staining, sensitivity to O/129 vibriostatic disc, and API 20 E identification system according to the technique of **Buller (2004)**. The stock cultures of the isolates were stored in tryptone soy broth (TSB), supplemented with 2.5% salt and 10% glycerol at –80°C for further analysis (**El-Adawy et al., 2021**).

Antibiotic susceptibility testing

Antibiotic sensitivity testing of the retrieved isolates was performed on Mueller-Hinton agar (Oxoid™) using the disc diffusion method according to **Bauer *et al.* (1966)**. Each isolate was tested against seven antibiotics (Oxoid™); namely, tetracycline 30µg, ciprofloxacin 5µg, florfenicol 30µg, trimethoprim/sulfamethoxazole 1.25/23.75µg, amoxicillin 30µg, ampicillin 10µg and gentamycin 10µg. Briefly, bacterial isolates were inoculated into tryptic soy broth supplemented with 2 % NaCl and incubated overnight at 30°C. An amount of 250µl broth culture was aliquoted onto Mueller Hinton agar plates in triplicates and streaked using L shaped disposable sterile plastic spreader. Streaked plates were left for a while to dry, and then antibiotic discs were placed on the agar surface using an antibiotic stamp dispenser. The stamped plates were incubated for 24h at 30C, and the size of the inhibition zones was measured using standard caliber and matched against standard inhibition zones reported for the suspect *Vibrio* isolates. Isolates were categorized as susceptible (S), intermediate (I) and resistant (R).

Molecular typing of the retrieved isolates

Bacterial DNA was extracted from overnight incubated pure isolates in broth, using the Qiagen DNA extraction kit (Qiagen) following the instruction of the manufacturer. The PCR amplification of the 16S rRNA gene was performed using the universal primer pairs (16S-F: 5'-AGAGTTTGATCCTGGCTCAG-3') and (16S-R: 5'-GGTACCTTGTTACGACTT-3'), following the method of **Weisburg *et al.* (1991)**. PCR was performed in a reaction volume of 50µl consisting of 25µl of Emerald Amp MAX PCR master mix (Takara Bio, Kusatsu, Japan), 5ul DNA template, 1ul of each primer, and 18ul of water. PCR condition started at 94°C for 6 min as an initial denaturation; succeeded by 35 cycles of 94°C for 30 seconds (denaturation), 50°C for 30 seconds (annealing) then 72°C for 45 seconds (extension) and finished at 72°C for 10min as a final extension step. The amplicons were purified from the reaction, using the QIA quick PCR purification kit (Qiagen) according to the manufacturer's guidelines. The amplicons were electrophoresed in 1.5% agarose dissolved in Tris-EDTA buffer with ethidium bromide (0.5µg/ml). PCR products were submitted to Sigma Scientific Services Laboratory (Cairo, Egypt) to be directly sequenced in both directions using the ABI 3730xl DNA sequencer (Applied Biosystems™, USA).

The obtained nucleotides were assembled and edited using the BioEdit program (**Hall, 1999**). The assembled sequences were compared to those available in the database of GenBank using BLASTN search (NCBI) (**Abdelsalam *et al.*, 2009a, 2009b**). The sequences were submitted to GenBank for issuing the accession numbers. The molecular identification of bacteria is mainly based on its 16S rRNA sequence index designated by **Drancourt *et al.* (2000)**. The bacterial species identity is confirmed at $\geq 99\%$ similarity score.

The phylogenetic tree was constructed using MEGA X through the use of the neighbor-joining method with 1000 bootstrap replicates (**Kumar *et al.*, 2018**). The

phylogenetic analysis was conducted, based on the 16S rRNA gene sequences recovered from *V. alginolyticus* and *V. parahaemolyticus* strains isolated from moribund shrimp and aligned with the other related 16S rRNA gene sequences obtained from GenBank. *Listeria monocytogenes* was selected as the out-group.

Histopathological examination

Hepatopancreatic tissue and muscle samples were collected from moribund shrimp on spot at the farm. Samples were fixed in Davidson's fixative solution for 48 hours then placed in 50% ethanol, embedded in paraffin and sectioned. Tissue sections were stained with Hematoxylin and Eosin (H and E) and then examined microscopically at scale bars of 100 μm according to **Bancroft and Gamble (2008)**.

Treatment trial

A treatment strategy comprising two waterborne drugs and one oral antibiotic was adopted to stop mortalities and enhance shrimp health within the affected shrimp ponds. 1.5ml / m³ of hydrogen peroxide (40 % solution) was slowly infused into the pond's water day after day, alternated with 250 ml/acre of *Yucca schidigera* extract solution (Sanolife[®] AFM) after the sunrise together with Florfenicol powder (Aquaflor[®]) at a dosage of 25 mg / Kg shrimp biomass in the diet for 1 week according to **Eissa et al. (2021)**.

RESULTS

Clinical examination

Obviously, the majority of shrimps were lethargic and stunted in growth. Externally, moribund shrimps exhibited reddening/ congestion of almost all body appendages and telson (Fig. 1). Internally, the gastrointestinal tract was empty, and the hepatopancreas was pale and friable.

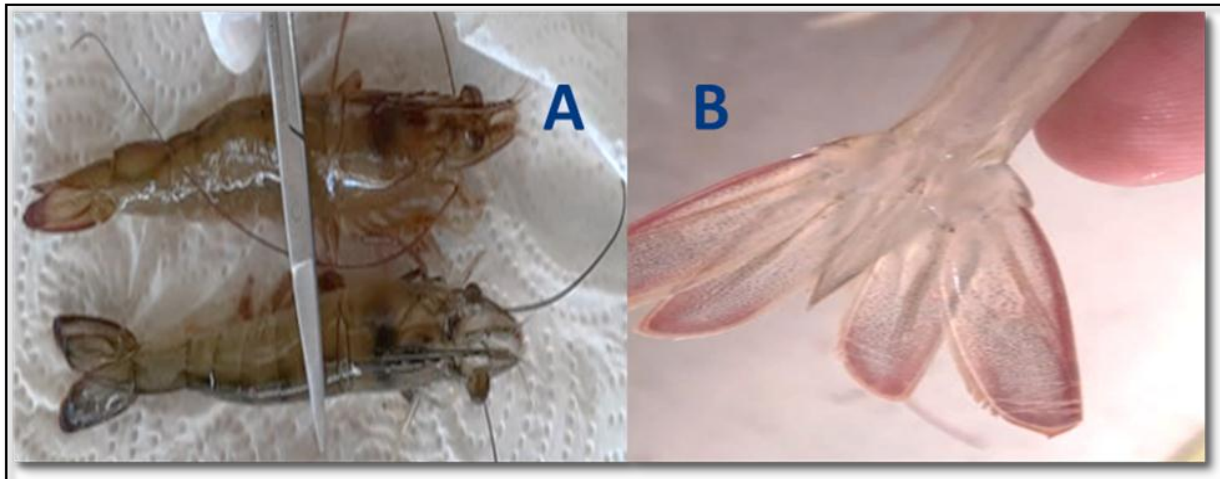


Fig. 1. A. Shrimp exhibiting congestion among all body appendages and telson. **B.** Congestion and redness in the uropod's and telson of clinically infected shrimp.

Bacteriological examination

A total of 53 *Vibrio* isolates were retrieved from the investigated shrimp samples. Circular, green/yellow colonies, were produced on TCBS agar. Medium to large-sized creamy whitish colonies were achieved on marine agar. Phenotypically, isolates were short Gram-negative rods, oxidase-positive and sensitive to 0/129 vibriostatic disc. The API20E identification system grouped the isolates into *V. parahaemolyticus* (30 isolates) and *V. alginolyticus* (23 isolates). The API 20E codes of the retrieved *V. parahaemolyticus* were 4156106, 4156104, 4146006, and 4147106, while that of *V. alginolyticus* were 4156124, 4146124, and 4156124.

Table 1. Antibiotic susceptibility testing of retrieved *Vibrio* spp.

Item		standard		<i>V. parahaemolyticus</i> (30)	<i>V. alginolyticus</i> (23)	Total
Ampicillin 10µg	CLSI (2004)	S	≥17	0	0	0
		IM	14-16	0	0	0
		R	≤13	30	23	53
Amoxicillin 30µg	CLSI (2010)	S	≥18	0	0	0
		IM	14–17	0	0	0
		R	<13	30	23	53
Gentamycin 10µg	CLSI (2010)	S	≥15	5	0	5
		IM	13–14	0	4	4
		R	≤12	25	19	44
Trimethoprim 1.25µg / Sulfamethoxazole 23.75µg	CLSI (2010)	S	≥16	23	16	39
		IM	11–15	2	4	6
		R	≤10	5	3	8
Ciprofloxacin 5µg	CLSI (2010)	S	≥21	20	15	35
		IM	6–20	7	7	14
		R	≤15	3	1	4
Florfenicol 30µg	CLSI (2004)	S	≥18	25	20	45
		IM	13-17	5	3	8
		R	≤12	0	0	0
Tetracycline 30µg	CLSI (2010)	S	≥15	19	10	29
		IM	12–14	4	8	12
		R	≤11	7	5	12

Antibiotic sensitivity testing

The uppermost resistance of *Vibrio* isolates (100%) was noticed against ampicillin 10 μ g and amoxicillin 30 μ g, followed by Gentamycin 10 μ g and tetracycline 30 μ g, which showed 83% and 15% resistances, respectively. On the contrary, florfenicol 30 μ g was the most effective antibiotic (100% susceptibility) (**Table 1**)

Histopathological examination

Histological examination of the hepatopancreas (HP) of moribund *Litopenaeus vannamei* shrimps revealed the presence of mild necrotic and severe degenerative changes (vacuolar degeneration), together with sloughing of HP tubule epithelial cells (Fig. 2). Muscle fibers of moribund shrimps were heavily infiltrated with inflammatory hemocytes (Fig. 3). It is worth mentioning that, no evidence of inclusion bodies suggestive of viral infections was detected.

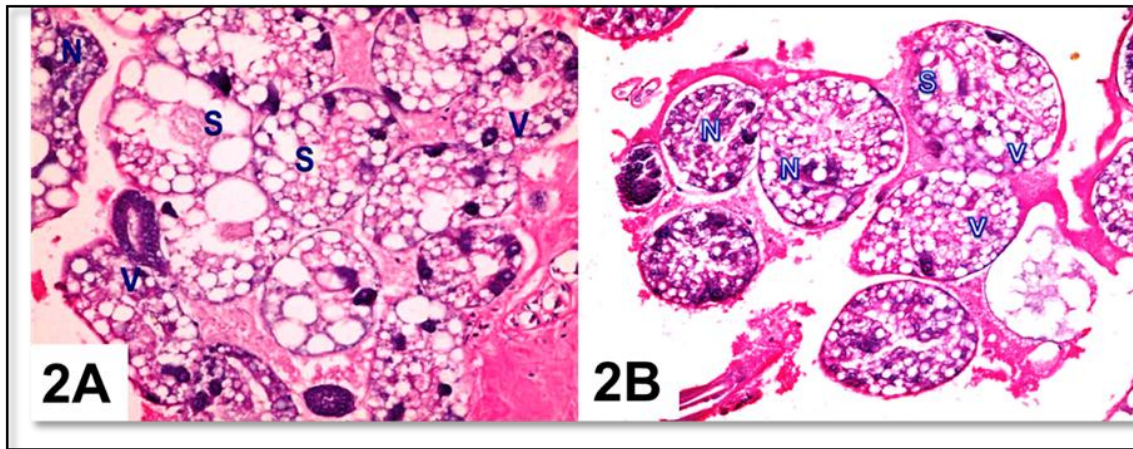


Fig. 2. The hepatopancreas (HP) of shrimp showed mild necrotic (N), severe degenerative changes (vacuolar degeneration) (V) and sloughing of HP cells (S). Scale bars = 100 μ m.

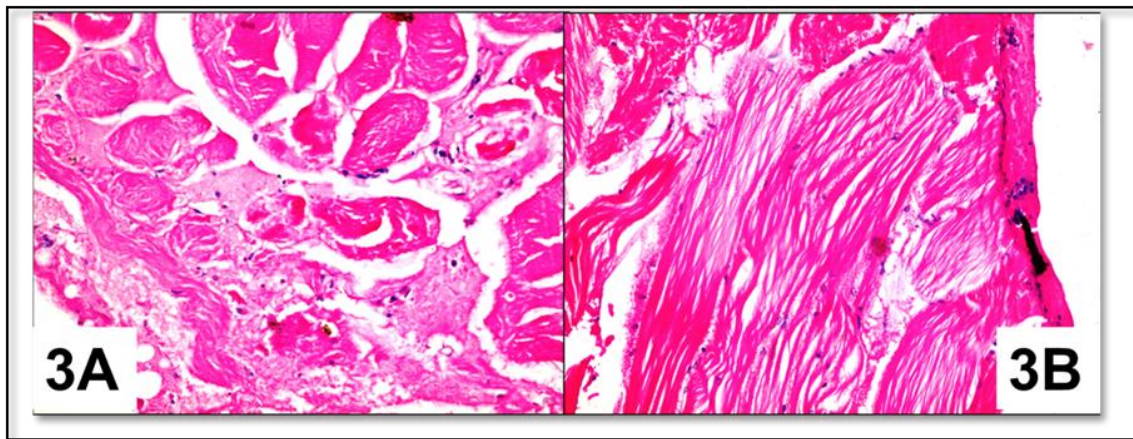


Fig. 3. Muscle fibers of shrimp exhibiting heavy infiltration by inflammatory hemocytes. Scale bars = 100 μ m.

Molecular typing of retrieved isolates

The pathogenic bacterial isolates were molecularly typed by sequencing the 16S rRNA gene. The BLAST analysis of sequenced genes proved that the 6 bacterial isolates were deeply embedded in *Vibrio* spp. group. The similarity index of the 16S rRNA sequences revealed that 6 bacterial isolates were identified as *V. alginolyticus* (3 isolates) and *V. parahemolyticus* (3 isolates). The accession numbers of sequenced genes from the 3 isolates of *V. alginolyticus* were OL619049, OL619050, and OL619051. The alignment of these sequences exhibited 99.85 - 100% 16S rRNA homology with *V. alginolyticus* (MN945277.1; MN938893.1; MN843961.1; MK102576.1; MH879822.1; and MK308627.1). The intra-species similarity was 99.78–100% for the 3 isolates of *V. alginolyticus* recovered from shrimp, with nucleotide differences ranging from 1-3 base pairs.

The GenBank accession numbers of sequenced genes from the 3 isolates of *V. parahemolyticus* were OL619052, OL619053, and OL619054. The alignment of these sequences exhibited 99.86 – 99.93% 16S rRNA similarity to the accession number of *V. parahemolyticus* (CP014046.2; CP006005.1; MW182301.1; NR_041838.1; and NR_114631.1). The intra-species similarity was 99.71–100% for the 3 isolates of *V. parahemolyticus* recovered from shrimp, with nucleotide differences ranging from 3 -4 base pairs.

The phylogenetic tree was constructed to compare the sequences of 16S rRNA of the 6 *Vibrio* isolates, with 27 various accession numbers documented from *V. alginolyticus*, *V. parahemolyticus*, *V. fluvialis*, *V. vulnificus* and *L. monocytogenes*. The phylogenetic analysis demonstrated two main lineages. The first clade was subdivided into two subclades. The first subclade involved *V. alginolyticus* and *V. parahemolyticus* isolates, separated into two branches forming a discrete phylogenetic subclade with a 98% bootstrap value. The isolates of *V. alginolyticus* (3 isolates) and *V. parahemolyticus* (3 isolates) are embedded among other interrelated bacterial isolates and separated from *V. fluvialis*, *V. vulnificus* and *L. monocytogenes* isolates (Fig. 4).

Field treatment trial

Interestingly, the combined treatment strategy comprising two waterborne drugs and one oral antibiotic was effective in sharply reducing the mortalities and enhancing shrimp health within the affected shrimp ponds. The adopted 1.5ml/m³ dosage of hydrogen peroxide of 40% solution day after day alternated with a 250ml/acre dosage of *Yucca schidigera* extract solution (Sanolife[®] AFM) after the sunrise, together with a dosage of 25mg florfenicol (Aquaflor[®]) / Kg shrimp biomass in the diet for 1 week were capable of reducing mortality rates from 60 % to 5 % by the 7th day of treatment. Further, general health was evidently improved, as indicated by the increased shrimp swimming activity, shrimp color restoration and the increased feed intake.

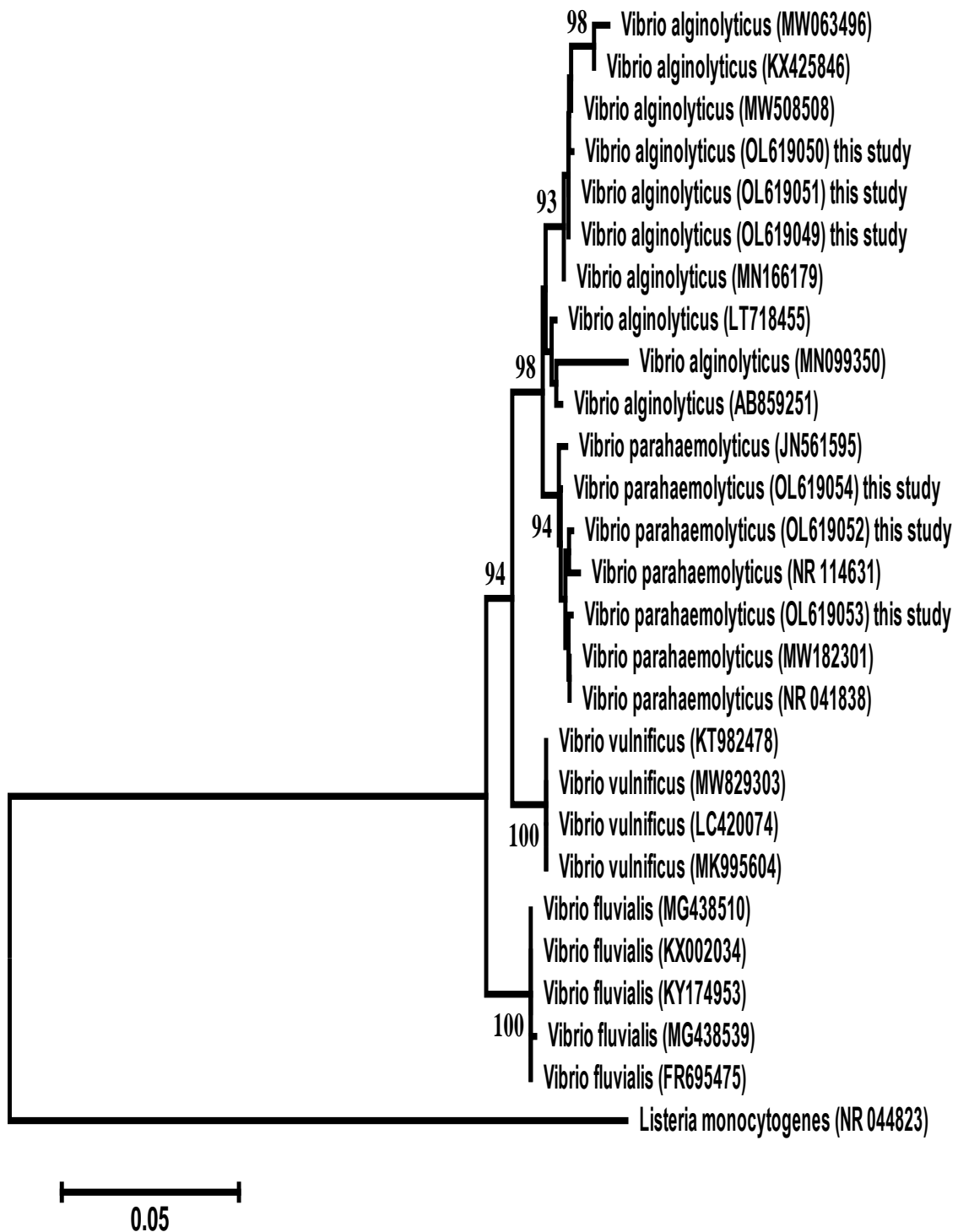


Fig. 4. Phylogenetic analysis based on the 16S rRNA gene sequences of *V. alginolyticus* and *V. parahemolyticus* isolated in this study. The tree was constructed and analyzed by the neighbor-joining method.

DISCUSSION

It is known that the native fish and shellfish farmers in the coastal areas around Egyptian Lake Manzala adopt some erratic management regimes, including high stocking densities, raw trash fish feeding, and minimal rates of water exchange (Eissa *et al.*, 2020; EI-Jakee *et al.*, 2020; Abdelsalam *et al.*, 2021; Attia *et al.*, 2021a, 2021b; Elgendy *et al.*, 2022a, 2022b). This combined bundle of erratic regimes in hot summer months could trigger frequent killer bacterial diseases outbreaks, including streptococcosis, aeromonads, mycoplasmosis, and vibriosis as the most suggested threat (Abdelsalam *et al.*, 2015a, 2015b; Elgendy *et al.*, 2017; Eissa *et al.*, 2021a; Eissa *et al.*, 2021b; Ragab *et al.*, 2022; Sherif *et al.*, 2022). Furthermore, the chemical nature of water at these Egyptian northern coastal water bodies is rich in iron, which indirectly favors enhanced pathogenicity of members of the bacterial family Vibrionaceae including *Vibrios* (siderophores), especially in the hot summer (Eissa *et al.*, 2020). In the current study, the shrimp mass kills were reported during the hot months of summer (July & August) and early autumn (September), where water temperatures exceeded 30°C, DO was as less as 3 mg/L, and toxic ammonia was more than 15 folds as the maximum permissible limits. All these deteriorated water quality parameters are frequently considered as a primary trigger of siderophores pathogenic invasion in marine aquaculture environments as well as the shallow open marine water environments (Liu & Chen, 2004). Thus, the given predisposing factors, the high mortality rates, and the reported clinical signs together with such characteristic behavioral changes among moribund shrimps are all consistent with acute vibriosis outbreaks during those hot months of the year.

The prolonged existence of shrimps in such bad environmental circumstances weakens shrimp immune defense mechanisms and potentiates numerous bacterial invasions (Elgendy *et al.*, 2015). Although higher densities like those seen in the investigated shrimp farm should result in higher output, yet it has numerous negative impacts on farmed shrimp, such as unfavorable interactions among individuals, water quality degradation, and deterioration of shrimp health (Bardera *et al.*, 2021). *Litopenaeus vannamei* aggressive behaviors increases at higher shrimp density when food sources are limited (Ruiz-Velazco *et al.*, 2010). Overcrowding and prolonged contact periods between individuals create perfect conditions for transmitting infectious diseases among farmed shrimp (Ruiz-Velazco *et al.*, 2010).

Vibrios can be transmitted via direct contact and through water, causing outbreaks in natural fisheries and aquaculture (Moustafa *et al.*, 2010; 2014; 2015). *Vibrios* are ubiquitous and prevailing in those marine water bodies with shallow nature or poor exchange rates. Elgendy *et al.* (2015, 2016) reported that, the aquatic environment acts as a big reservoir for numerous strains of *vibrios* and initiates infections upon the establishment of unfavorable aquatic conditions similar to circumstances noticed in the present study. *Vibrios* harbor numerous virulence mechanisms, including many extracellular enzymes and several fitness genes potentiating their pathogenicity (Gennari

et al., 2012). Moreover, the environmental vibrio strains possess different antibiotic resistance genes that can transmit between bacteria in the aquatic environment threatening the health of aquatic animals and humans (**Gennari *et al.*, 2012**).

Moribund shrimps exhibited typical signs of vibriosis, lethargy, poor growth, an empty stomach, and pale hepatopancreas, which are all consistent with vibriosis in shrimp (**Soto-Rodriguez *et al.*, 2015**). The bacteriological examination supported the presumed clinical signs, suggesting that *V. parahaemolyticus* and *V. alginolyticus* pathogens are blamed for the mass shrimp mortalities. A total of 53 vibrio isolates were retrieved from the investigated shrimp specimens. All vibrio isolates produced typical phenotypic traits on TCBS agar; a result which agrees with that of **Elgendy *et al.* (2015)**. Identities of isolates were confirmed via API 20E and 16SrRNA sequence analysis. The pathogenic bacterial isolates were genetically identified by sequencing the 16S rRNA gene. The BLAST analysis of sequenced genes proved that the 6 bacterial isolates were deeply embedded in *Vibrio* spp. group.

Histopathologically, all reported degenerative, necrotic, and cell sloughing lesions throughout the hepatopancreas of moribund shrimps could be attributed to the lytic effects of hemolysins, proteases, and other potential toxins secreted by the highly virulent vibrios (**Aguirre-Guzmán *et al.*, 2004; Kumar *et al.*, 2021**). Additionally, the inflammatory response caused by vibrios in different tissues, including muscles could be attributed to the high doses of secreted toxins favored by high iron concentrations and high water temperatures (**Aguirre-Guzmán *et al.*, 2004; Eissa *et al.*, 2015; Kumar *et al.*, 2021**). The haemocytic infiltration within muscles is a regular crustacean cell-mediated immune response in case of systemic bacterial invasion.

Vibrio parahaemolyticus, *V. alginolyticus*, and *V. harveyi* are the most prevalent pathogens in shrimp farming, causing severe infections and economical losses (**Zhou *et al.*, 2012; Nunan *et al.*, 2014**). Similarly, **Elgendy *et al.* (2015)** identified *V. alginolyticus*, *V. vulnificus*, *V. harveyi*, and *V. mimicus* from a high mortality outbreak affecting maricultured shrimp *Penaeus indicus* erupted after a period of unstable climatic conditions and have caused significant economic losses. Vibriosis outbreaks relevant to *V. parahaemolyticus* are extremely serious in shrimp farming, causing fatal infections, particularly at the larval stages, causing a disease called infectious pancreatic necrosis (**Kongrueng *et al.*, 2015**). The disease is repeatedly reported in black tiger shrimp, *Penaeus monodon* and *L. vannamei* (**Soto-Rodriguez *et al.*, 2015**). *Vibrio* infections upset the health status of shrimp due to the existence of several virulence factors such as adhesins, biofilm, hemolysins and secretion systems (**Kongrueng *et al.*, 2015**).

Vibrio isolates showed the highest resistance (100%) against ampicillin and amoxicillin. Further, isolates showed 83% and 15% resistance against gentamycin and tetracycline, respectively. The highest susceptibility was noticed against florfenicol at 100% and ciprofloxacin at 92%. The improper use of antibiotics in aquaculture to treat diseases affecting fish and shellfish causes the emergence of resistant strains (**Serrano,**

2005). Depending on the results of the antibiotic susceptibility, florfenicol was used in a field treatment trial with H₂O₂. The health and survival of shrimp in the treated earthen pond improved. Mortality is reduced to 5% after treatment. The efficiency of florfenicol against vibriosis infections have been confirmed in previous studies (Samuelsen & Bergh, 2004). However, the combined treatment regimen had multiple therapeutic pathways, where hydrogen peroxide worked as an oxidizer for unionized ammonia and free iron in water, preventing vibrios from using them as pathogenic mechanism triggers. In addition, a numerous count of vibrios in bacterial load would extensively burst under the the sterilizing effects of released atomic oxygen (Zou *et al.*, 2020). Notably, the *Yucca schidigera* extract solution improves the functional performance of shrimp gills through direct and indirect means by removing the toxic ammonia from pond's water, which further improves the functional performance of gills during gas exchange (Wang *et al.*, 2020). Finally, florfenicol, with its efficient bactericidal effect arising from prolonged existence in shrimp tissues (high bioavailability), would decisively destroy vibrios in shrimp haemolymph and systemic tissues.

CONCLUSION

Vibriosis caused by *V. parahaemolyticus* and *V. alginolyticus* is a significant bacterial disease affecting farmed shrimp. The disease is extremely fatal at the larval stages. Vibrio infections are triggered by environmental deterioration and erratic rearing practices. High temperature, low dissolved oxygen, elevated unionized ammonia levels, and overstocking render farmed shrimp susceptible to infections. Vibrio infections were resistant to some antibiotics, perhaps due to their irregular application in aquaculture. Vibriosis infections in farmed shrimp can be successfully treated with florfenicol and H₂O₂. Good management practices are necessary for avoiding vibrio infections affecting farmed *L. vannamei*.

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REFERENCES

- Abdelsalam, M.; Chen, S. C. and Yoshida, T. (2009a): Surface properties of *Streptococcus dysgalactiae* strains isolated from marine fish. Bull. Eur. Assoc. Fish Pathol., **29**: 15-23.
- Abdelsalam, M.; Eissa, A.E. and Chen, S.C. (2015a): Genetic diversity of geographically distinct *Streptococcus dysgalactiae* isolates from fish. Journal of advanced research, **6(2)**: 233-238.

- Abdelsalam, M.; Fujino, M.; Eissa, A.E.; Chen, S.C. and Warda, M. (2015b):** Expression, genetic localization and phylogenetic analysis of NAP_{lr} in piscine *Streptococcus dysgalactiae* subspecies *dysgalactiae* isolates and their patterns of adherence. *Journal of advanced research*, **6(5)**: 747-755.
- Abdelsalam, M.; Nakanishi, K.; Yonemura, K.; Itami, T.; Chen, S. C. and Yoshida, T. (2009):** Application of Congo red agar for detection of *Streptococcus dysgalactiae* isolated from diseased fish. *Journal of Applied Ichthyology*, **25(4)**: 442-446.
- Abdelsalam, M.; Ewiss, M.A.; Khalefa, H.S.; Mahmoud, M.A.; Elgendy, M.Y. and Abdel-Moneam, D.A (2021):** Coinfections of *Aeromonas* spp., *Enterococcus faecalis*, and *Vibrio alginolyticus* isolated from farmed Nile tilapia and African catfish in Egypt, with an emphasis on poor water quality. *Microbial Pathogenesis*. 160 (2021). 105213 <https://doi.org/10.1016/j.micpath.2021.105213>.
- Attia, M.M.; Elgendy, M.Y.; Abdelsalam, M.; Hassan, A.; Prince, A.; Salaeh, N.M.K. and Younis, N.A. (2021b):** Morpho-molecular identification of Heterophyes heterophyes encysted metacercariae and its immunological and histopathological effects on farmed Mugil cephalus in Egypt. *Aquaculture International*. **29**: 1393–1407. <https://doi.org/10.1007/s10499-021-00708-3>
- Attia, M.M.; Elgendy, M.Y.; Prince, A.; El-Adawy, M.M. and Abdelsalam, M. (2021a):** Morphomolecular identification of two trichodinid coinfections (Ciliophora: Trichodinidae) and their immunological impacts on farmed Nile Tilapia. *Aquac Res.* 52:4425-4433. <https://doi.org/10.1111/are.15281>.
- Aguirre-Guzmán, G.; Mejia Ruíz, H.; and Ascencio F. (2004):** A review of extracellular virulence product of *Vibrio* species important in diseases of cultivated shrimp. *Aquaculture Res.*, **35(15)**: 1395-1404.
- Bancroft, J. D. and Gamble, M. (2008):** Theory and practice of histological techniques. 6th Edition, Churchill Livingstone, Elsevier, China.
- Bardera, G.; Owen, M. A.; Facanha, F. N.; Alcaraz-Calero, J. M.; Alexander, M. E. and Sloman, K. A. (2021):** The influence of density and dominance on Pacific white shrimp (*Litopenaeus vannamei*) feeding behaviour. *Aquaculture*, **531**: 735949.
- Bauer, A.; Kirby, W.; Sherris, J. C. and Turck, M. (1966):** Antibiotic susceptibility testing by a standardized single disk method. *American J. Clinical Pathol.*, 45:493.
- Bondad-Reantaso, M. G.; Subasinghe, R. P.; Josupeit, H.; Cai, J. and Zhou, X. (2012):** The role of crustacean fisheries and aquaculture in global food security: past, present and future. *J. Invertebrate Pathol.*, **110**: 158–165.

- Buller, N. B. (2004):** Bacteria from Fish and Other Aquatic Animals: A Practical Identification Manual, CABI Publishing, Cambridge.
- CLSI (2004):** Clinical and Laboratory Standards Institute, Methods for antimicrobial disk susceptibility testing of bacteria isolated from aquatic animals; Proposed guideline, M42-P. CLSI, Wayne, PA, USA.
- CLSI (2010):** Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement M100-S20, CLSI, Wayne, Pa, USA.
- Drancourt, M., Bollet, C., Carlouz, A., Martelin, R., Gayral, J. P. and Raoult, D. (2000):** 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *J. Clin. Microbiol.*, **38(10)**: 3623-3630.
- Eissa, A. E.; Abdelsalam, M.; Abumhara, A.; Kammon, A.; Gammoudi, F. T.; Ben Naser, K. M. and Asheg, A. (2015):** First record of *Vibrio vulnificus/Anisakis pegreffii* concurrent infection in black scorpionfish (*Scorpaena porcus*) from the south Mediterranean basin. *Res. J. Pharm. Biol. Chem. Sci.*, **6(3)**: 1537-1548.
- Eissa, A. E.; Abolghait, S. K., Younis, N. A.; Dessouki, A. A.; El-Lamie, M. M.; Abu Mhara, A. A. and Abdelsalam, M. (2020):** *Myxobolus episquamalis* infection in farmed flathead grey mullet *Mugil cephalus* L. and thin-lipped mullet *Liza ramada*. *Aquacult Int.*, **28**:363–376. <https://doi.org/10.1007/s10499-019-00467-2>
- Eissa, A. E.; Abou-Okada, M.; Alkurdi, A. R.; El Zlitne, R. A.; Prince, A., Abdelsalam, M. and Derwa, H. (2021a):** Catastrophic mass mortalities caused by *Photobacterium damsela* affecting farmed marine fish from Deeba Triangle, Egypt. *Aquaculture Research*, **52 (9)**: 4455 – 4466.
- Eissa, A.E.; Attia, M.A., Elgendy, M.Y; Ismail, G.A.; Sabry, N.M.; Prince, A.; Mahmoud, M.A.; El-Demerdash, G.O.; Abdelsalam, M. and Derwam H.IM. (2021b):** Streptococcus, *Centrocestus formosanus* and *Myxobolus tilapiae* concurrent infections in farmed Nile tilapia (*Oreochromis niloticus*). *Microbial Pathogenesis*, 160: (2021) 105213. <https://doi.org/10.1016/j.micpath.2021.105084>.
- El-Adawy, M. M.; Eissa, A. E.; Shaalan, M.; Ahmed, A. A.; Younis, N. A.; Ismail, M. M. and Abdelsalam, M. (2021):** Green synthesis and physical properties of Gum Arabic-silver nanoparticles and its antibacterial efficacy against fish bacterial pathogens. *Aquaculture Research*, **52(3)**: 1247-1254.
- Elgendy, M. Y.; Abdelsalam, M.; Mohamed, S. A. and Ali, S. E. (2022a):** Molecular characterization, virulence profiling, antibiotic susceptibility, and scanning electron microscopy of *Flavobacterium columnare* isolates retrieved from Nile tilapia (*Oreochromis niloticus*). *Aquaculture International*, **30**: 845–862.
- Elgendy, M. Y.; Kenawy, A. M. and Noor El-Deen, A. E. (2016):** *Gyrodactylus anguillae* and *Vibrio vulnificus* infections affecting cultured eel, *Anguilla anguilla*. *Comunicata Scientiae* **7**: 1-11.

- Elgendy, M. Y.; Shaalan, M.; Abdelsalam, M.; Eissa, A. E.; El-Adawy, M. M. and Seida, A. A. (2022b):** Antibacterial activity of silver nanoparticles against antibiotic-resistant *Aeromonas veronii* infections in Nile tilapia, *Oreochromis niloticus* (L.), in vitro and in vivo assay. *Aquaculture Research*, 53(3): 901-920.
- Elgendy, M. Y.; Soliman, W. S.; Hassan, H. A.; Kenawy, A. M. and Liala, A. M. (2015):** Effect of Abrupt Environmental Deterioration on the Eruption of Vibriosis in Mari-Cultured Shrimp, *Penaeus indicus*, in Egypt. *Journal of Fisheries and Aquatic Science*, 10: 146-158.
- Elgendy, M.Y.; Soliman, W.S.; Abbas, W.T.; Ibrahim, T.B.; Younes, A.M. and Omara, S.T. (2017):** Investigation of some Virulence Determents in *Aeromonas hydrophila* Strains Obtained from Different Polluted Aquatic Environments. *Jordan J Biol Sci*, 10: 265-272.
- EI-Jakee, J.; Elshamy, S.; Hassan, A.W.; Abdelsalam, M.; Younis, N.; El-Hady, M.A. and Eissa, A.E. (2020):** Isolation and characterization of mycoplasmas from some moribund Egyptian fishes. *Aquaculture International*, 28(3): 901-912.
- FAO, (2018):** The State of World Fisheries and Aquaculture, Meeting the Sustainable Development Goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.
- Gennari, M.; Ghidini, V.; Caburlotto, G. and Lleo, M. M. (2012):** Virulence genes and pathogenicity islands in environmental *Vibrio* strains nonpathogenic to humans. *FEMS Microbiol. Ecol.*, 82: 563–573.
- Hall, T. A. (1999):** BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41: 95-98.
- Kumar, S.; Stecher, G.; Li, M.; Knyaz, C. and Tamura, K. (2018):** MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.*, 35(6):1547-1549.
- Kumar, V.; Roy, S.; Behera, B. K.; Bossier, P. and Das, B. K. (2021):** Acute hepatopancreatic necrosis disease (AHPND): virulence, pathogenesis and mitigation strategies in shrimp aquaculture. *Toxins*, 13(8): 524.
- Kongrueng, J.; Yingkajorn, M.; Bunpa, S.; Sermwittayawong, N.; Singkhamanan, K. and Vuddhaku, V. (2015):** Characterization of *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease in southern Thailand. *Journal of Fish Diseases*, 38: 957–966.
- Liu, C. H. and Chen, J. C. (2004):** Effect of ammonia on the immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus*. *Fish and Shellfish Immunology*, 16(3): 321-334.
- Moustafa, M.; Laila, A. M.; Mahmoud, M. A.; Soliman, W. S. and Elgendy, M. Y. (2010):** Bacterial infections affecting marine fishes in Egypt. *J. Am. Sci.*, 6: 603-612.

- Moustafa, M.; Eissa, A. E.; Laila, A. M.; Gaafar, A. Y.; Abumourad, I. M. and Elgendy, M. Y. (2014):** Mass mortalities in mari-cultured European sea bass (*Dicentrarchus labrax*) at northern Egypt. Res. J. Pharm. Biol. Chem. Sci., **5**: 95–109.
- Moustafa, M.; Eissa, A. E.; Laila, A. M.; Gaafar, A. Y.; Abumourad, I. M. and Elgendy, M. Y. (2015):** Investigations into the potential causes of mass kills in mari-cultured gilthead sea bream (*Sparus aurata*) at northern Egypt. Res. J. Pharm. Biol. Chem. Sci., **6**: 466–477.
- Nunan, L.; Lightner, D.; Pantoja, C. and Gómez-Jiménez, S. (2014):** Detection of acute hepatopancreatic necrosis disease (AHPND) in Mexico. Dis. Aquat. Org., **111**: 81-86.
- Ragab, R. H.; Elgendy, M. Y.; Sabry, N. M.; Sharaf, M. S.; Attia, M. M.; Korany, R. M.; Abdelsalam, M.; Eltahan, A. S.; Eldessouki, E. A.; El-Demerdash, G.O. and Khalil, R. H. (2022):** Mass kills in hatchery-reared European seabass (*Dicentrarchus labrax*) triggered by concomitant infections of *Amyloodinium ocellatum* and *Vibrio alginolyticus*. International Journal of Veterinary Science and Medicine, **10(1)**: 33-45.
- Ruiz-Velazco, J. M.; Hernandez-Llamas, A. and Gomez-Munoz, V. M. (2010):** Management of stocking density, pond size, starting time of aeration, and duration of cultivation for intensive commercial production of shrimp *Litopenaeus vannamei*. Aquac. Eng., **43**: 114–119.
- Sadek, S.; Rafael, R.; Shakouri, M.; Rafomanana, G.; Ribeiro, F. L. and Clay, J. (2002):** Shrimp Aquaculture in Africa and the Middle East: The Current Reality and Trends for the Future. Report prepared under the World Bank, NACA, WWF and FAO Consortium Program on Shrimp Farming and the Environment. Work in Progress for Public Discussion. Published by the Consortium, 42 pages.
- Samuelsen, O. and Bergh, Ø. (2004):** Efficacy of orally administered florfenicol and oxolinic acid for the treatment of vibriosis in cod (*Gadus morhua*). Aquaculture. **235**: 27-35.
- Serrano, P. H. (2005):** Responsible use of antibiotics in aquaculture. Fisheries technical paper, 469, pp. 110. Food and Agriculture organization of the United Nations, Rome, 2005, FAO fisheries.
- Sherif, A.H.; Abdelsalam, M.; Ali, N.G. and Mahrous, K.F. (2022).** Zinc Oxide Nanoparticles Boost the Immune Responses in *Oreochromis niloticus* and Improve Disease Resistance to *Aeromonas hydrophila* Infection. Biological Trace Element Research, pp.1-10.
- Soto-Rodriguez, S. A.; Gomez-Gil, B.; Lozano-Olvera, R.; Betancourt-Lozano, M. and Morales-Covarrubias, M. S. (2015):** Field and experimental evidence of *Vibrio parahaemolyticus* as the causative agent of Acute Hepatopancreatic Necrosis

Disease (AHPND) of cultured shrimp (*Litopenaeus vannamei*) in north-western Mexico. Appl. Environ. Microbiol., **81**: 1689-1699.

Wang, L.; Wu, D.; Fan, Z.; Li, H.; Li, J.; Zhang, Y.; Xu, Q.; Wang, G. and Zhu, Z. (2020): Effect of *Yucca schidigera* extract on the growth performance, intestinal antioxidant status, immune response, and tight junctions of mirror carp (*Cyprinus carpio*). Fish and Shellfish Immunology, **103**: 211-219.

Weisburg, W. G.; Barns, S. M.; Pelletier, D. A. and Lane, D. J. (1991): 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol., **173(2)**: 697-703.

Zhou, J.; Fang, W.; Yang, X.; Zhou, S.; Hu, L.; Li, X.; Qi, X.; Su, H. and Xie, L. (2012): A nonluminescent and highly virulent *Vibrio harveyi* strain is associated with Bacterial white tail disease" of *Litopenaeus vannamei* shrimp. PLoS ONE, **7**: e29961.

Zou, P.; Yang, Q.; Wang, H.; Xie, G.; Cao, Z.; Chen, X. and Huang, J. (2020): In vitro disinfection efficacy and clinical protective effects of common disinfectants against acute hepatopancreatic necrosis disease (AHPND)-causing *Vibrio* isolates in Pacific white shrimp *Penaeus vannamei*. J. Microbiol., **58(8)**: 675-686.