Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(3): 3533 – 3561 (2025)
www.ejabf.journals.ekb.eg

Advanced Diagnosis on Vibriosis in Egyptian Mariculture, Fish and Shrimp

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ARTICLE INFO
Article History:
Received: April 18, 2025
Accepted: June 16, 2025
Online: June 30, 2025

Keywords:
Vibriosis,
Antibiotic susceptibility,
Egyptian mariculture,
Gilthead sea bream,
Whiteleg shrimp,
Thinlip mullet,
Molecular diagnosis

ABSTRACT

Vibriosis, caused by various *Vibrio* species, poses a major threat to Egyptian mariculture, particularly affecting *Sparus aurata*, *Mugil capito*, and *Litopenaeus vannamei*. This study, conducted in November 2023, aimed to identify and characterize the *Vibrio* species involved using biochemical and molecular methods. *Vibrio* spp. were isolated from 45% of the samples examined, with the highest prevalence in *S. aurata* (58.33%), followed by *M. capito* (43.33%) and *L. vannamei* (33.33%). Three species were identified: *V. alginolyticus*, *V. cholerae*, and *V. mimicus*. All *S. aurata* isolates were *V. alginolyticus*, while *M. capito* showed a mixture of all three. In shrimp, *V. alginolyticus* and *V. cholerae* were found. Antibiotic susceptibility testing revealed general sensitivity to most drugs tested, although *V. alginolyticus* showed resistance to penicillin. These findings support the need for targeted diagnostics and biosecurity to manage Vibriosis in aquaculture.

INTRODUCTION

Marine and brackish water aquaculture are experiencing significant expansion throughout the Mediterranean basin, driven by Egypt's rising need for high-quality protein, which has propelled its aquaculture development over the past forty years **(Soliman & Yacout, 2016)**.

Egypt ranks ninth globally and first in Africa in fish production from aquaculture **(Da Silva, 2016)**. Like other marine aquaculture initiatives in the Mediterranean, Egypt has emphasized the cultivation of the economically significant gilthead seabream (*Sparus aurata*) and thinlip mullet (*Mugil capito*). Egypt commenced shrimp aquaculture in 1985 **(Sadek *et al.*, 2002)** due to the significant contribution of shrimp farming to global food security and economic development **(Bardera *et al.*, 2021)**. Since then, the sector has steadily expanded, with numerous large-scale projects focusing on building a resilient shrimp farming industry **(Elgendy *et al.*, 2015)**. Today, aquaculture accounts for about

2% of Egypt's total shrimp production, reaching approximately 123 metric tons **(FAO, 2018)**. The species' adaptability to varying salinities and temperatures **(Bondad-Reantaso *et al.*, 2012; FAO, 2019)** makes it well-suited to Egypt's environmental conditions. However, the rapid expansion of the gilthead seabream, thinlip mullet and shrimp farming has not been matched by effective health management strategies, leaving the industry vulnerable to risks from introduced and endemic pathogens.

The increasing occurrence of infectious diseases in the farmed gilthead seabream, thinlip mullet, and shrimp is frequently associated with several detrimental factors, such as inadequate water quality, the buildup of organic waste, ineffective management practices, and unusual behaviors like cannibalism, particularly in shrimp and the wild sourcing of the thinlip mullet seeds **(Eissa *et al.*, 2015; Elgendy *et al.*, 2015)**. Collectively, these elements diminish the host's immune defence mechanisms, heightening their vulnerability to pathogens. *Vibrio* species are prevalent in aquatic environments and are known to act as opportunistic pathogens, potentially causing illness when environmental conditions worsen and the host's immune system is weakened by physical or environmental stressors **(Chien *et al.*, 2002)**.

Vibrio species are halophilic, Gram-negative, facultatively anaerobic bacteria that are typically located in seawater and brackish environments, with certain species also flourishing in freshwater **(Stephen, 2022)**. Notably, *V. alginolyticus*, prevalent in Egypt **(Austin & Austin, 2007)**, and *V. mimicus* are significant pathogens in aquaculture, affecting marine fish and shellfish **(Raja *et al.*, 2017)**. Pathogenic *Vibrio* species, including *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus*, have the potential to infect humans, presenting a spectrum of severity **(Baker-Austin *et al.*, 2018)**. In aquaculture, the application of antimicrobials is common for infection management; however, this practice presents significant challenges, such as the emergence of antibiotic-resistant bacteria and possible public health risks associated with residuals **(BondadReantaso *et al.*, 2023)**. This study sought to determine the effect of *Vibrio* species on mariculture of gilthead seabream, thinlip mullet, and whiteleg shrimp within earthen pond-based mariculture in Egypt. The study encompassed the phenotypic, genotypic, and molecular characterization of isolated pathogens, as well as an assessment of their antibiotic susceptibility, yielding important data for public health in Egypt.

MATERIALS AND METHODS

Fish and shrimp sampling

In November 2023, a total of 180 live aquatic specimens comprising the gilthead seabream (*Sparus aurata*), thinlip mullet (*Mugil capito*), and whiteleg shrimp (*Litopenaeus vannamei)* were randomly sampled from aquaculture farms in North-East Egypt (Table 1). Each specimen was individually enclosed in a sealed plastic bag containing a mixture of water and oxygen to preserve optimal transport conditions. The specimens were swiftly conveyed to the Animal Health Research Institute in Ismailia

Governorate. Upon arrival, all fish and shrimp underwent thorough clinical examination to detect any external abnormalities or pathological signs.

Table 1. Examined cultured fish species and shrimp, number, average length and body weight

Fish species & Shrimp	**Number of examined fish & shrimp**	**Average body weight (g)**	**Average length (cm)**
Gilthead seabream *(Sparus aurata*)	60	142 ± 30	15 ± 2
Thinlip mullet (*Mugil capito*)	60	310 ± 7	35 ± 2
Whiteleg shrimp *(Litopenaeus vannamei)*	60	17 ± 3	13±2

Isolation and identification of bacteria

Following **Eissa *et al.* (2019)**, samples from *Sparus aurata* and *Mugil capito* were aseptically obtained from the gills, skin ulcers, liver, kidneys, and spleen of examined fish, regardless of their condition alive, moribund, or recently deceased. Similarly, for *Litopenaeus vannamei*, internal samples from the hepatopancreas and musculature were aseptically collected after external washing with water, surface sterilization with alcohol, and burning of the carapace, as detailed by **Fadel and El-Lamie (2019)**. The obtained samples were inoculated into alkaline peptone water (APW) with 3% NaCl and incubated at 37°C for 24 hours. After enrichment, the cultures were streaked over thiosulfate citrate bile salts sucrose (TCBS) agar and incubated under the same conditions. Colonies exhibiting yellow or green pigmentation were chosen, purified, and analyzed morphologically and biochemically following the methodology of **Buller (2004)**. Biochemically identified isolates were further validated by molecular approaches to detect both *Vibrio* species and their corresponding virulence genes, adhering to the protocol established by **Ibrahim *et al.* (2018)**.

Molecular analysis

Molecular analysis was conducted based on morphological and biochemical identification. DNA was extracted from samples using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH), with modifications to the manufacturer's instructions. The quality of DNA was evaluated using electrophoresis in a 0.8% agarose gel with ethidium bromide staining. PCR was conducted using the GeneAmp® PCR System 2720 thermal cycler (Thermo Fisher Scientific, Bremen, Germany) with the DreamTaq Green PCR

Master Mix (2X) (Thermo Scientific™, Bremen, Germany), resulting in a total reaction volume of 25µL. Oligonucleotide primers specific to the genes were utilized as outlined in Table (1). Each reaction included 0.25µM of each primer and 30ng of DNA. The cycling parameters are detailed in Table (2). The PCR product was analyzed using a 1.5% agarose gel with ethidium bromide and visualized under UV light. The resulting amplicons were sent to Colors Laboratory in Cairo, Egypt for PCR purification and sequencing.

Initial analyses of the obtained sequences were conducted to determine their identity with GenBank accessions utilizing the NCBI nucleotide BLAST® tool **(Altschul *et al.*, 1997)**. All sequences were aligned using the ClustalW program integrated within BioEdit® 7.0.5.3. The initial tree(s) for the heuristic search were generated automatically through the application of neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated via the Maximum Composite Likelihood (MCL) method, followed by the selection of the topology with the highest log likelihood value. The trees were accurately scaled, with branch lengths quantified in terms of substitutions per site. Phylogenetic analyses utilized MEGA X software **(Kumar *et al.*, 2018)**. Table (2) shows primers sequences, target genes, amplicon sizes and cycling conditions used in molecular analysis.

Table 2. Primers sequences, target genes, amplicon sizes and cycling conditions

Target gene	Primers sequences 5'-3'	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
V. cholerae sodB	AAG ACC TCA ACT GGC GGT A GAA GTG TTA GTG ATC GCC AGA GT	248	94˚C 5 min.	94˚C 30 sec.	57˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Tarr *et al.* (2007)**
V. cholerae ctxAB	CC GGG TTG TGG GAA TGC TCC AAG GCC ATA CTA ATT GCG GCA ATC GCA TG	536	94˚C 5 min.	94˚C 30 sec.	59˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**De Menezes *et al.*(2014)**
V. alginolyticus collagenase	CGAGTACAGTCACTTGAAA GCC CACAACAGAACTCGCGTTA CC	737	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	**Abu-Elala *et al*.(2016)**
V. alginolyticus tdh	CCATCTGTCCCTTTTCCTGC CCAAATACATTTTACTTGG	373	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	**Mustapha *et al.* (2013)**
blaTEM	ATCAGCAATAAACCAGC	516	94˚C	94˚C	54˚C	72˚C	72˚C	**Colom *et al.***

	CCCCGAAGAACGTTTTC		5 min.	30 sec.	40 sec.	45 sec.	10 min.	**(2003)**
V. mimicus sodB	CAT TCG GTT CTT TCG CTG AT GAA GTG TTA GTG ATT GCT AGA GAT	121	94˚C 5 min.	94˚C 30 sec.	57˚C 40 sec.	72˚C 30 sec	72˚C 7 min.	**Tarr *et al.* (2007)**

Antibiotic sensitivity test

Antibiotic susceptibility testing (AST) of *Vibrio alginolyticus, V. cholerae*, and *V. mimicus* isolates was conducted with the disk diffusion method (Kirby-Bauer test), adhering to the protocol established by **Balouiri *et al.* (2016)**. The isolates were evaluated against a selection of antibiotics: Gentamicin (10 µg), Ampicillin (10 µg), Ciprofloxacin (5 µg), Trimethoprim-Sulfamethoxazole (25 µg), Tetracycline (30 µg), Piperacillin (µg), and Chloramphenicol (30 µg). Colonies cultivated on TCBS agar at 37°C for 24 hours were suspended in sterile saline to achieve a 0.5 McFarland standard concentration. The bacterial suspension was uniformly distributed on Mueller-Hinton agar plates with sterile cotton swabs, followed by the application of antibiotic disks. Plates were incubated at 37°C for 24 hours, and the diameters of the inhibitory zones were measured. Results were classified as sensitive (S), intermediate (I), or resistant (R) in accordance with **CLSI (2018)**.

RESULTS

1. Clinical picture

In the present study, naturally infected cultured gilthead seabream (*Sparus aurata*), thinlip mullet (*Mugil capito*), and whiteleg shrimp (*Litopenaeus vannamei*) exhibited a variety of clinical abnormalities in November, coinciding with non-gradual and irregular water temperature fluctuations ranging from 25.5 to 18.0°C. *S. aurata* showed signs including exophthalmia, abdominal distension, and hemorrhages near the pectoral fins (Fig. 1), along with internal lesions such as gill congestion, hepatic hemorrhages, congested kidneys and spleen, and engorged ovarian blood vessels (Fig. 2). *M. capito* commonly displayed hemorrhages on the body surface, especially around the eyes and pectoral fins, along with ocular opacity, fin erosion, and sharply demarcated deep ulcers (Fig. 3). The gills, liver, spleen, and kidneys exhibited congestion and hemorrhage (Fig. 4). In *L. vannamei*, external manifestations included black spots on the cuticle, carapace, telson, uropods, pleopods, and discolored gills (Fig. 5). Some individuals showed reddish or black discoloration on the uropods and carapace (Fig. 6a), and in certain cases, the hepatopancreas appeared congested and friable (Fig. 6b).

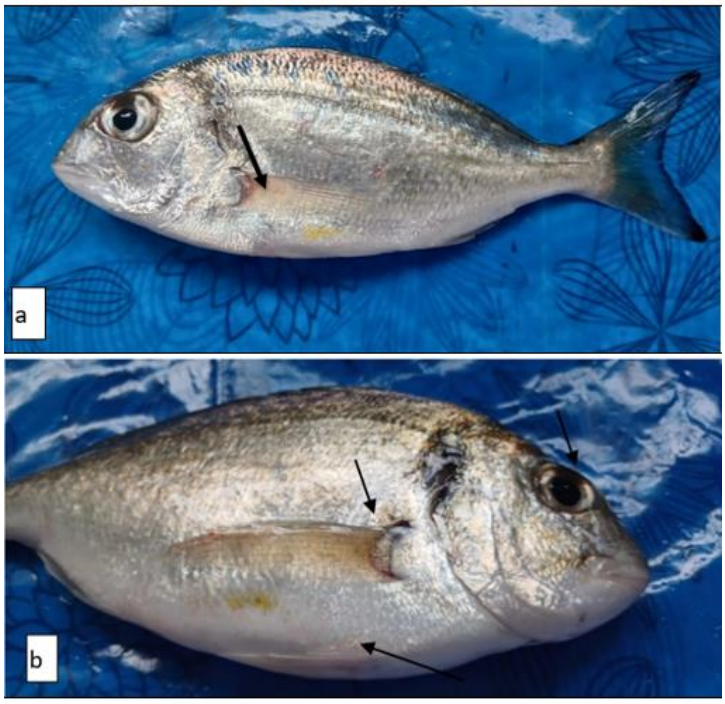

Fig. 1. Seabream (*Sparus aurata*) exhibit: a and b- Swollen abdomens, and hemorrhages near the pectoral fins

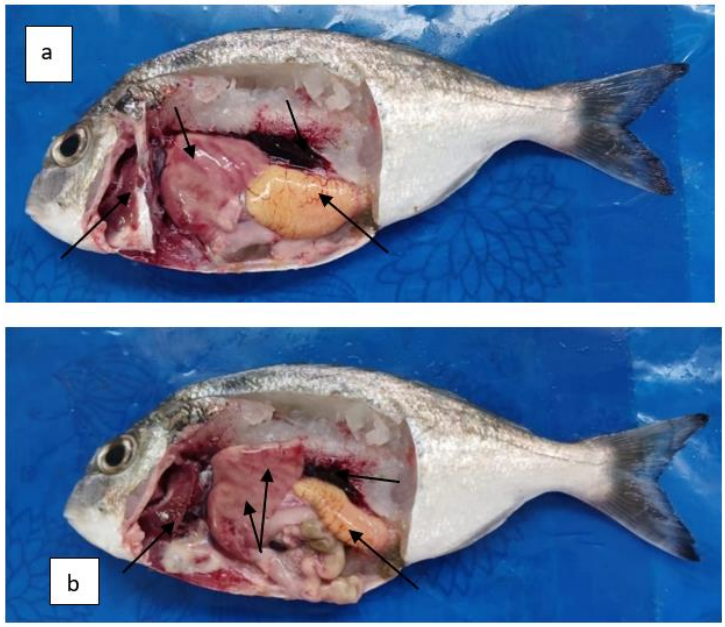

Fig. 2. Seabream (*Sparus aurata* L*)* show: a and b- Congested gills, hemorrhagic liver patches, congested kidneys, spleen, and ovarian blood vessels

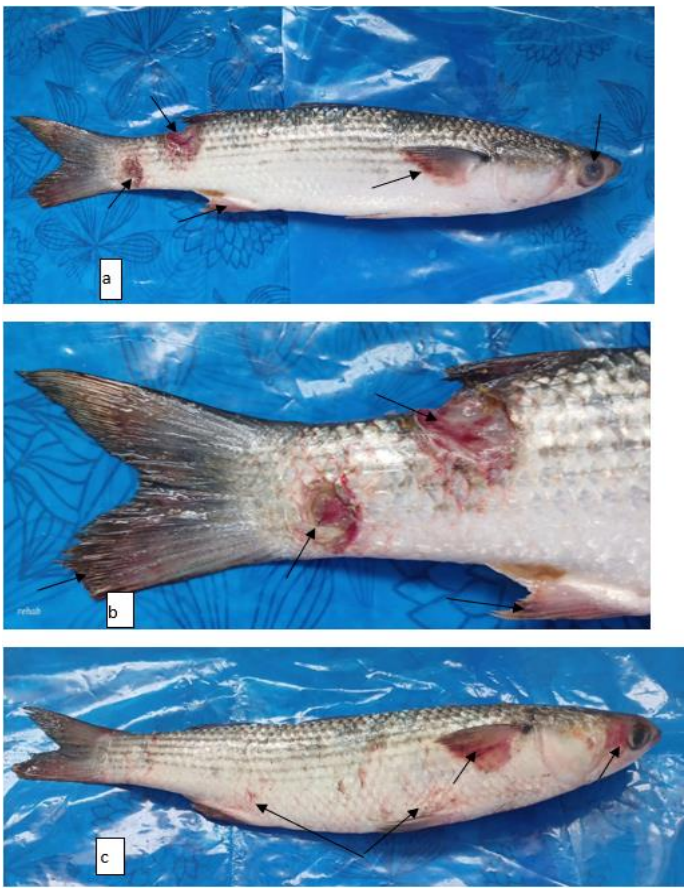

Fig. 3. Thinlip mullet *(Mugil capito)* shows: a and b- Deep ulcers with defined margins, fin erosions and, ocular opacity. c- Body surface hemorrhages, particularly near the eyes and pectoral fins

Fig. 4. Thinlip mullet *(Mugil capito)* shows: a and b- Hemorrhagic and congested gills, liver, spleen, and kidneys

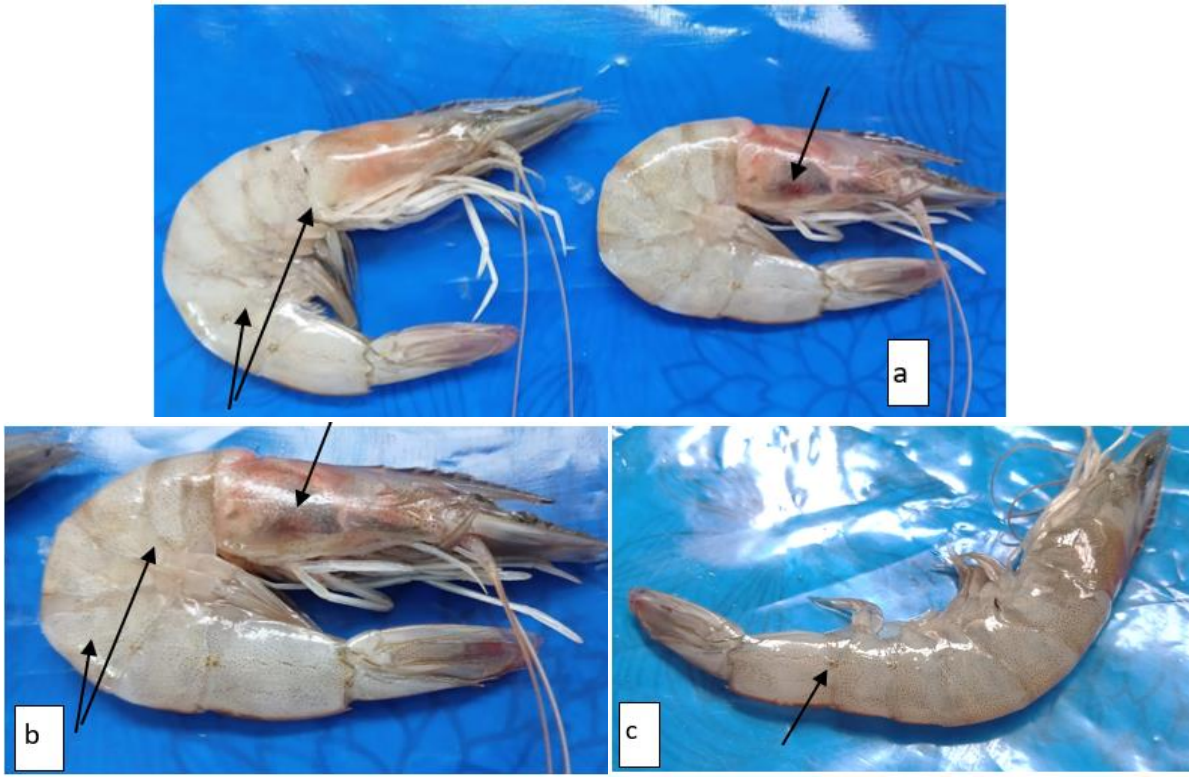

Fig. 5. White Shrimp (*Litopenaeus vannamei)* show: a, b and c- Black spots on their cuticles, carapaces, telsons, uropods, pleopods, and gills exhibited black colorations

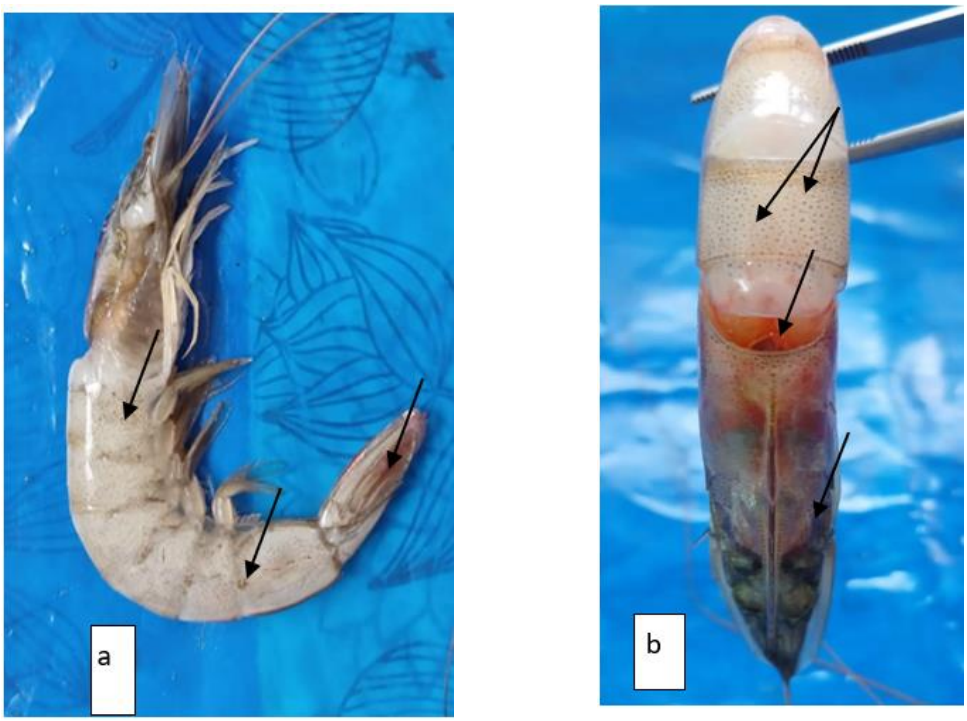

Fig. 6. White shrimp (*Litopenaeus vannamei)* showing: a- Black or reddish discoloration on their uropods and carapaces. b- Congested and tender hepatopancreas

2. Bacterial examination

The predominant *Vibrio* species identified in naturally infected gilthead seabream (*Sparus aurata*), thinlip mullet (*Mugil capito*), and whiteleg shrimp (*Litopenaeus vannamei*) were *V. alginolyticus*, *V. cholerae*, and *V. mimicus*. The identification of these species was achieved through the analysis of distinct colony morphology, followed by confirmation via biochemical profiling (Table 3).

Table 3. Biochemical and cultural characteristics of isolated *Vibrio* from examined cultured fish and shrimp

Test	*Vibrio cholerae*	*Vibrio alginolyticus*	*Vibrio mimicus*
Growth on TCBS	Yellow	Yellow	Green
Gram-stain	-ve	-ve	-ve
Shape	Curved rod	Curved rod	Curved rod
Motility	+	+	+
Catalase	+	+	+
Oxidase	+	+	+
H_2S on triple sugar iron (TSI)	K/A	- A/A	K/A
Citrate	+	-	-
Indole	+	+	+
Methyl red	-	+	-
Vogaus Proskauer	-	+	-
Urease production	-	-	-
Growth on 0% Nacl	+	-	+
Growth on 3 % Nacl	+	+	+
Growth on 6.5 % Nacl	-	+	-

-ve: Negative; +ve: Positive. H2S (TSI) = generation of H2S from triple sugar iron, A/A = Acid/Acid, K/A = Alkaline/Acid.

3. Molecular analysis

3.1.Results of molecular identification of different species of Vibrio isolates and their virulence genes

A bacteriological examination was performed on 180 live, moribund, or freshly dead specimens of fish and shrimp, specifically including the gilthead seabream (*Sparus aurata*), thinlip mullet (*Mugil capito*), and whiteleg shrimp (*Litopenaeus vannamei*). A total of 81 colonies exhibited traits indicative of *Vibrio* species, particularly *V. alginolyticus, V. cholerae,* and *V. mimicus*. Polymerase Chain Reaction (PCR) analysis was conducted to verify the presence of *Vibrio* species and their associated virulence genes. The positive isolates that were identified underwent classification by species, and a representative isolate was chosen for sequencing.

3.1.1. Molecular identification of *Vibrio alginolyticus*

Fig. (7) shows the existence of the collagenase gene in four chosen isolates of *Vibrio alginolyticus*, while Fig. (8) displays the existence of the tdh virulence gene in three of the four selected *V. alginoltyticus* positive strains.

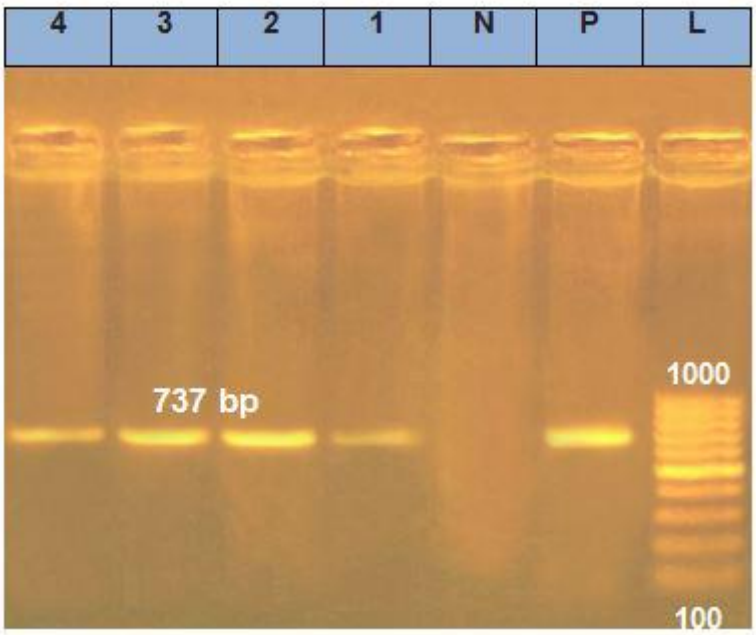

Fig. 7. Molecular detection of collagenase (737 bp) gene of *Vibrio alginolyticus* by PCR. Lane L: 100-1000 bp DNA ladder; Lane P: positive control; Lane N: negative control; Lanes1-4: *V. alginolyticus* shows aband of 737 bp

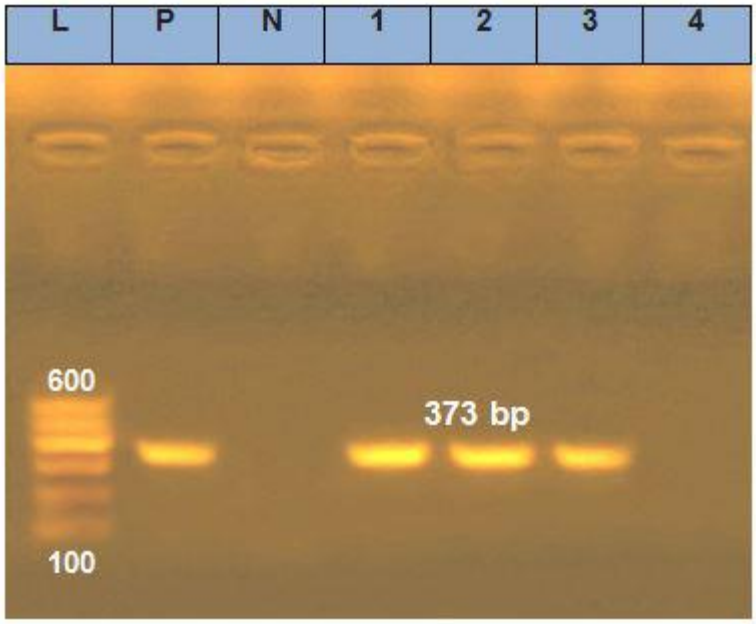

Fig. 8. Identification of the *Vibrio alginolyticus* virulence gene tdh (373 bp) using PCR. Lane L: 100- 600 bp DNA ladder; Lane P: positive control; Lane N: negative control. Lanes 1, 2, and 3: tdh gene-positive strains exhibiting bands at 373 bp; Lane 4: tdh gene-negative strain

3.1.2. Molecular identification of *Vibrio cholerae*

Fig. (9) demonstrates that the sodB gene was present in three out of four selected *Vibrio cholerae* isolates, and Fig. (10) validates the existence of the ctxAB virulence gene in one of the three positive *V. cholerae* isolates.

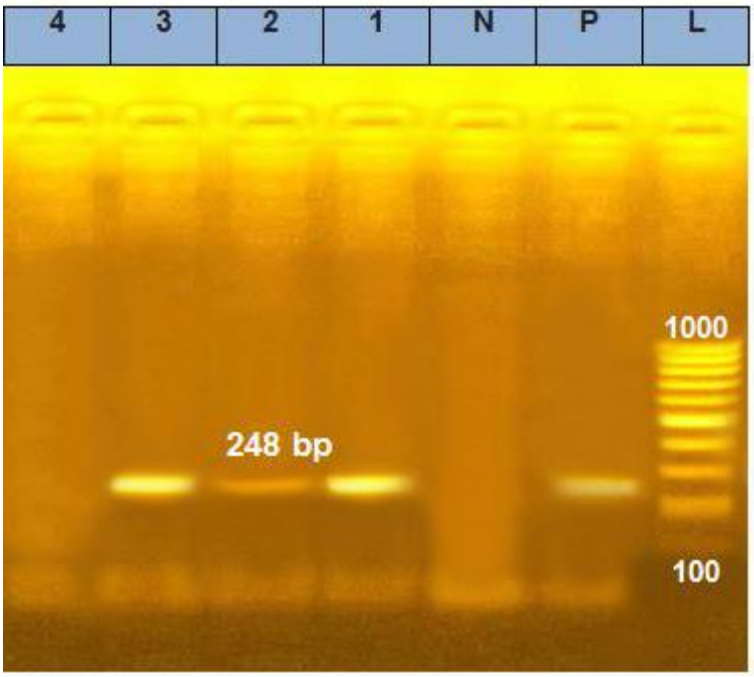

Fig. 9. PCR used for identifying the sodB gene of *Vibrio cholerae*, which is at 248 bp. Lane L: 100-1000 bp DNA ladder; Lane P: positive control; Lane N represents the negative control, while lanes 1-3 show bands at 248 bp for *V. cholerae*

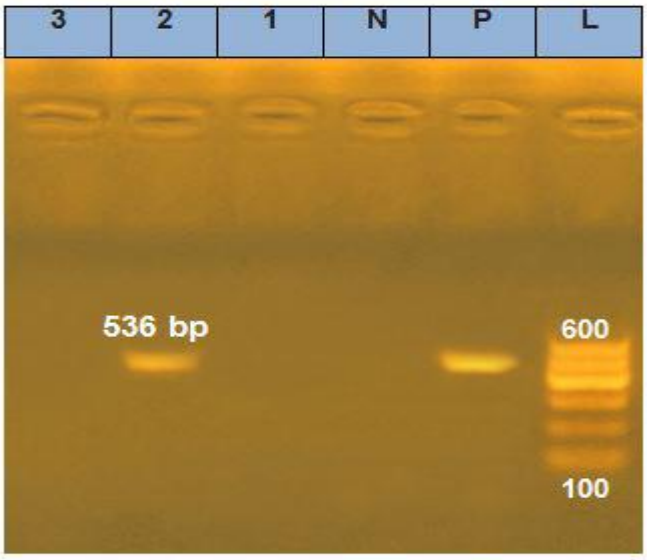

Fig. 10. Identification of the *Vibrio cholerae* ctxAB virulence gene (536 bp) using PCR. Lane L: 100- 600 bp DNA ladder; Lane P: positive control; Lane N, the negative control is shown, while Lane 2 exhibits the *V. cholerae* band at 536 bp.

3.1.3. Molecular identification of *Vibrio mimicus*

Fig. (11) demonstrates that the sodB gene was present in one of the three selected *Vibrio mimicus* isolates.

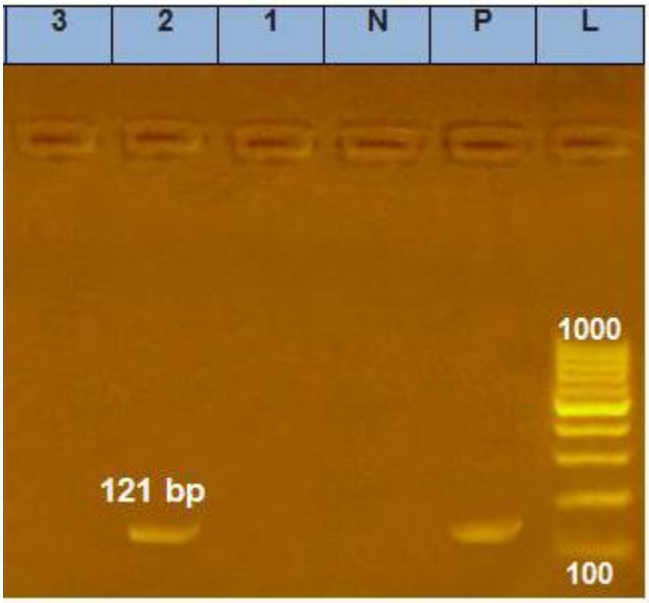

Fig. 11. The identification of the *Vibrio mimicus* sodB gene (121 bp.) using PCR. Lane L: 100-1000 bp DNA ladder; Lane P: positive control; Lane N represents the negative control, while Lane 2 exhibits the *V. mimicus* band at 121 bp

3.2. Result of molecular detection of antimicrobial resistance genes

Fig. (12**)** shows all the four selected isolates of *Vibrio alginolyticus* had β-lactam resistance gene (blaTEM).

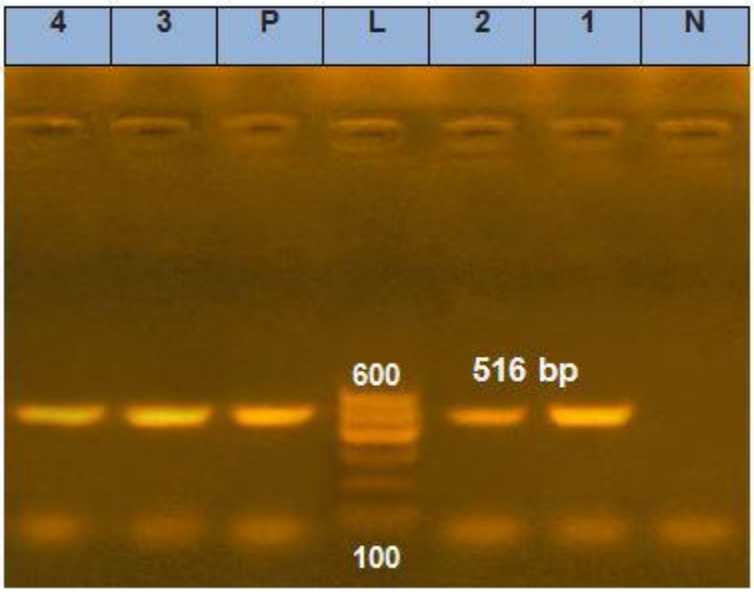

Fig. 12. The presence of the *Vibrio alginoltyticus* blaTEM resistence gene (516 bp) using PCR. In Lane N, the negative control is shown, while Lane 1-4: exhibits the *alginoltyticus* blaTEM resistence gene band at 516 bp

3.3. Gene sequencing and phylogenetic analysis

The molecular characterization of the pathogenic bacterial isolates involved sequencing the sodB gene for *Vibrio cholerae* and *Vibrio mimicus*, as well as the collagenase gene for *Vibrio alginolyticus*. BLAST analysis of the obtained sequences confirmed that the isolates were firmly grouped within the *Vibrio* genus. The similarity index of the *sodB* gene sequences from isolates obtained from the thinlip mullet and the whiteleg shrimp confirmed their identity as *V. cholerae* (Fig. 13), while the *sodB* gene sequences from the thinlip mullet isolates were identified as *V. mimicus* (Fig. 14). Similarly, the *collagenase* gene sequences from the thinlip mullet, whiteleg shrimp, and gilthead seabream confirmed the identity of these isolates as *V. alginolyticus* (Fig. 15).

The *sodB* gene sequences of *V. cholerae* isolates from the thinlip mullet and whiteleg shrimp exhibited 100% nucleotide identity with reference *V. cholerae* strains. The *sodB* gene sequence of *V. mimicus* from the thinlip mullet also showed 100% identity with known *V. mimicus* strains. Furthermore, the collagenase gene sequences of *V. alginolyticus* isolates from the three species—the thinlip mullet, whiteleg shrimp, and gilthead seabream—were determined to be 100% identical to the established sequences of *V. alginolyticus*. The nucleotide sequences acquired in this study have not been submitted to the NCBI GenBank database as of now.

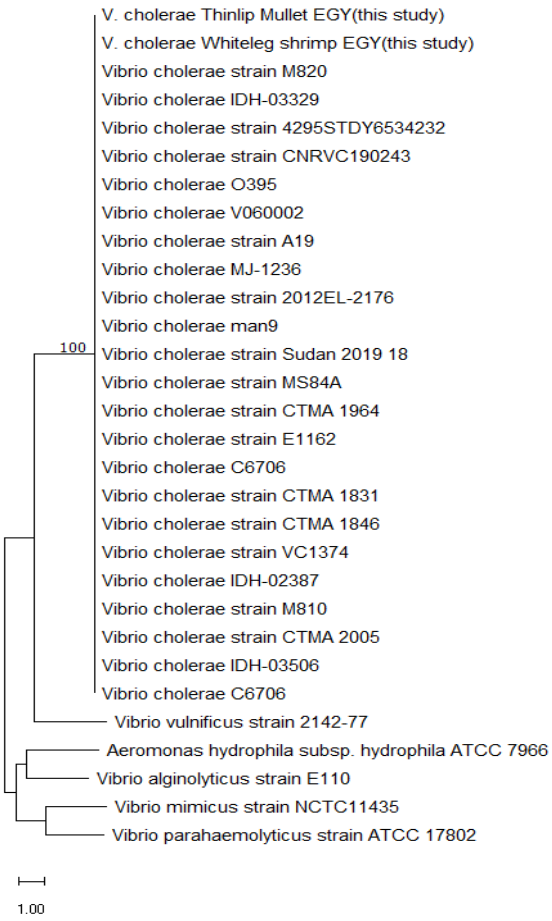

Fig. 13. Phylogenetic tree derived from SodB gene sequences of *Vibrio cholerae* isolates collected from thinlip mullet and whiteleg shrimp in this research. The phylogenetic tree was generated with the neighbor-joining method, incorporating additional species as outgroups

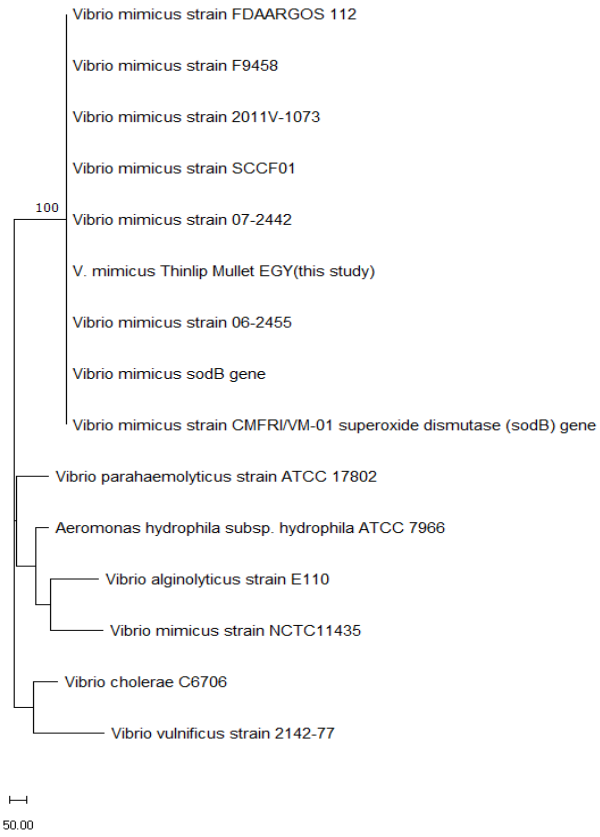

Fig. 14. A phylogenetic tree constructed from the SodB gene sequences of *Vibrio mimicus* isolates sourced from the thinlip mullet. The tree was constructed utilizing the neighbor-joining method, incorporating additional species as well

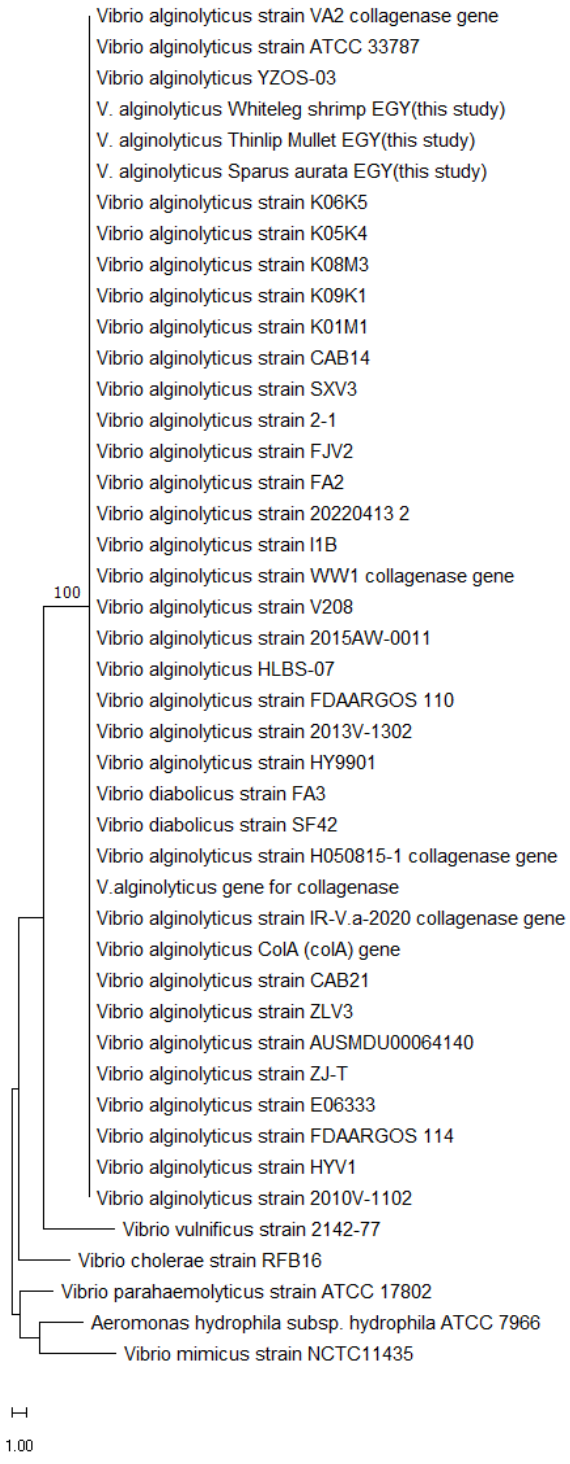

Fig. 15. phylogenetic tree derived from the collagenase gene sequences of *Vibrio alginolyticus* isolates obtained from the thinlip mullet, whiteleg shrimp, and gilthead seabream. The phylogenetic tree was generated employing the neighbor-joining method, incorporating additional species as outgroups for comparative analysis

4. Prevalence of *Vibrio* isolates from naturally infected cultured fish and shrimp

The present investigation carried out in November 2023 revealed that *Vibrio* spp. were isolated from 45% of the analyzed samples of cultured *Sparus aurata* , *Mugil capito*, and *Litopenaeus vannamei*. The highest prevalence was observed in *Sparus aurata* at 58.33%, followed by *Mugil capito* at 43.33% and *Litopenaeus vannamei* at 33.33% (Table 4). In the analysis of *Mugil capito* isolates, *Vibrio alginolyticus* emerged as the predominant species, accounting for 57.14% of the detections, while *V. cholerae* and *V. mimicus* were identified at prevalence of 28.57% and 14.28%, respectively. Interestingly, all *Vibrio*-positive *Sparus aurata* samples were infected with *V. alginolyticus*, indicating a strong association between this species and seabream infections. In contrast, *Litopenaeus vannamei* samples showed a mixed infection pattern, with 60% of isolates identified as *V. alginolyticus* and 40% as *V. cholerae* (Table 5).

Table 4. Prevalence of *Vibrio* spp. isolated from naturally infected cultured fishes and shrimp

Fish spp. & Shrimp	Number of examined fish & shrimp (No.)	Infected fish & shrimp with *Vibrio* spp.	
		No.	%
Mugil capito	60	35	58.33
Sparus aurata	60	26	43.33
Litopenaeus vannamei	60	20	33.33
Total	180	81	45

Table 5. *Vibrio* spp. isolated from naturally infected cultured fishes and shrimp

Fish species & Shrimp	No. of Infected fish & shrimp (Positive cases for *Vibrio* spp.)	*Vibrio* species	Positive cases	
			No.	%
Thinlip Mullet (*Mugil capito*)	35	*V.alginolyticus*	20	57.14
		V. cholerae	10	28.57
		V. mimicus	5	14.28
Seabream *(Sparus aurata*)	26	*V. alginolyticus*	26	100
White shrimp *(Litopenaeus vannamei)*	20	*V. alginolyticus*	12	60
		V. cholerae	8	40

4.1. Prevalence of Vibrio species isolates from different organs of naturally infected cultured fish and shrimp

Fig. (16) illustrates the isolation of *Vibrio* spp. from different internal organs and tissues of naturally infected cultured *Sparus aurata* and *Mugil capito*. The liver exhibited the highest prevalence (38.46 and 37.14%,), followed by the kidneys (26.92 and 25.71%) and spleen (19.32 and 17.14%). Lower prevalence rates were observed in the gills (11.54 and 8.75%) and skin/ulcers (3.84 and 11.43%), respectively (Fig. 16). In *Litopenaeus vannamei*, *Vibrio* spp. were isolated from the hepatopancreas and musculature, with corresponding prevalence rates of 75 and 25% (Fig. 17).

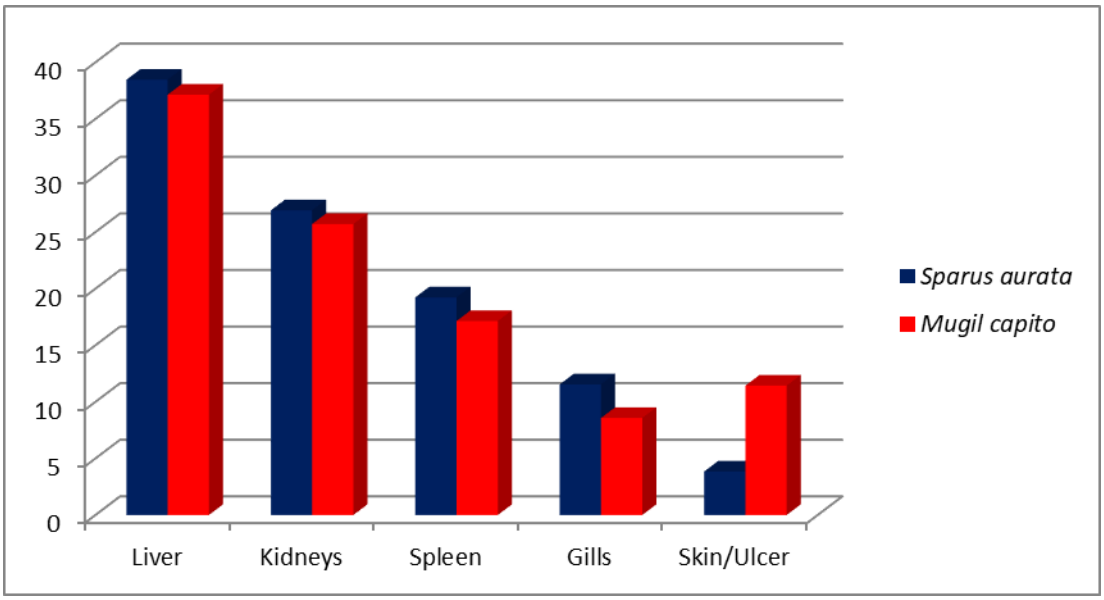

Fig. 16. Prevalence of *Vibrio* spp. isolates from different organs and tissues of naturally infected cultured *Sparus aurata L.* and *Mugil capito*

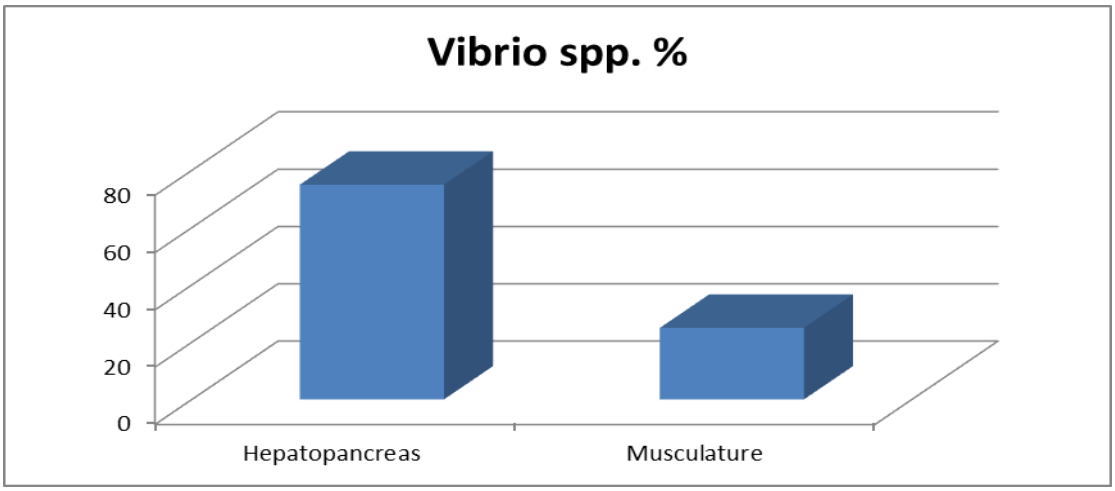

Fig. 17. *Litopenaeus vannamei* shrimp *Vibrio* spp. prevalence in the hepatopancreas and musculature

5. Antibiotic sensitivity test

Isolates of *Vibrio alginolyticus*, *V. cholerae*, and *V. mimicus* isolated from the gilthead seabream (*Sparus aurata*), thinlip mullet (*Mugil capito*), and whiteleg shrimp (*Litopenaeus vannamei*) were evaluated for their antibiotic susceptibility profiles, as presented in Table (6).

Table 6. Effect of different antibiotics on *Vibrio* spp. isolates from naturally infected cultured fish and shrimp

Antimicrobial Class	Antibiotics	Code	Doses of antibiotic (µg)	*Vibrio cholerae*	*Vibrio alginolyticus*	*Vibrio mimicus*
Aminoglycosides	Gentamicin	CN	10 ug	S	S	S
Tetracyclines	Tetracycline	T	30 ug	S	S	S
Phenicols	Chloramphenicol	C	30 ug	S	S	S
Beta lactams	Ampicillin	AM	10 ug	S	S	S
	Penicillin	P	10 ug	S	R	S
Folate pathway inhibitors	Trimethoprim-Sulfamethoxazole	SXT	30 ug	S	S	S
Fluoroquinolone	Ciprofloxacin	CIP	5 ug	S	S	S

S: Sensitive I: Intermadiate R: Resistant

DISCUSSION

Infectious disease outbreaks remain a significant challenge for the aquaculture sector, resulting in substantial economic losses attributed to morbidity and mortality **(Rohani *et al.*, 2022)**. Vibriosis stands out as one of the most prevalent and damaging bacterial infections, particularly affecting farmed shrimp, fish, and shellfish. This phenomenon is notably prevalent in hatcheries and grow-out systems, where the juvenile stages exhibit heightened vulnerability. The occurrence and intensity of Vibriosis are affected by various factors, including the origin of the cultured species, environmental conditions like water quality, farm management practices, and the pathogenicity of the *Vibrio* strains involved **(Ina-Salwany *et al.*, 2019)**.

During the present investigation, cultured *Sparus aurata*, *Mugil capito*, and *Litopenaeus vannamei* naturally infected with *Vibrio* spp. exhibited a range of characteristic clinical signs. *S. aurata* presented with exophthalmia, abdominal distension, and hemorrhages around the pectoral fins, along with internal findings such as congested gills, hepatic hemorrhagic patches, and congestion of the kidneys, spleen, and ovarian blood vessels. Similarly, *M. capito* commonly displayed surface hemorrhages, especially near the eyes and pectoral fins, in addition to ocular opacity, fin erosion, and well-defined deep ulcers. Internally, the gills, liver, spleen, and kidneys also showed marked congestion and hemorrhages. The clinical observations align with the findings of **El-Hady *et al.* (2015)**, **El-Bouhy *et al.* (2016)** and **Ismail *et al.* (2024a)**. Pathogenesis generally begins with bacterial adhesion to host tissues, facilitated by various virulence

factors, followed by proliferation and systemic dissemination via the bloodstream **(Manchanayake *et al.*, 2023)**.

In this study, some *Litopenaeus vannamei* shrimp displayed black spots on their cuticles, carapaces, telsons, uropods, pleopods, and gills, with some exhibiting black or reddish discoloration on the uropods and carapaces. The hepatopancreas exhibited congestion and tenderness in specific instances. The findings align with earlier studies conducted by **El Zlitne *et al.* (2022)**, **Ismail *et al.* (2024b)** and **Shimaa *et al.* (2024)**. Prolonged exposure to adverse environmental conditions diminishes the immune defences of shrimp, consequently heightening their vulnerability to bacterial infections **(Elgendy *et al.*, 2015)**. *Vibrio* species utilize various virulence factors, including extracellular enzymes and specific fitness genes, to enhance their ability to infect and cause disease **(Gennari *et al.*, 2012)**. The observed shell discoloration aligns with descriptions of shell disease syndrome, commonly referred to as "brown spot" or "black spot disease." This condition typically begins when damage to the exoskeleton allows opportunistic pathogens to invade **(Radhakrishnan & Kizhakudan, 2019)**. Melanization on the outer shells of crustaceans is a well-documented immune response in both marine and freshwater species **(Cuéllar-Ánjel *et al.*, 2014)**.

Fluctuations in water temperature, ranging erratically from 25.5 to 18.0°C, may have significantly increased fish susceptibility to opportunistic bacterial infections, particularly those caused by *Vibrio* species. Sudden thermal changes are known stressors in aquatic systems, capable of compromising immune function and disrupting mucosal barriers—the primary defense against invading pathogens **(Bowden, 2008; Scharsack *et al.*, 2022)**. *Vibrio* spp. are opportunistic pathogens that can rapidly exploit host vulnerability under environmental stressors such as thermal shock or unstable temperatures **(Austin & Austin, 2016; Lages *et al.*, 2019)**. Moreover, temperature fluctuations may directly enhance the virulence and proliferation of *Vibrio* spp., thereby increasing their pathogenicity. Accordingly, the irregular temperature patterns observed during the study likely created favorable conditions for bacterial colonization and disease development.

Bacteriological analysis identified the predominant *Vibrio* species in naturally infected *Sparus aurata* (gilthead seabream), *Mugil capito*, and *Litopenaeus vannamei* (whiteleg shrimp) as *V. alginolyticus*, *V. cholerae*, and *V. mimicus*. These species were Gram-negative, motile, halophilic, curved rods and tested positive for catalase and oxidase. Colony morphology on TCBS agar aided differentiation: *V. mimicus* colonies appeared smooth and green (sucrose-negative), while *V. cholerae* and *V. alginolyticus* colonies were smooth and yellow (sucrose-positive).

Biochemical tests revealed that *V. cholerae* isolates were indole and citrate positive but negative for methyl red, Voges-Proskauer, and urease, with alkaline/acid reactions in TSI. *V. alginolyticus* tested positive for indole, methyl red, and Voges-Proskauer, but negative for citrate and urease, showing an acid/acid TSI reaction. *V. mimicus* was negative for all these biochemical tests and showed an alkaline/acid reaction in TSI.

These findings are consistent with previous studies **(Ibrahim *et al.*, 2018; El Sayed *et al.*, 2021; Hernández-Robles *et al.*, 2021; Gobarah *et al.*, 2022, 2023; Mansour & El-Shaer, 2023; Hend *et al.*, 2024; Ismail *et al.*, 2024a; Shimaa *et al.*, 2024)**.

PCR was used to confirm species identification and assess virulence gene profiles. The *sodB* gene was amplified in three out of four *V. cholerae* isolates (248 bp), with one also carrying the *ctxAB* gene (536 bp). All four *V. alginolyticus* isolates amplified the collagenase gene (737 bp), and three also carried the *tdh* gene (373 bp). One of three *V. mimicus* isolates tested positive for *sodB* (121 bp). These results align with previous work identifying *Vibrio* spp. in various aquatic organisms using PCR **(Abd El Tawab *et al.*, 2018; Ibrahim *et al.*, 2018; Sadat *et al.*, 2020; Gobarah *et al.*, 2022; Zobayda *et al.*, 2023; Ismail *et al.*, 2024a; Shimaa *et al.*, 2024)**.

Resistance gene analysis revealed the presence of the *blaTEM* gene (516 bp) in all four tested *V. alginolyticus* isolates, supporting findings by **Gobarah *et al.* (2023)**, who observed widespread *blaTEM* detection in *Vibrio* spp. from infected fish. Similarly, **Silvester *et al.* (2019)** reported high *blaTEM* prevalence in isolates from estuarine water, seafood, and shrimp farms. This highlights the growing issue of antimicrobial resistance in aquaculture and the urgent need for prudent antibiotic use.

Molecular identification was confirmed through phylogenetic analysis and gene sequencing. *sodB* gene sequences from *Mugil capito* and *Litopenaeus vannamei* matched *V. cholerae*, while the *sodB* gene from *Mugil capito* matched *V. mimicus*, confirming their taxonomic classification. Likewise, the collagenase gene sequences from *V. alginolyticus* isolates showed complete identity with reference strains, suggesting its predominance and potential host adaptation in Egyptian mariculture. Although these sequences matched known *Vibrio* species, they have not yet been submitted to public databases, underscoring the need for ongoing molecular surveillance and data sharing.

In samples collected in November 2023, *Vibrio* spp. were identified in 45% of naturally infected *Sparus aurata*, *Mugil capito*, and *Litopenaeus vannamei*. Prevalence rates were 58.33% in *S. aurata*, 43.33% in *M. capito*, and 33.33% in *L. vannamei*. In *M. capito*, *V. alginolyticus* was most prevalent (57.14%), followed by *V. cholerae* (28.57%) and *V. mimicus* (14.28%). All *S. aurata* samples tested positive for *V. alginolyticus*. In *L. vannamei*, 60% were positive for *V. alginolyticus*, and 40% for *V. cholerae*. These findings align with previous studies **(Abdel-Aziz *et al.*, 2013; El-Bouhy *et al.*, 2016; Ismail *et al.*, 2024a)**.

Findings for shrimp also align with **Yu *et al.* (2023)**, who reported *V. alginolyticus* in 28.6% of shrimp, 55.1% of breeding water, and 56% of biological feeds. **Elgendy *et al.* (2015)** identified multiple *Vibrio* spp. during a severe outbreak in *Penaeus indicus*. El **Zlitne *et al.* (2022)** reported *V. parahaemolyticus* and *V. alginolyticus* in clinically affected *L. vannamei* shrimp from earthen ponds.

Differences in *Vibrio* species prevalence across studies may result from varying sample sizes, host susceptibility, environmental factors (e.g., salinity), and sources of larvae or water **(Al-Taee *et al.*, 2017; Gobarah *et al.*, 2022)**.

The liver exhibited the highest prevalence of *Vibrio* spp. in both *S. aurata* (38.46%) and *M. capito* (37.14%), followed by the kidneys (26.92%, 25.71%), spleen (19.32%, 17.14%), gills (11.54%, 8.75%), and skin/ulcers (3.84%, 11.43%). These findings mirror previous research **(Abdel-Aziz *et al.*, 2013; Abd El Tawab *et al.*, 2018)**, which found the liver and kidneys to be primary infection sites due to their roles in detoxification and immune defense.

In *L. vannamei*, *Vibrio* spp. were detected in the hepatopancreas (75%) and muscles (25%). This agrees with **Shanmugasundaram *et al.* (2015)** and **Shimaa *et al*. (2024)**, who observed multiple *Vibrio* spp. in the hepatopancreas of infected shrimp. As a major site of metabolism and immunity, the hepatopancreas is a key target for bacterial infection **(Zhao *et al*., 2017)**.

Antibiotic susceptibility testing showed that all isolates were sensitive to gentamicin, tetracycline, ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and ciprofloxacin. However, *V. alginolyticus* showed resistance to piperacillin, a β-lactamase inhibitor. This is consistent with findings from **Sadat *et al.* (2020)** and **Gobarah *et al.* (2023)**, who noted similar resistance profiles, particularly to ampicillin and penicillin. Differences in resistance patterns may be influenced by antibiotic usage practices across aquaculture facilities.

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CONCLUSION

In conclusion, this study reveals that *Vibrio alginolyticus*, *V. cholerae*, and *V. mimicus* are the main pathogens affecting *Sparus aurata*, *Mugil capito*, and *Litopenaeus vannamei*, with *V. alginolyticus* being the most prevalent. Infections were tissue-specific, primarily targeting the liver and kidneys in fish, and the hepatopancreas and musculature in shrimp. Most isolates were susceptible to Gentamicin, Tetracycline, and Ciprofloxacin, though *V. alginolyticus* showed resistance to Piperacillin. These findings point to the increasing challenge of antibiotic resistance in aquaculture, highlighting the necessity for proactive monitoring and sustainable health management.

REFERENCES

Abd El Tawab, A.; Ibrahim, A.M. and Sittien, A. (2018). Phenotypic and Genotypic characterization of *Vibrio* species isolated from marine fishes. Benha Vet. Med. J., 34(1): 79–93.

Abdel-Aziz, M.; Eissa, A.E. and Hanna, 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. Int. J. Vet. Sci. Med.,1(2):87–95.

Abu-Elala, N.M.; Abd-Elsalam, R.M.; Marouf, S.; Abdelaziz, M. and Moustafa, M. (2016). Eutrophication, Ammonia Intoxication, and Infectious Diseases: Interdisciplinary Factors of Mass Mortalities in Cultured Nile Tilapia. J. Aquat. Anim. Health, 28(3):187–198.

Al-Taee, A.M.R.; Khamees, N.R. and Al-Shammari, N.A.H. (2017). *Vibrio* Species Isolated from Farmed Fish in Basra City in Iraq. J. Aquac. Res. Development, 8: 472.

Altschul, S.F.; Madden, T.L.; Schäffer, A.A.; Zhang, J.; Zhang, Z.; Miller, W. and Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic. Acids. Res., 25(17):3389-402.

Austin, B. and Austin, D.A. (2007). Bacterial fish pathogens, Diseases of farmed and wild fish. Fourth Edition, Praxis publishing Ltd, Chichester, UK.

Austin, B. and Austin, D.A. (2016). Bacterial Fish Pathogens: Disease of Farmed and Wild Fish. Springer.

Baker-Austin, C.; Oliver, J.D.; Alam, M.; Ali, A.; Waldor, M.K.; Qadri, F. and Martinez-Urtaza, J. (2018). *Vibrio* spp. infections. Nature Reviews Disease Primers. 4(8):1–19.

Balouiri, M.; Sadiki, M. and Ibnsouda, S.K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. J. Pharm. Anal., 6(2): 71-79.

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. Aquac., 531: 735949.

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.

Bondad-Reantaso, M.G.; MacKinnon, B.; Karunasagar, I.; Fridman, S.; Alday-Sanz, V.; Brun E.; Groumellec, M.Li.A.; Surachetpong, W.; Karunasagar, I.; Hao, B.; Dall'Occo, A.; Urbani, R. and Caputo, A. (2023). Review of alternatives to antibiotic use in aquaculture. Rev. Aquac. 15:1421–1451.

Bowden, T.J. (2008). Modulation of the immune system of fish by their environment. Fish Shellfish Immunol., 25(4): 373–383.

Buller, N.B. (2004). Bacteria from Fish and Other Aquatic Animals. A Practical Identification Manual , CABI Publishing, Wallingford, UK. Aquac., 240(1-4):626-627.

Chien, J.; Shih, J.; Hsueh, P.; Yang, P. and Luh, K. (2002). *Vibrio alginolyticus* as the cause of pleural empyema and bacteremia in an immunocompromised patient. Eur. J. Clin. Microbiol. Infect. Dis., 21(5):401-403.

CLSI, (2018). Performance standards for antimicrobial susceptibility testing, 28th Edition. CLSI supplement M100, Wayne. Clinical and Laboratory Standards Institute.

Colom, K.; Pèrez, J.; Alonso, R.; Fernández-Aranguiz, A.; Lariňo, E. and Cisterna, R. (2003). Simple and reliable multiplex PCR assay for detection of *bla*$_{\text{TEM}}$,*bla*$_{\text{SHV}}$ and *bla*$_{\text{OXA-1}}$ genes in Enterobacteriaceae. FEMS Microbiol. Letters, 223: 147-151.

Cu´ellar-Anjel, J.; Corteel, M.; Galli, L.; Alday-Sanz, V. and Hasson, K.W. (2014). Principal shrimp infectious diseases, diagnosis and management. In: Alday-Sanz, V. The Shrimp Book. Nottingham University Press, UK. 517-622.

Da Silva, J.G. (2016). The state of world fisheries and aquaculture**.** In Contributing to Food Security and Nutrition for All; Food and Agriculture Organization of the United Nations Press: Rome, Italy, 1–204.

De Menezes, F.G.R.; Neves, S.D.; de Sousa, O.V.; Vila-Nova, C.M.V.M.; Maggioni, R.; Theophilo, G.N.D.; Hofer, E. and Vieira, R.H.S.D. (2014). Detection of virulence genens in environmental strains of *Vibrio cholerae* from estuaries in north eastern Brazil. Rev. Inst. Med. Trop. Sao Paulo,, 56(5):427-432.

Eissa, A. E.; Abdelsalam, M.; Abumhara, A.; Kammon, A.; Gammoudi, F. T.; Ben Naser, K.M.; Borhan, T. and Asheg, A. (2015). First Record of *Vibrio vulnificus* / Anisakis pegreffi Concurrent Infection in Black scorpionfish (Scorpaena porcus) from the South Mediterranean Basin. Research J. Pharm. Biol. Chem. Sci., 6(3): 1537-1548.

Eissa, I.; Abou-Elgheit, S. N.; Dessuki, A. A.; Hassanin, A. and Mostafa, L. T. (2019). Advanced diagnosis of vibriosis among some marine fishes in lake Temsah, Egypt. J. Hellenic Vet. Med. Soc., 70(1): 1339–1346.

El-Bouhy, Z.; El-Nobi, G. and El-Murr, A. (2016). Study on vibriosis in *Mugil Capito* in El-Dakahlia and Damitta governorates. Egypt. Abbassa Int. J. Aqua.,9(1):19–35.

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. J. Fish. Aquat. Sci., 10: 146-158.

El-Hady, M.A.; El-khatib, N.R. and Abdel- Aziz, E.S. (2015). Microbiological studies on *Vibrio* species isolated from some cultured fishes. Anim. Health Res. J., 3(1): 12-19.

EL Sayed, M.R.; Osman, A.E.; Emam, R.M.; Abo EL-Gglil, M.A.A. and Sayed, H. (2021). Studies on *vibrio alginolyticus* infection among some red sea fishes at hurghada. Assiut. Vet. Med. J., 67(170): 37-50.

El Zlitne, R.; Eissa, A.E.; Elgendy, M.Y.; Abdelsalam, M.; Sabry, N.M.; Sharaf, M.S.; Eltahan, A.S.; Mahmoud, A.E.; El Moghazi, D.F.; Ismail, M.M.; Abu Mhara, A.; Ismail, E.M.; Zaki, M.M. and Abdelbaky, A.A. (2022). Vibriosis triggered mass kills in Pacific white leg shrimp (*Litopenaeus vannamei*) reared at some Egyptian earthen pond-based aquaculture facilities. Egypt. Aquat. Biol. Fish. , 26(3): 261 – 277.

Fadel, H.M. and El-Lamie, M.M. (2019). Vibriosis and Aeromonas infection in shrimp: isolation, sequencing and control. Int. J. One Health, 5(1).

FAO (2018). The State of World Fisheries and Aquaculture, Meeting the Sustainable Development Goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.

FAO (2019). *Sparus aurata* (Linnaeus, 1758). Cultured Aquatic Species Information Programme. FAO Fisheries and Aquaculture Department. [(accessed on 9 November 2019)].

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.

Gobarah, D.E.A.; Helmy, S.M.; Mahfouz, N.B.; Fahmy, H.A. and Abou Zeid, M.A.M. (2022). Virulence genes and antibiotic resistance profile of *Vibrio* species isolated from fish in Egypt. Vet Res Forum., 15;13(3):315–321.

Gobarah, D.E.A.; Helmy, S.M.; Mahfouz, N.B.; Fahmy, H.A.; Abou Zeid, M.A.M. and Moustafa, E.M. (2023). Molecular Characterization of Antimicrobial Resistance of *Vibrio* Species Isolated from Fish in Egypt. J. Hellenic Vet. Med. Soc., 74 (1): 5101-5110.

Hend, M.M.; Soad, M.; EL ALEM, M.A.; SALEM, H.S.S. and Bakry, M.A. (2024). Trials for control of *vibrio alginolyticus* infection in cultured Seabream using probiotics. Assiut Vet. Med. J., 70(180): 203-217.

Hern´andez-Robles, M.F.; Natividad-Bonifacio, I.; lvarez-Contreras, A.K.A.; Tercero-Alburo, J.J.; Quiñones-Ramı´rez, E.I. and Va´zquez-Salinas, C. (2021). Characterization of Potential Virulence Factors of *Vibrio mimicus* Isolated from Fishery Products and Water. Int. J. Microbiol., (6):1-7.

Ibrahim, H. M.; Reham, A.A.; Nesreen, Z. E. and Hanan, R. M. G. (2018). *Vibrio* Species in Fish and Shell Fish. Benha Vet. Med. J., 34(2): 246-254.

Ina-Salwany, M.Y.N.; Al-saari, A.M.; Mursidi, F.A.; Mohd-Aris, A.; Amal, M.N.A.; Kasai, H.; Mino, S.; Sawabe, T. and Zamri-Saad, M. (2019). Vibriosis in fish: a review on disease development and prevention. J. Aquat. Anim. Health, 31(1): 3-22.

Ismail, E.T.; El-Son, M.A.M.; El-Gohary, F.A. and Zahran, E. (2024a). Prevalence, genetic diversity, and antimicrobial susceptibility of *Vibrio* spp. infected gilthead sea breams from coastal farms at Damietta, Egypt. BMC Vet. Res., 20(1):129.

Ismail, M.M.; ElBanna, N.I.; Qorany, R.A.; Assy, A.M. and Ahmed, A.A. (2024b). Comprehensive Assessment of Bacterial Diseases in the Shrimp: Clinical, Phenotypic, Genotypic, and Histopathological Approaches. Egypt. J. Aquat. Biol. Fish., 28(4): 2147 – 2165.

Kumar, S.; Stecher, G.; Michael, L.; Christina, K. and Koichiro, T. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol. Biol. Evol., 35(6): 1547–1549.

Lages, M.A.; Balado, M. and Lemos, M.L. (2019). The Expression of Virulence Factors in *Vibrio anguillarum* Is Dually Regulated by Iron Levels and Temperature. Front Microbiol., 5(10):2335.

Manchanayake, T.; Salleh, A.; Amal, M.N.A.; Yasin, I.S.M. and Zamri-Saad, M. (2023). Pathology and pathogenesis of Vibrio infection in fish: A review. Aquac. Reports, 28(10):101459.

Mansour, S.M. and El-Shaer, W. (2023). Studies on the most prevailing bacterial diseases in *Trachurus indicus* fish. Egypt. J. Aquat. Biol. Fish., 27(3): 163 – 179.

Mustapha, S.; Mustapha, E.M. and Nozha, C. (2013). *Vibrio alginolyticus:* An Emerging Pathogen of Foodborne Diseases. Int. J. Sci. Technol (IJST), 2(4): 302-309.

Radhakrishnan, E.V. and Kizhakudan, J.K. (2019). Health Management in Lobster Aquaculture. In: Lobsters: Biology. Fisheries and Aquaculture. Springer, Singapore, 571–601.

Raja, R.A.; Panigrahi, A.; De, D. and Kumar, S. (2017). Investigations on white spot disease outbreak in *Penaeus monodon*. Indian J. Fish., 64(3): 56–60.

Rohani, M.F.; Islam, S.M.; Hossain, M.K.; Ferdous, Z.; Siddik, M.A.; Nuruzzaman, M.; Padeniya, U.; Brown, C. and Shahjahan, M. (2022). Probiotics, Prebiotics and Synbiotics Improved the Functionality of Aquafeed: Upgrading Growth, Reproduction, Immunity and Disease Resistance in Fish. Fish Shellfish Immunol., 120:569–589.

Sadat, H.; El-Sherbiny, A.; Zakaria, H.; Ramadan, H. and Awad, A. (2020). Prevalence, antibiogram and virulence characterization of Vibrio isolates from fish and shellfish in Egypt: a possible zoonotic hazard to humans. J. Applied Microbiol., 131(1): 485—498.

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.

Scharsack, J.P. and Franke F. (2022). Temperature effects on teleost immunity in the light of climate change. J Fish Biol.,101(4):780-796.

Shanmugasundaram, S.; Mayavu, P.; Manikandarajan, T.; Suriya, M.; Eswar, A. and Anbarasu, R. (2015). Isolation and identification of *Vibrio* spp. in the Hepatopancreas of cultured white pacific shrimp (*Litopenaeus vannamei*) . Int. Letters Natural Sci., 46: 52-59.

Shimaa, M. M.; Rehab, E. M. G. and Walaa, A. El-Shaer (2024). Insights on Vibriosis in white shrimp (*Metapenaeus stebbingi*): prevalence, virulence genes, and potential limitations of Existence in the meat. Egypt.J. Anim. Health, 4 (3): 76-96.

Silvester, R.; Pires, J.; Van Boeckel, T.P.; Madhavan, A.; Meenakshikutti, A.B. and Hatha, M. (2019). Occurrence of b-Lactam Resistance Genes and Plasmid-Mediated Resistance Among Vibrios Isolated from Southwest Coast of India. Microb. Drug Resist., 25(9):1306-1315.

Soliman, N.F. and Yacout, D.M. (2016). Aquaculture in Egypt: status, constraints and potentials. Aquac. Int., 24: 1201-1227.

Stephen, G. (2022). An update on vibriosis, the major bacterial disease shrimp farmers face. Responsible Seafood Advocate,1-8.

Tarr, C. L.; Patel, J. S.; Puhr, N. D.; Sowers, E. G.; Bopp, C. A. and Strockbine, N. A. (2007). Identification of Vibrio isolates by a multiplex PCR assay and rpoB sequence determination. J. Clin. Microbiol., 45(1): 134-140.

Yu, Y.; Tang, M.; Wang, Y.; Liao, M.; Wang, C.; Rong, X.; Li, B.; Ge, J.; Gao, Y.; Dong, X. and Zhang, Z. (2023). Virulence and antimicrobial resistance characteristics assessment of Vibrio isolated from shrimp (*Penaeus vannamei*) breeding system in south China. Ecotoxicol. Environ. Saf., 252,114615.

Zhao, W.; Wang, L.; Liu, M.; Jiang, K.; Wang, M.; Yang, G.; Qi, C. and Wang, B. (2017). Transcriptome, antioxidant enzyme activity and histopathology analysis of hepatopancreas from the white shrimp Litopenaeus vannamei fed with aflatoxin B1(AFB1). Dev. Comp. Immunol., 74:69-81.

Zobayda, F.H.; Saiful Islam, M.D.; Al Momen Sabuj, A.; Pondit, A.; Kumar Sarkar, A.; Golzar Hossain, M.D. and Saha, S. (2023). Molecular Detection and Antibiotic Resistance of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio alginolyticus* from Shrimp (*Penaeus monodon*) and Shrimp Environments in Bangladesh. Aquac. Res., 11.