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Polyphasic Characterization of an Emerging *Vibrio harveyi* Strain Associated with Mortalities in European Seabass (*Dicentrachus Labrax*) in Rearing Mariculture Floating Cages

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ABSTRACT

Vibrio harveyi is a motile marine bacterium frequently linked to fish and shellfish diseases in aquaculture, particularly vibriosis, which leads to high mortality rates in marine species. This pathogen also poses a public health concern due to its potential to contaminate seafood and cause gastrointestinal infections in humans. The growing antibiotic resistance underscores the importance of effective monitoring and management strategies to safeguard both aquatic life and human health. In this study, 120 specimens of the European seabass (*Dicentrachus labrax*) and water waere collected from mariculture floating net cages during episodes of widespread fish mortality in marine fish farms. The moribund fish European seabass were thoroughly examined to assess clinical, post-mortem, and bacteriological aspects of the disease, thus providing evidence for the occurrence of a septicemic-hemorrhagic bacterial disease. Identification of the bacterium included morphological analysis using the API 20E system, 16S rRNA characterization through PCR and gene sequencing, and testing against Vibriostat O/129. Antibacterial susceptibility tests and histopathological examinations revealed an immune cell infiltration in the interstitial tissue, indicating a defensive response to infection. In addition, *Vibrio harveyi* strain could resist several antibiotics, resulting in difficulties in fish treatment. The study aimed to enhance understanding of the disease affecting seabass and explore potential therapeutic interventions to mitigate its impact.

INTRODUCTION

Egypt's brackish water aquaculture accounted for 16.6% of the total aquaculture production among Mediterranean and Black Sea countries during 2020–2021 **(FAO, 2022a, 2023)**. However, the country's aquaculture industry has faced significant challenges, including mass mortality of cultured marine and freshwater fish. Septicemic

bacterial pathogens, particularly *Vibrio* species, have caused severe losses in the aquaculture sector, posing a substantial threat to fish production. *Vibrio*, a genus of pathogenic bacteria, is a major cause of disease outbreaks globally, with infections being especially common in aquaculture. This has prompted the development of various strategies for disease prevention and management **(Sanches-Fernandes *et al*., 2022)**.

Vibriosis has been reported at all stages of the seabass production chain **(Muniesa *et al*., 2020)** and is a significant pathogen responsible for high mortality rates in marine organisms, severely affecting aquaculture production. The disease is characterized by clinical signs such as hemorrhages on the fins, particularly around the operculum and vent, eye lesions or blindness, abdominal swelling, gastroenteritis, muscle necrosis, skin ulcers, and tail rot disease **(Zhang *et al*., 2020; Zhou *et al*., 2024)**.

V. harveyi is a motile bacterium ubiquitous in the marine environment and widely distributed across the globe. Once regarded as an opportunistic pathogen, it has now become a major threat to the fishing and aquaculture industries. It causes systemic infections that, if untreated, can quickly result in high mortality rates and substantial economic losses. Managing infections caused by this bacterium is particularly challenging due to its adaptability and ability to thrive in diverse environmental conditions **(Samsing *et al*., 2023)**.

The bacteria are commonly found in marine and estuarine waters, with their prevalence closely linked to seasonal variations, particularly during the warmer months from June to November **(Cascarano *et al*., 2021)**. This pathogen has been linked to mass mortalities in larvae and juveniles of the farmed gilthead seabream in Malta **(Haldar *et al*., 2010)**. It has also been detected in the European seabass and gilthead seabream farmed at various aquaculture facilities along the Mediterranean coast of Spain **(Pujalte *et al*., 2003)**. According to **Vendramin *et al*. (2016**), *V. harveyi* has been identified as an emerging threat to seabass in a survey of the major pathogens threatening the Mediterranean aquaculture.

The objective of this study was to investigate the biochemical and molecular characteristics of the *V. harveyi* strain, providing valuable insights for the development of strategies to control mortality rates in sea bass aquaculture. This detailed analysis aimed to identify key features of the strain, which will allow the formulation of targeted approaches to mitigate its impact and improve fish health management in aquaculture systems.

MATERIALS AND METHODS

1. Fish sampling

A private European seabass (*Dicentrarchus labrax*) mariculture facility with floating net cages (30m × 10m × 2.5m) located in the coastal region of Alexandria, Egypt,

reported a mortality rate of 50% over a 10-day period, with approximately 120 fish dying per day. One hundred and twenty moribund fish, each weighing 100±5g, were transported in aerated water to the wet laboratory of the Fish Disease Department at AHRI for further analysis. Clinical and post-mortem examinations were performed as described by **Austin and Austin (2012)**, along with bacteriological and histopathological evaluations.

2. Analysis fish water farm

Four water samples were collected in sterile polyethylene bottles from various designated floating cages for analysis, following the protocols of **Boyd (1990)**, **APHA (2012)** and the **Canadian Council on Animal Care (2005)**. Measurements of key water quality parameters, including pH (S.N.B0021750), dissolved oxygen (Aqualytic, OX24), temperature, salinity (HANNA Instruments, Romania), and concentrations of un-ionized ammonia, nitrate, nitrite, and sulfate were performed on site. Samples were transported on ice to the Animal Health Research Institute and analyzed using a Thermo-Spectronic 300 UV/Visible spectrophotometer.

3. Bacteriological examination

Gill, liver, kidney, spleen and brain samples were plated under aseptic conditions on tryptic soy agar (TSA) containing 3% NaCl and thiosulfate citrate bile salts sucrose (TCBS) agar media. Plates were incubated at 25°C for 18-24 hours. A single suspect colony, which appeared as a shiny colony on TSA and a yellow colony on TCBS, was subjected to further analysis, including Gram staining, motility and oxidase tests **(Buller, 2004)**, API 20E system (bioMérieux), and a Vibriostatic O/129 (150 μg) susceptibility test (Oxoid).

4. Genotypic identification

The *V. harveyi* isolate was identified through species-specific PCR targeting the conserved 16S rRNA gene of Vibrio species, followed by sequencing for further confirmation.

4.1. DNA extraction

DNA extraction from pure bacterial colonies was carried out using the QIAamp DNA Mini Kit (Qiagen, Germany), with modifications to the manufacturer's protocol. The oligonucleotide primers used in this study were supplied by Metabion (Germany) and are listed in Table (1).

Table 1. Primers sequences, 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		
16S rRNA	CGGTGAAATGCGTAGAGAT	663	94˚C 5 min.	94˚C 30 sec.	56˚C 45 sec.	72˚C 45sec.	72˚C 10 min.	**Tarr *et al.*, 2007**
	TTACTAGCGATTCCGAGTTC							

4.2. PCR amplification

PCR amplification was performed using primers in a 25µL reaction mixture containing 12.5µL EmeraldAmp Max PCR Master Mix (Takara, Japan), 1µL of each primer (20 pmol), 7.5µL water, and 3µL DNA template. The reaction was performed in an Applied Biosystems 2720 thermal cycler. PCR products were analyzed by electrophoresis on a 1.5% agarose gel (AppliChem, Germany, GmbH).

4.3. DNA sequencing and phylogenetic analysis

DNA sequences were obtained using an Applied Biosystems 3130 Genetic Analyzer (HITACHI, Japan). Sequence identity was determined through BLAST® (Basic Local Alignment Search Tool) analysis **(Altschul *et al.*, 1990)** by comparing the sequences with GenBank accessions. A phylogenetic tree was constructed using the MegAlign module of BioEdit, and phylogenetic analyses were performed using the neighbor-joining method in MEGA 7 **(Tamura *et al.*, 2013)**.

5. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *V. harveyi* isolate was performed using the disk diffusion method on Mueller-Hinton agar (Oxoid, UK) supplemented with 3% (w/v) NaCl, according to the protocol described by **Quinn *et al.* (2002)**. Commercial antimicrobial discs (6 mm diameter, Oxoid) were used, including ampicillin (10µg, AM 10), ciprofloxacin (5 µg, CIP 5), gentamicin (10µg, GN 10), oxytetracycline (30µg, TE 30), lincomycin (2µg, L2), nalidixic acid (30µg, NA 30), oxolinic acid (2µg, OA 2), erythromycin (15µg, E 15), trimethoprim/sulfamethoxazole (25µg, SXT 25), and tetracycline (30µg, T 30). Inhibition zone diameters were interpreted as susceptible, intermediate, or resistant according to **CLSI (2010)** guidelines.

6. Histopathological examination

Tissue samples from the liver, brain, kidney, and spleen of naturally infected seabass were fixed in 10% buffered formalin. The tissues were carefully sectioned, washed, and dehydrated through a graded series of ethyl alcohol, followed by clearing in xylene. The samples were then embedded in paraffin and sectioned into thin slices (approximately 4–6 microns thick). Sections were stained with hematoxylin and eosin (H&E) for microscopic examination **(Bancroft *et al*., 2012)**.

7. Biosafety measures

Biosafety measures were, throughout the study, strictly adhered to the pathogen safety data sheets for *Vibrio parahaemolyticus* and the guidelines of the Pathogen Regulation Directorate of the **Public Health Agency of Canada (2010)**.

RESULTS

1. Result of clinical examination

Infected fish exhibited symptoms such as lethargy, loss of appetite, open mouths (indicative of anorexia), corneal opacity, excessive mucus production, and deteriorating tails. Hemorrhagic patches and detached scales were observed in some fish (Fig. 1), along with abdominal distention (swelling), ascites (fluid accumulation in the abdomen), and protrusion of the anal opening.

Necropsy findings revealed the presence of serosanguinous (blood-tinged) fluid in the abdominal cavity. The liver appeared either pale or congested and enlarged, while both the kidney and spleen were congested and enlarged. An enlarged gallbladder was also noted in some cases (Fig. 2).

Control measures

Topical disinfectants were applied. Fish density was reduced. Dead fish were promptly removed and disposed of outside the cages.

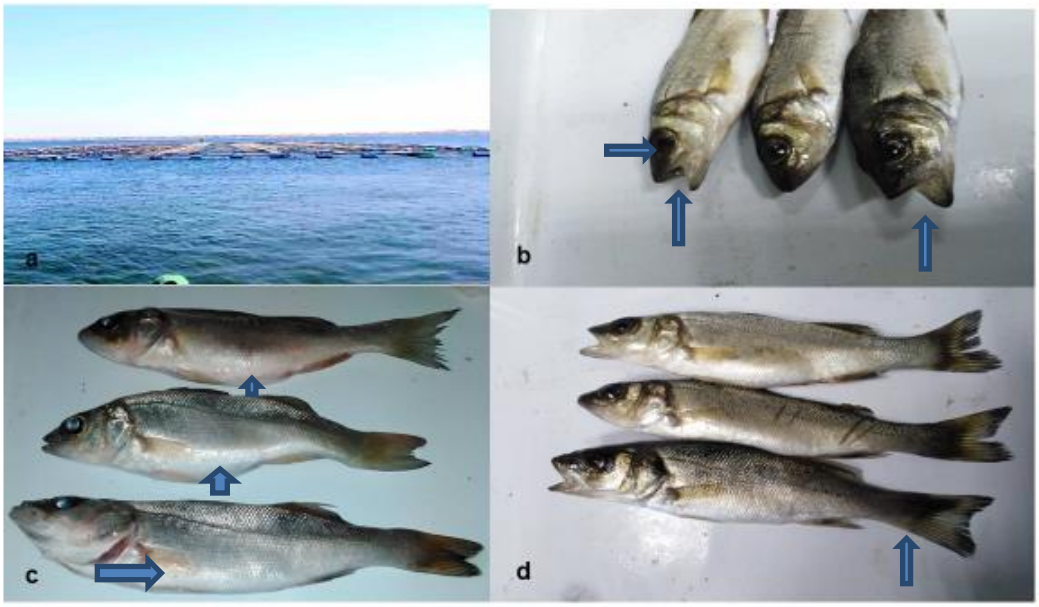

Fig. 1. (**a**) Floating net cages of fish farm. Clinical observations of infected seabass showing: (**b**) Anorexia, exophthalmia and detached scales, (**c**) Lethargy bilateral destination of abdomen, (**d**) Skin darkness dark pectoral fins and tail

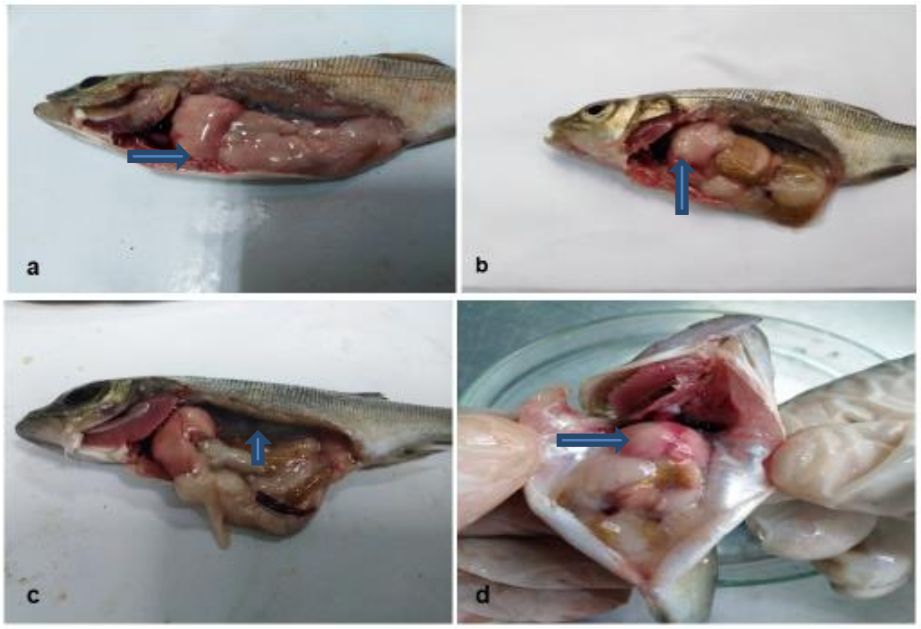

Fig. 2. Necropsy findings showed: **a**) Blood-tinged fluid in the abdominal cavity, **b**) The liver appeared enlarged and pale, **c**) The kidney and spleen were enlarged and congested, enlargement of the gallbladder, **d**) moribund pale, friable liver and enlarged with serosanguinous fluid and empty stomach

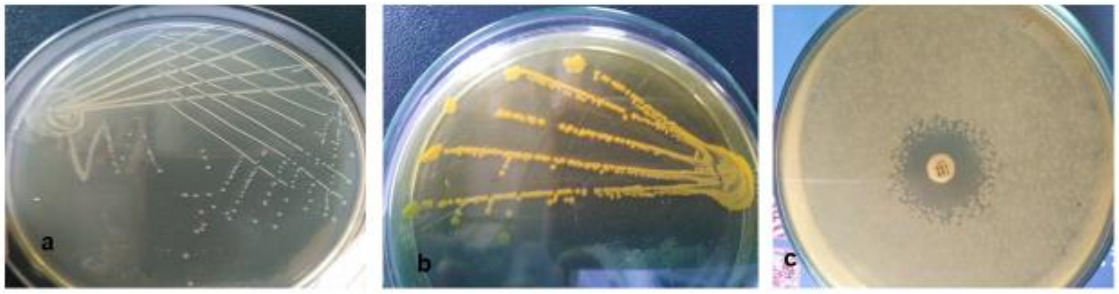

Fig. 3. Shiny colonies of *Vibrio harveyi* on **(a)** TSA with 3% NaCl, **(b)** yellow colonies on TCBS, **(c)** *V. haveyi* was positive to Vibriostat O/129 (150µg)

2. Phenotypic characteristics of *V. harveyi*

The isolated *Vibrio harveyi* was identified as a facultative, aerobic, Gram-negative, short rod-shaped bacterium that exhibited luminescence, motility, and fermentation capability. It formed yellow colonies on TCBS agar (Fig. 3a, b) and tested positive for both oxidase and catalase. *V. harveyi* was the only bacterial species isolated from the infected seabass. Additionally, the isolate tested positive for Vibriostat O/129 (150µg) (Fig. 3c). The biochemical identification of *V. harveyi* using the API 20E system is summarized in Table (2).

Table 2. The phenotypic characterization of *V. harveyi* by API 20E system

Biochemical	Result
ONPG	-
Arginine diydrolase	-
Lysine decarboxylase	+
Ornithine decarboxylase	+
Citrate	-
H2S	-
Urease	+
Tryptophane deaminase	-
Indole	+
Vogus Proskauer	-
Gelatinase	+
Glucose	+
Mannitol	+
Inositol	-
Sorbitol	-
Rhaminose	-
Sucrose	-

Melobinose	-
Amygdalin	+
Arabinose	-
oxidase	+

3. Water quality of infected seabass cages farm

Four water samples from the affected seabass cages were analyzed, with the following results: temperature ranged from 28 to 30°C, salinity levels were 28, 28, 28.5, and 28.6g/ L, pH values were 8.45, 8.40, 8.48, and 8.37, and dissolved oxygen (DO) levels ranged from 2.5 to 4.1mg/ L. Ammonia (NH_3) concentrations were 0.006, 0.003, 0.004, and 0.003mg/ L, nitrite (NO_2^-) was 0.05mg/ L, nitrate (NO_3^-) was 8mg/ L in all samples, and sulfate levels were 1060, 1174, 1096, and 1225mg/ L.

4. Molecular characterizations and sequence analysis of *16S rRNA* gene

The *V. harveyi* isolates exhibited a specific band at 663bp (Fig. 4). Phylogenetic analysis was performed by sequencing the 16S rRNA gene, and the sequence was submitted to GenBank. The *V. harveyi* strain identified in this study (accession no. MW241595.1) showed a 100% match with other relevant *V. harveyi* 16S rRNA gene sequences retrieved from the GenBank database (Fig. 5 & Table 3).

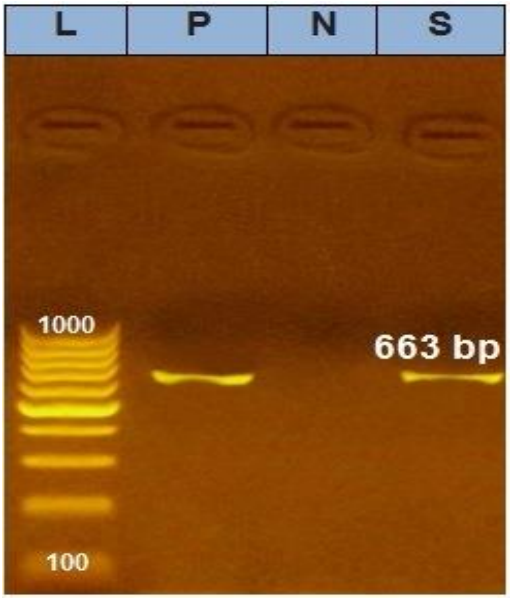

Fig. 4. Agarose gel electrophoresis showing amplification of 16S rRNA. L: 1 kb Ladder, P: positive control, N: Negative control, S: Positive sample 663bp

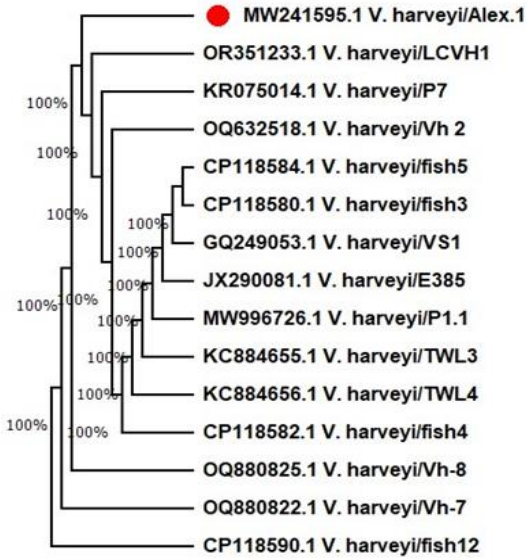

Fig. 5. *V. harveyi* isolate's phylogenetic tree of the 16srRNA gene (Red circle)

Table 3. The identity percentage between *V. harveyi* isolate and other references isolates

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
1		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	1	MW241595.1 V. harveyi/Alex.
2	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	2	OR351233.1 V. harveyi/LCVH
3	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	3	OQ880825.1 V. harveyi/Vh-8
4	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	4	KR075014.1 V. harveyi/P7
5	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	5	OQ880822.1 V. harveyi/Vh-7
6	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	6	OQ632518.1 V. harveyi/Vh 2
7	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	7	CP118584.1 V. harveyi/fish5
8	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	8	CP118590.1 V. harveyi/fish12
9	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	9	CP118580.1 V. harveyi/fish3
10	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	10	CP118582.1 V. harveyi/fish4
11	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	11	KC884656.1 V. harveyi/TWL4
12	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	12	KC884655.1 V. harveyi/TWL3
13	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	13	MW996726.1 V. harveyi/P1.1
14	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	14	JX290081.1 V. harveyi/E385
15	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		15	GQ249053.1 V. harveyi/VS1
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		

5. Anti-bacterial sensitivity

The *V. harveyi* strain demonstrated general sensitivity to oxytetracycline, ciprofloxacin, and sulfamethoxazole-trimethoprim, but exhibited inconsistent resistance to other tested antibiotics. This variability in resistance may be attributed to factors such as genetic mutations, resistance mechanisms, or the presence of resistant strains. The selection of appropriate antibiotic treatment should be guided by these findings and the clinical context. The data are summarized in Table (4).

Table 4. Antimicrobial sensitivity of the isolated *V. harveyi* strain

Antibiotic disc	**Inhibition zone diameter standard(mm)**			**Result**	
	S	**I**	**R**		
Ampicillin 10 mcg	≤13	14-16	≥17	0	R
Ciprofloxacin 5 µg	≤15	16-20	≥21	22	S
Erythromycin 15 µg	≤13	14-22	≥23	12	R
Lincomycin 2 µg	≤11	12-16	≥17	12	I
Gentamycin 10 µg	≤12	13-14	≥15	10	R
Nalidixic Acid 30 µg	≤13	14-18	≥19	15	I
Oxolinic acid 2 µg	≤10	11-12	≥30	10	R
Oxytetracycline30µg	≤14	15-18	≥19	21	S
Trimethoprime /sulfamethoxazole 25µg	≤10	11-15	≥16	23	S
Tetracycline 30 µg	≤14	15-18	≥19	10	R

6. Histopathological results

Histopathological examination of infected seabass tissues revealed significant damage across multiple organs. The liver exhibited severe hepatocellular necrosis, with cells appearing fragmented or faded and often losing their typical structure (Fig. 6a). Hemorrhaging was also evident, marked by red blood cells leaking from compromised blood vessels, creating a striking contrast against surrounding tissues. In the spleen, activation of melanomacrophage centers was observed along with the depletion of white pulp (Fig. 6b). The brain showed diffuse areas of malacia associated with a significant accumulation of inflammatory cells, congested blood vessels, and perivascular edema, as well as disorganization of the granular layer (Fig. 6c). Kidney tissues (Fig. 6d) exhibited epithelial vacuolation and degeneration within the renal tubules, interstitial edema leading to tissue swelling, dilated blood vessels with red blood cell extravasation, and infiltration of inflammatory cells into the interstitial tissue.

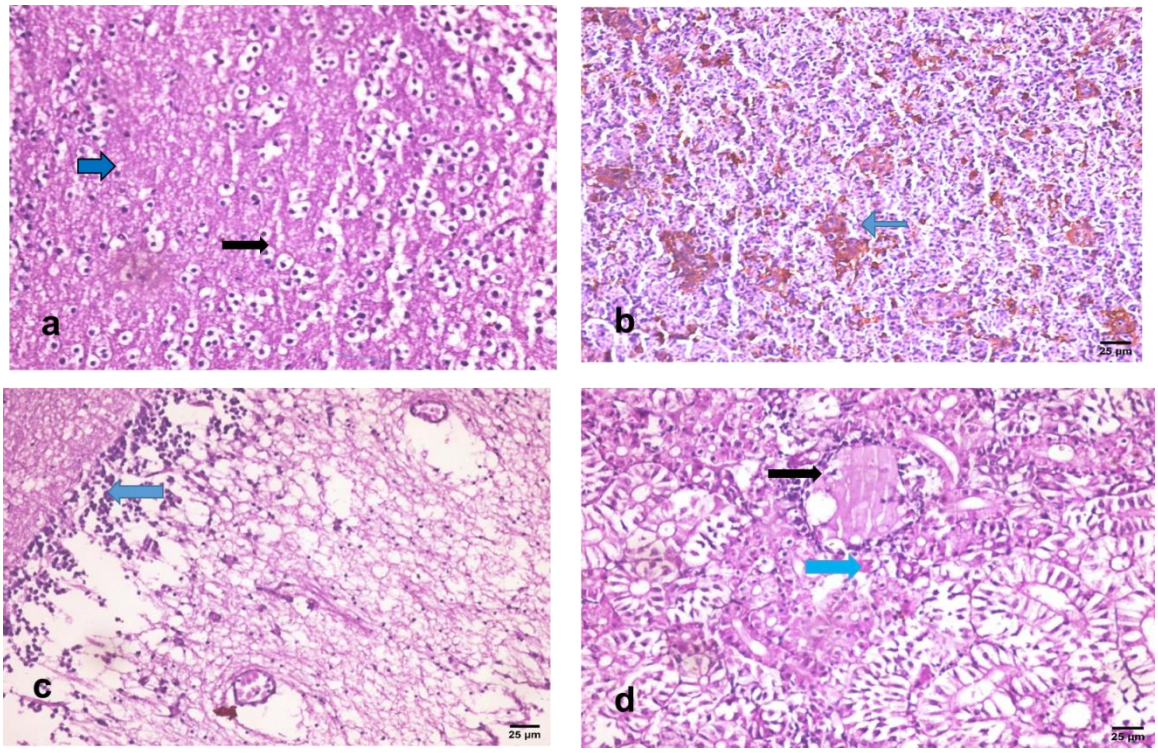

Fig. 6. Pathological changes in naturally infected seabass by *V. harveyi*: **(a)** Liver showing severe hepatocellular necrosis (black arrow), with area of acellular lytic tissues (blue arrow). (**b**) Spleen showing activation of melano-macrophage centers. **(c)** Brain showing diffuse area of malacia associated with huge accumulation of inflammatory cells (blue arrow) with congestion blood vessels. **(d)** Kidney showing area of caseated necrosis (black arrow), associated with interstitial inflammatory cells infiltration (blue arrow), H&E, X400

DISCUSSION

Vibrio harveyi has emerged as a significant pathogen affecting the health of aquatic species in marine environments **(Samsing *et al.*, 2023)**. The susceptibility of aquaculture species to vibriosis is often exacerbated by stressful conditions such as transportation, handling, low dissolved oxygen levels, and overcrowding **(Austin & Austin, 1993; Inglis *et al.*, 1993)**. High stocking densities in aquaculture systems are frequently associated with elevated organic loads and fluctuating salinity levels, contributing to the outbreak of diseases **(Akayh & Timur, 2002)**. Disease outbreaks pose a major constraint to the economic sustainability and growth of aquaculture production **(Meyer, 1991)**.

In the present study, a natural concurrent infection of *V. harveyi* in the cage-cultured European seabass (*Dicentrarchus labrax*) was addressed. Infected fish exhibited clinical signs such as lethargy, loss of appetite, open mouths (indicative of anorexia), corneal opacity, excessive mucus production, tail rot, and hemorrhagic patches on the external

body surface, which ultimately led to high mortality rates. Necropsy examinations revealed the presence of serosanguinous fluid in the abdominal cavity, pale and enlarged liver, and congested kidneys and spleen. These pathological findings align with those reported in previous studies **(Haenen *et al*., 2014; Moustafa *et al*., 2014; Zhang *et a*l., 2014; Eissa *et al*., 2017; Mohamad *et al*., 2019; Zang *et al*., 2020; Samsing *et al*., 2023; Aly *et al*., 2024; Zhou *et al*., 2024)**.

Over the past decade, global climatic changes have significantly impacted Mediterranean marine aquaculture, leading to substantial economic losses due to the emergence of new bacterial pathogens, including *V. harveyi* **(Amoro *et al*., 2020)**. Egypt, as one of the southern Mediterranean countries, has experienced indirect effects of global warming on aquaculture operations, particularly through the transmission of bacterial infections that result in high mortality rates **(Kaleem & Sabi, 2021; Mehrim & Refaey, 2023; Tawfeek *et al*., 2024)**.

In the present study, mortalities recorded in seabass rearing cages along the coastal region of Alexandria province were associated with changes in water quality parameters during the summer season. The observed decrease in dissolved oxygen levels and the increase in water temperature provided favorable conditions for *V. harveyi* to thrive, leading to severe fish losses under stressful conditions **(Moriarty, 1997; Rameshkumar *et al*., 2014)**. Environmental stress not only heightened the susceptibility of fish to bacterial infections but also contributed to the emergence of disease outbreaks. Additionally, climatic changes have been linked to the expression of virulence genes, the emergence of novel toxin-producing *Vibrio* strains, and the enhanced horizontal transmission of *Vibrio* species in aquaculture systems **(Leon Robles *et al*., 2013)**.

The biochemical assays conducted in this study confirmed the presence of *V. harveyi*, consistent with the phenotypic characteristics described by **Mohamad *et al.* (2019)** and **Samsing *et al*. (2023)**. Molecular techniques, particularly 16S rRNA gene sequencing, have become essential for species identification and phylogenetic analysis of *Vibrio* species. In this study, 16S rRNA sequencing further validated the identity of the isolated *V. harveyi* strain. The high nucleotide identity and phylogenetic analysis provided robust support for accurate strain identification **(Soliman *et al*., 2021; Aly *et al.*, 2024)**. The study isolate (*V. harveyi*/Alex.1, GenBank accession no. MW241595.1) exhibited 100% clustering with other reference strains from fish, including *V. harveyi*/fish5 (CP118584.1), *V. harveyi*/fish12 (CP118590.1), *V. harveyi*/fish3 (CP118580.1), and *V. harveyi*/fish4 (CP118582.1).

Our findings suggest that antibiotics can be effective in controlling vibriosis; however, the prolonged and uncontrolled use of antibiotics in aquaculture may contribute to the development of antimicrobial resistance **(Aisyhah *et al.*, 2015; Salama *et al*., 2016; Soliman *et al.*, 2021)**. In this study, the isolated *V. harveyi* strain was susceptible to

oxytetracycline, ciprofloxacin, sulfamethoxazole-trimethoprim, and vibriostatic agent O/129 (150µg) but exhibited resistance to the remaining tested antimicrobial agents. These findings align with previous studies **(Ransangan & Mustafa, 2009; Ransangan *et al*., 2012; Pavlinec *et al*., 2022)**. **Pavlinec *et al*. (2022)** also highlighted the need for standardized testing protocols and harmonized interpretive criteria, noting that susceptibility studies for *V. harveyi* remain limited **(CLSI, 2020)**. **Musa *et al*. (2008)** reported that *V. harveyi* isolates from the black tiger shrimp were resistant to ampicillin (90%) but highly susceptible to chloramphenicol, tetracycline, and furazolidone. Conversely, **Mohamad *et al.* (2019)** found that *V. harveyi* isolates from the hybrid groupers in Malaysia were sensitive to oxytetracycline and tetracycline. Antibiotic resistance poses significant risks to human health and can exacerbate the progression of infectious diseases **(Aly, 2013)**. Strengthening fish immunity through dietary supplements such as probiotics and nonspecific immunostimulants, while avoiding antimicrobial misuse, is critical for effective disease control **(Zhang *et al*., 2020)**.

Histopathological examinations in this study revealed that liver, kidney, brain, and spleen damage caused by *V. harveyi* significantly impaired essential metabolic functions. The dissemination of bacterial toxins through the bloodstream can lead to systemic infections with severe consequences, often resulting in mortality if untreated **(Kimura & Kusuda, 1979; Woo, 2011)**. In response to *V. harveyi* infection, the immune system mobilized inflammatory cells, which clustered around necrotic areas. These immune cells, visible as small, darkly stained nuclei, were either grouped or dispersed throughout the affected organs, consistent with findings from previous studies **(Moustafa *et al*., 2014)**.

CONCLUSION

This study highlights the increasing frequency of *Vibrio harveyi* infections in *Dicentrarchus labrax* within mariculture settings in recent years, underscoring the rapid emergence of this pathogen. Additionally, the study elucidates the phenotypic and genomic characteristics of the isolated strain, along with its antimicrobial resistance profile and associated pathological changes.

To mitigate the impact of *V. harveyi* infections, it is recommended to implement robust biosecurity measures and to enforce strict veterinary hygiene protocols within aquaculture facilities. The prudent use of antibiotics in the fisheries industry is essential to prevent the accumulation of antibiotic residues in aquatic environments and to reduce the significant losses associated with multidrug-resistant *V. harveyi* strains.

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