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Studies on the most Prevailing Bacterial Diseases in *Trachurus indicus* Fish

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ABSTRACT

Sixty freshly captured wild *Trachurus indicus* fish were randomly selected during the summer season of 2022 from the Suez Canal and examined clinically and bacteriologically to detect the most prevailing bacterial diseases affecting it. Infected fish revealed hemorrhages at the operculum, base of the pectoral and pelvic fins and congestion in different internal organs. Motile Aeromonas septicemia and vibriosis were detected as the most prevalent and virulent bacteria isolated and identified by the traditional techniques; namely, *Aeromonas hydrophila* and *Vibrio algynolyticus* with a prevalence of 40% and 30%, respectively. PCR was used for validation of the isolated pathogenic *Aeromonas hydrophila* and Vibrio species using the 16s rRNA gene and the virulence genes. All bacterial isolates were recovered from different internal organs and gills with the highest prevalence from the liver (45, 43.33 %) followed by the kidney (30, 33.33%), spleen (17.5, 16.67%), and gills (7.5, 6.67 %), respectively.

INTRODUCTION

In Egypt, fisheries play an important role in the national income structure **(Ellis, 1999)**. Fish are considered very important in human diet, forming a main source of high quality animal protein and beneficial omega-3 polyunsaturated fatty acids in comparison to red meat **(FAO, 2020)**.

Horse mackerel was considered the main fish species in both the catch of the Gulf of Suez and the whole Egyptian Red Sea fisheries **(Sahar *et al.*, 2018)**. It belongs to the genus Trachurus in the family Carangidae and had the ability to adapt well to its culture environment, so it can be used in aquaculture **(Er *et al.*, 2021)**.

Bacterial agents are the main cause of high mortalities in marine fish as they are responsible for a variety of marine fish diseases **(Eissa *et al.*, 2018)**. They are normally found and typically prevalent in fish environments and in some particular unfavorable environmental conditions which were thought to be the main risk factors for disease induction and significant economic losses **(Shawna & Brian, 2020)**, as disease outbreaks were caused due to a complex interactions between fish, pathogens and the aquatic environment **(Escobar *et al.*, 2018)**. These pathogens had a major impact on fish production **(Dissasa *et al.*, 2022)**. Gram-negative bacteria such as Aeromonas species,

Flavobacterium spp., Acinetobacter spp., Edwardsiella spp. and Pseudomonas spp. were considered the main bacterial fish pathogens affecting fish production and causing severe economic losses **(Hala *et al.*, 2021)**. **Boran *et al.* (2013)** reported that, bacterial pathogens as *Photobacterium damselae damselae*, *Aeromonas hydrophila, Vibrio vulnificus* and *Vibrio alginolyticus* can be isolated from horse mackerel fish.

Molecular techniques (PCR) were used to provide rapid, sensitive and accurate data to identify specific pathogens without the need for time-consuming traditional biochemical methods (**Abdelsalam *et al*., 2022**).

This study's objectives was to investigate the clinical picture of the most prevailing bacterial diseases affecting wild *Trachurus indicus* fish collected from the Suez Canal, bacterial isolation and identification using traditional and advanced techniques such as PCR, identification of bacterial virulence genes responsible for pathogenicity, and recording the prevalence of isolated disease-causing bacteria.

MATERIALS AND METHODS

Naturally infected fish

Sixty (60) freshly and randomly captured *Trachurus indicus* fish, with weights' range of 60 ±10g were randomly gathered from the Suez Canal in Ismailia governorate during summer season 2022. All moribund and freshly dead fish were carried in an ice box to the wet lab of the Department of Fish Diseases and Management, Animal Health Research Institute, Ismailia Branch. Freshly dead fish were examined clinically, postmortem and bacteriologically using the procedure described by **Noga (2010)**.

Bacterial isolation and identification

Fish lesions on the gills, liver, kidney and spleen of naturally infected *Trachurus indicus* fish were sampled under strict aseptic conditions. They were inoculated into nutrient broth at 30°C for 24 hours, cultured in nutrient agar and tryptic soy agar containing 2% NaCl and incubated at 30°C for 24 hours. Re-inoculation of cultured bacteria was performed until separate colonies were obtained. These isolated colonies were collected and sub- cultured on special medium for further identification as Thiosulphate citrate bile salt sucrose agar (TCBS, Oxoid), Pseudomonas base agar and Aeromonas base agar medium, supplemented with ampicillin (5 mg/L) at 30°C for 24h according to **El-Dakroury *et al*. (2020)**. The suspected purified colonies were selected and identified by studying morphological characteristics of the colonies as Gram's stain and motility test and biochemically, using different biochemical tests like cytochrome oxidase (Biomerieux, Marcy-l'Etoile, France), catalase, glucose fermentation, indole and methyl red, Voges Proskauer, urease test and sensitivity against different concentrations of sodium chloride (0–6.5%) and vibriostatic agent (150 µg) according to **Quinn *et al.* (2002)** and **Austin and Austin (2012)**. PCR was used as a confirmatory identification and for the detection of virulence genes of the isolated bacteria according to **El-Dakroury *et al.* (2020)** and **Abd El Tawab *et al*. (2021**).

Polymerase chain reaction

Polymerase chain reaction (PCR) was used as a confirmatory identification method for biochemically identified bacterial isolates and identification of its virulence genes using species-specific genes.

A) DNA extraction: A modified version of the manufacturer's instructions was used to extract DNA from samples using the QIAamp DNA Mini kit from Qiagen, Germany, GmbH. Table (1) shows the PCR conditions used for the detection of different bacteria and some virulence genes.

B) Oligonucleotide Primer: The primers that Metabion (Germany) provided for use are listed in Table (2).

C) PCR amplification: For PCR, on an applied biosystem 2720 thermocycler, the reaction was carried out.

D) Analysis of the PCR Products: The PCR products were separated by electrophoresis employing gradients of 5V/cm in 1x TBE buffer at room temperature on a 1.5% agarose gel (Applichem, Germany, GmbH). The PCR products were put into each well of the gel slot in amounts of 20 l for gel examination. The fragment sizes were calculated using the Generuler 100 bp ladder (Fermentas, Thermo Scientific, Germany). Alpha Innotech, Biometra, a gel documentation system, was used to photograph the gel, and software was used to evaluate the data.

Table 1. PCR conditions for detection of *Aeromonas hydrophila,* Vibrio spp. and *Vibrio alginolyticus* and some virulence genes

Target bacteria	**Target gene**	**Amplified segment (bp)**	**Primary denatur-ation**	**Amplification (35 cycles)**			**Final extension**	**Reference**
				Secondary denaturati-on	**Anneal-ing**	**Extens-ion**		
Aeromonas hydrophila	*16S rRNA*	685	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	***Gordon et al.* (2007)**
	AeroA	326	94˚C 5 min.	94˚C 30 sec.	52˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	**Singh *et al.* (2008)**
	Hly A	592	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Rozi *et al.* (2017)**
Vibrio spp.	*16S rRNA*	663	94˚C 5 min.	94˚C 30 sec.	56˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Tarr *et al.* (2007)**
Vibrio alginolyticus	*Collagenase*	737	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Abu-Elala *et al.* (2016)**
	Tdh	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)**

Table 2. Primers sequences for detection of *Aeromonas hydrophila* ,Vibrio spp. and *Vibrio alginolyticus* and some virulence genes

Target bacteria	Target gene	Primers sequences	Reference
Aeromonas hydrophila	*16S rRNA*	GAAAGGTTGATGCCTAATACGTA	**Gordon *et al*. (2007)**
		CGTGCTGGCAACAAAGGACAG	
	Aero A	CACAGCCAATATGTCGGTGAAG	**Singh *et al.* (2008)**
		GTCACCTTCTCGCTCAGGC	
	Hly A	GGCCGGTGGCCCGAAGATACGGG	**Rozi *et al.* (2017)**
		GGCGGCGCCGGACGAGACGGGG	
Vibrio spp.	*16S rRNA*	CGGTGAAATGCGTAGAGAT	**Tarr *et al.* (2007)**
		TTACTAGCGATTCCGAGTTC	
Vibrio alginolyticus	*Collagenase*	CGAGTACAGTCACTTGAAAGCC	**Abu-Elala *et al*. (2016)**
		CACAACAGAACTCGCGTTACC	
	Tdh	CCATCTGTCCCTTTTCCTGC	**Mustapha *et al.* (2013)**
		CCAAATACATTTTACTTGG	

RESULTS

Clinical picture

Most of the naturally infected *Trachurus indicus* fish showed hemorrhages at the operculum, at the base of the pectoral and pelvic fins (Fig. 1). Postmortem findings showed congestion and hemorrhages in different internal organs such as kidney, spleen and liver (Fig. 2).

Bacterial examination

Table (3) shows the morphological and biochemical characters of both *A. hydrophila* and *V. alginolyticus*, isolated from naturally infected *Trachurus indicus* fish. *A.hydrophila* colonies were round, flat, pale in shape and translucent on MacConkey agar; while on Aeromonas agar, they were green with a black center. *V. alginolyticus* produced pale colonies on MacConkey agar while they produced yellow colonies on TCBS medium. *A. hydrophila* was differentiated from *V. alginolyticus* by its resistance to O/129 and 6.5% sodium chloride that inhibit the ability of its growth.

Result of molecular identification of the most prevalent bacterial isolates by polymerase chain reaction (PCR)

A) Molecular identification of *Aeromonas hydrophila*

Fig. (3) exhibits the presence of 16s rRNA and aerolysin virulence gene in four selected isolates of *A. hydrophila*, while hemolysin virulence gene was detected in only two of four *A. hydrophila* isolates.

B) Molecular identification of *Vibrio alginolyticus*

Fig. (4) shows the presence of 16s rRNA gene in four selected isolates of Vibrio species, while Fig. (5) and Fig. (6) show the presence of collagenase gene and tdh virulence gene in four selected isolates of *V. alginoltyticus* .

Prevalence of bacterial isolates in naturally infected *Trachurus indicus* fish

The total prevalence of bacterial pathogens isolated from *Trachurus indicus* fish in the summer season of 2022 was 70% including *Aeromonas hydrophila* which is considered the most predominant and prevalent species of bacterial isolates, with a rate of 40%, followed by *Vibrio alginolyticus* with a rate of 30% (Table 4).

Prevalence of suspected bacteria isolated from various internal organs of naturally infected *Trachurus indicus* fish

Fig. (7) shows *Aeromonas hydrophila* and *Vibrio alginolyticus* isolated from various internal organs and tissues of naturally infected *Trachurus indicus*, with high prevalence from liver (45 , 43.33 %), followed by kidney (30 , 33.33%) and spleen (17.5, 16.67%), while the lowest prevalence was from the gills (7.5 , 6.67 %), respectively.

Fig. 1. Naturally infected *Trachurus indicus* fish showing hemorrhages at the operculum, at the base of the pectoral and pelvic fins

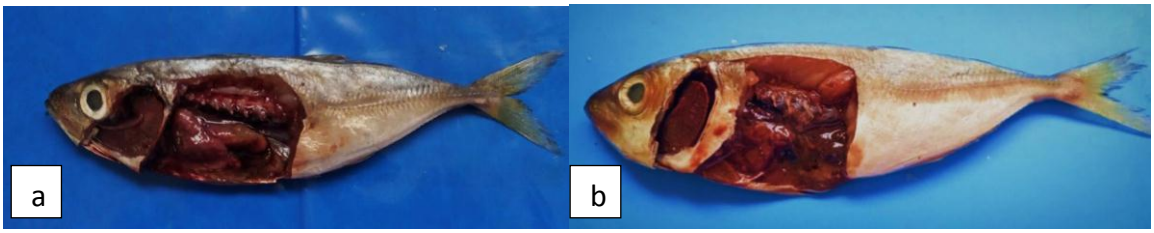

Fig. 2. Postmortem examination of naturally infected *Trachurus indicus* fish showing: **a)** and **b)** hemorrhages in various internal organs

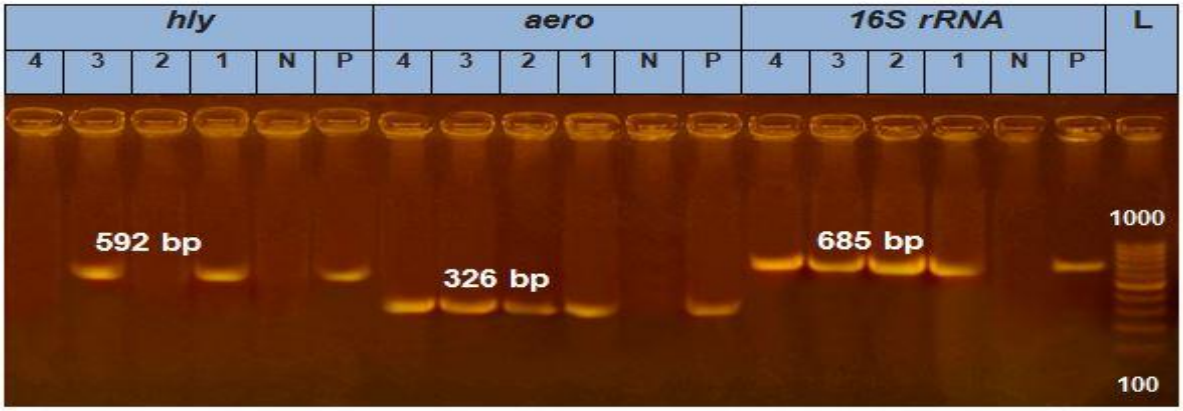

Fig. 3. Detection of 16S rRNA (685 bp), aer A (326 bp) and hly A (592 bp) virulence genes to characterize *Aeromonas hydrophila* by PCR. Lanes L: 100-1000 bp ladder, N: negative control, P: positive control; lanes 1-4: *A. hydrophila* positive strains for 16S rRNA gene; Lanes 1-4: positive strain for the aer A gene; Lanes 1 and 3: positive strains for the *hly A* gene.

Fig. 4. Detection of vibrio spp. 16s rRNA (663bp) gene by PCR. Lanes L: 100-1000 bp ladder, N: negative control; P: positive control, lanes 1-4: Vibrio spp. showing bands at 663bp.

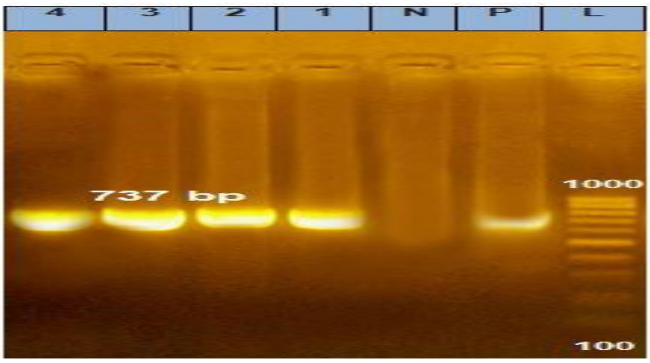

Fig. 5. Detection of *Vibrio alginolyticus* collagenase gene (737bp) by PCR. Lanes L: 100-1000 bp ladder, N: negative control; P: positive control, lanes 1-4: *Vibrio alginolyticus* showing bands at 737bp.

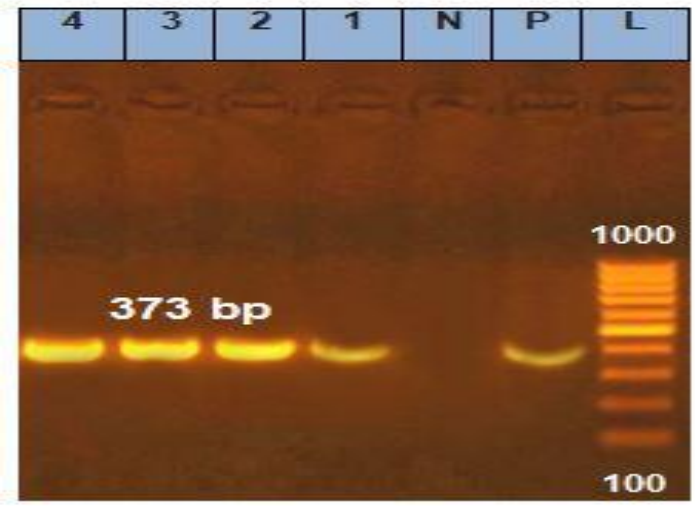

Fig. 6. Detection of *Vibrio alginolyticus* virulence gene tdh (373bp) by PCR. Lanes L: 100-1000 bp ladder, N: negative control; P: positive control, lanes 1-4: *V. alginolyticus* showing bands at 373bp.

Table 3. Biochemical characterization of bacteria isolated from naturally infected *Trachurus indicus* fish

Test	***Aeromonas hydrophila***	***Vibrio alginolyticus***
Gram-stain	-ve	-ve
Shape	Short rod	Curved rod
Motility	+	+
Cytochrom oxidase	+	+
Catalase	+	+
H_2S on triple sugar iron (TSI)	- A/A	- A/A
Indole	+	+
Citrate	+	-
Methyl red	+	+
Vogaus Proskauer	±	+
Urease production	-	-
Growth at 3.5% Nacl	+	+
Growth at 6.5% Nacl	-	+

-: Negative; +: Positive , ± =Variable result, H_2S (TSI)= production of H_2S from triple sugar iron, A/A= acid/ acid

Table 4. Prevalence of bacterial isolates in naturally infected *Trachurus indicus* fish

Type of bacterial pathogen	No. of examined fish	No. of infected fish	%
Aeromonas hydrophila	60	24	40
Vibrio alginolyticus		18	30
Total		42	70

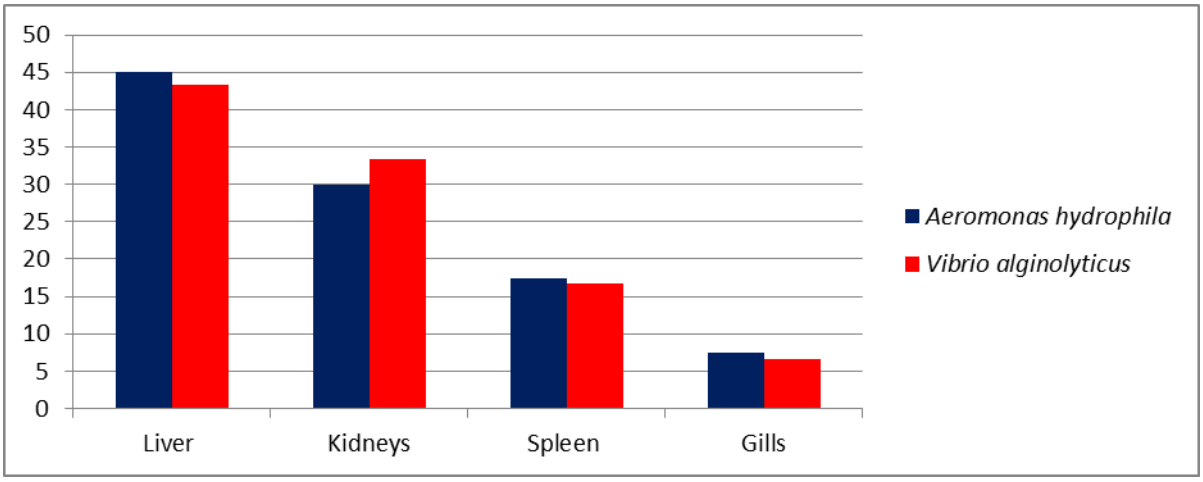

Fig. 7. Prevalence of bacteria isolated from various internal organs of naturally infected *Trachurus indicus* fish

DISCUSSION

Bacterial fish diseases are considered one of the most significant and important causes of high mortalities in both wild and cultured fish and sever economic loss **(Toranzo *et al.*, 2005)**. The most frequently observed clinical signs in naturally infected *Trachurus indicus* fish were hemorrhages at the operculum, the bases of the pectoral and pelvic fins at the mouth region, as well as redness around eyes. The current results are nearly identical to those recorded by **Boran *et al.* (2013)**, **Maather El-Lamie and Heba Adel-Mawla (2015)**, **Abdelaziz *et al.* (2017)**, **El-Dakroury *et al.* (2020)**, **Yen *et al.* (2022)** and **Noor El-Deen *et al.* (2023)**. These clinical signs appeared as a result of exposure of fish to poor environmental conditions and stressors, which affect fish immunity and cause multiplication of bacteria inside the intestine and secrete toxins and enzymes, which enable bacteria to invade the intestinal wall and passed in the blood stream to other various internal organs causing diseases **(Narvaez *et al.*, 2021)**.

In the current study, postmortem examination revealed hemorrhages in various internal organs such as liver, spleen, kidney and intestines. The current results are nearly

identical to those recorded by **Boran *et al.* (2013)**, **Maather El-Lamie and Heba Adel-Mawla (2015)**, **Abdelaziz *et al*. (2017)**, **Aly *et al.* (2019)**, **El-Dakroury *et al*. (2020)**, **Yen *et al.* (2022)** and **Noor El-Deen *et al.* (2023)**.

In the present study, bacteriological examination of the collected *Trachurus indicus* fish revealed Gram-negative bacteria, identified as *Aeromonas hydrophila* and *Vibrio alginolyticus*. All *A. hydrophila* isolates produced were pale-shaped, translucent colonies on MacConkey agar that did not contain lactose fermenters, whereas they produced green colonies with black centers on Aeromonas agar. These results coincide with those of previous studies **(Boran *et al.*, 2013; Noha *et al.*, 2018; El-Dakroury *et al*., 2020; Zorriehzahra e*t al.*, 2020**; **Hala *et al.*, 2021)**. While, all isolates of *V. alginolyticus* produced pale-shaped colonies on MacConkey agar as they were not lactose fermenters, while producing yellow colonies on TCBS (Thiosulfate Citrate Bile Salt agar) because they could ferment sucrose in the medium (sucrose positive). These results concur with those of **Boran *et al.* (2013)**, **Abdelaziz *et al*. (2017)**, **Ezzat *et al*. (2018)**, **El-Dakroury *et al*. (2020)**, **Dalia *et al*. (2022)**, **Hala *et al.* (2021)**, **Yen *et al.* (2022)** and **Noor El-Deen *et al.* (2023)**.

This study demonstrated that all the isolates of *Aeromonas hydrophila* were Gram-negative, rod-shaped, motile, positive to oxidase, catalase, indole production, citrate utilization and methyl red tests, variable for Vogues-Proskauer test and negative for urease production. These results agree with those of **Al-Maleky *et al.* (2011), Boran *et al.* (2013)**, **Noha *et al.* (2018)**, **Hamouda *et al*. (2019)**, **Mzula *et al*. (2019)**, **El-Dakroury *et al*. (2020)** and **Hala *et al.* (2021)**. While, *Vibrio alginolyticus* isolates were Gram-negative, curved rod-shaped, fermentative, motile, and responded positively to methyl red, oxidase, catalase, indole production and Vogues-Proskauer tests, whereas they were negative for urease production and citrate utilization tests. These results match with those of **Boran *et al.* (2013)**, **Maather El-Lamie and Heba Abdel-Mawla (2015)**, **Abdelaziz *et al*. (2017)**, **Ibrahim *et al*. (2018)**, **Aly *et al.* (2019)**, **El-Dakroury *et al.* (2020)**, **Dalia *et al.* (2022)**, **Yen *et al*. (2022)** and **Noor El-Deen *et al.* (2023)**.

For confirmation of the identified *Aeromonas hydrophila* and *Vibrio alginolyticus* and for the accurete dection of their virulence genes, we used polymerase chain reaction (PCR). Identification of *A. hydrophila* through the detection of 16s rRNA gene in four selected isolates gave bands at 685 bp. and identification of two virulence genes of *A. hydrophila* aer A (aerolysin) and hly A (hemolysin) genes gave a band at 326 bp. and 592 bp. genes in the selected four isolates and two out of four isolates, respectively. These outcomes were similar to **Abd El Tawab *et al*. (2021**) results who used PCR to detect aerolysin (aer A) and hemolysin (hlyA) genes in all random isolated *A. hydrophila* from marine fishes as they produced bands at 326 bp. for the aer A gene and 592bp. for the hlyA gene and **Hamouda *et al*. (2019)** who identified the 16S rRNA gene in six out of seven selected isolates as species specific gene to confirm the identification of *A.*

hydrophila isolates from *Oreochromis niloticus.* These results are similar to those of **Al-Gammal *et al.* (2020)** and **Sonkol *et al.* (2020)** who identified the aerolysin (aer A) gene in 100% of fish isolates. Additionally, PCR was used to identify vibrio species by detecting the 16s rRNA gene of four selected isolates, giving bands at 663 bp., detection of collagenase gene of *Vibrio alginolyticus* which gave bands at 737bp. and detection of *V. alginolyticus* virulence gene tdh, which gave bands at 373bp. in the selected four isolates of *V. alginolyticus.* These findings are nearly similar to that of **Abdelaziz *et al.* (2017)** who identified the 16S rRNA gene of vibrio species at 700 bp., collagenase gene at 737bp. and the tdh at 373bp. and similar to that of **Ezzat *et al*. (2018)** who detected the 16S rRNA gene for vibrio spp. at 663 bp. in the selected 13 isolates and the collagenase gene at 737 bp. in the tested 6 isolates from various marine fish. Notably, these results are similar to those of **Ibrahim *et al.* (2018)** who found the 16S rRNA gene at 663bp for Vibrio spp. Furthermore, **El-Dakroury *et al*. (2020)** identified the tdh virulence gene of *V. alginolyticus* isolated from marine fishes with a band at 373bp. in the selected three isolates and **Dalia *et al*. (*2022*)** identified the collagenase gene and the tdh virulence gene in the examined *V. alginolyticus,* with bands at 737bp. and 373bp., respectively.

The total prevalence of *Aeromonas hydrophila* and *Vibrio alginolyticus* isolated from the naturally infected *Trachurus indicus* fish in the summer season of 2022 was determined in the current study, with a rate of 40 and 30%, respectively. These results for *A. hydrophila* are nearly identical to those recorded in the study of **Ezzat *et al.* (2014)** who reported that, the most predominant and prevalent species of bacterial isolates from marine fishes of *Tilapia zillii* and *Mugil Capito* were recorded to *A. hydrophila,* with a rate of 39.39%. In addition to the previous study, **Al-Maleky (2011)** isolated *A. hydrophila* from *Platycephalus indicus* fish, with a prevalence rate of about 44.4%. A higher finding was recorded by **Eid *et al.* (2022)** who suggested that, *A. hydrophila* was the most common identified Aeromonas spp. (44%) isolated from *Mugil cephalus* (striped mullet), with a rate of 53.85% and **Abd El-Tawab *et al.* (2021**) who identified *A. hydrophila* isolates from mullet (*Mugil cephalus*) with total prevalence rate of about 54.2%. Lower observation was reported by **Hala *et al.* (2021)** who identified *A. hydrophila* from the Nile tilapia at a rate of 14.84%. In this context, **Eissa *et al*. (2015)** recorded that the most predominant bacterial species isolated from infected crayfish was *A. hydrophila,* with a prevalence rate of 35%. The results of these studies for *V. alginolyticus* were almost similar to those documented by **Abd El-Tawab *et al.* (2021**) who identified *V. alginolyticus* strains from different fishes, including O*reochromus niloticus* and *Mugil cephalus* fish with total prevalence of 33.8%. Higher observations were obtained by **Yen *et al.* (2022)** who revealed that *V. alginolyticus* found in 67% of the diseased *Sciaenops ocellatus* fish, whereas V. *fluvialis and V. orientalis* were isolated with an equal occurrence rate of 17% and **El-Bouhy *et al*. (2016)** who reported that prevalence values of *V. alginolyticus* and *V. parahemolyticus* in *Mugil capito* were 69.76% and 30.24%, respectively. Additionally, **Maather El-Lamie and Heba Abdel-**

Mawla (2015) mentioned that, the prevalence of *V. algynolyticus* isolated from *Trachurus indicus* infested with isopodes was 33.3%, while lower observations were reported by **Deng *et al*. (2020)** who found marine fish infected with *V. alginolyticus* and *V. parahaemolyticus*, with a prevalence of 14.29 and 4.29, respectively in South China. Identically, **Abd El Tawab *et al.* (2018)** found that *V. alginolyticus* isolated from marine fish was the dominant species with a prevalence of 16%, followed by *V. parahaemolyticus* at 5.33% and *V. cholerae* at 7.33%. Moreover, **Edris *et al.* (2013)** isolated *V. alginolyticus* from marine fish with a prevalence of 25.7%, and **El-Adawy (2010)** recorded that the total incidence rate of *V. alginolyticus* among *Mugil capito* was 14.61%. This difference in results regarding prevalence may be due to the difference of fish species and age, nature and number of tested fish, sampling techniques, water quality characteristics, in addition to time and area of the study.

In this study, all bacterial isolates of *Aeromonas hydrophila* and *Vibrio alginolyticus* were recovered from internal organs and tissues of naturally infected *Trachurus indicus* with high prevalence from liver (45 , 43.33 %), followed by kidney (30 , 33.33%) and then spleen (17.5, 16.67%). Whereas, the lowest prevalence values from gills were 7.5, 6.67 %, respectively. These results for *A. hydrophila* are almost similar to those recorded by **Hala *et al.* (2021)** who showed that the occurrence of Aeromonas species isolated from examined internal organs of *Oreochromis niloticus* was the highest in liver, followed by spleen and kidney. Similarly, **Ezzat *et al.* (2014)** found that *A. hydrophila* showed the highest prevalence in liver (44.23%), followed by kidney then spleen and gills (7.69%). The results for *V. alginolyticus* were almost similar to those recorded by **Ezzat *et al.* (2018)** who observed the highest prevalence in the liver of the tested marine fish (50% in mullet, 66.67% in sea bream, and 100% in seabass), compared to other internal organs. In this respect, **Abd El Tawab *et al.* (2018)** recorded *V. alginolyticus* in various internal organs of sea bream and *Mugil capito* with the highest prevalence from liver (35.3% , 33.33 %), followed by kidney (29.4, 30.30%), respectively. Moreover, **Ezzat *et al.* (2014)** isolated *V. alginolyticus* from different internal organs and gills of marine fishes, with high prevalence from liver (36.89%). These findings could be attributed to the consideration of the liver as one of the organs that were highly impacted by pollutants in the water and was also the most relevant for detoxification and biotransformation processes **(Camargo & Martinez, 2007)**. On the other hand, contrasting perspectives have been documented by **Eid *et al.* (2022)** who recovered *A. hydrophila* from gills in a higher prevalence than internal organs of *Mugil cephalus* as it was (54.17% and 53.85%, respectively). Another contradicted perspective was that of **Aly *et al.* (2019)** who recorded the highest prevalence of *V. alginolyticus* strains in the kidney and spleen (34.48%), followed by the liver (31.03%) of the tested gilthead sea bream.

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CONCLUSION

It could be concluded that the highest prevalence of bacterial isolates causing septicemic diseases in wild *Trachurus indicus* fish were caused by *Aeromonas hydrophila*, followed by *Vibrio alginolyticus*. Liver was the highest organ in prevalence for both bacteria, followed by kidney, spleen and gills. PCR is a quick, sensitive and accurate method for diagnosing *A. hydrophila* and *V. alginolyticus* and determining their virulence genes.

REFERENCES

Abdelaziz, M.; Ibrahim, M. D.; Ibrahim, M. A.; Abu-Elala, N. M. and Abdel-moneam, D. A. (2017). Monitoring of different Vibrio species affecting marine fishes in Lake Qarun and Gulf of Suez: phenotypic and molecular characterization. Egypt. J. Aquat. Res., 43(2): 141–146.

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.

Abd El-Tawab, A. A. A.; El-Hofy, F. I.; Hasb-Elnaby, G. R.; El-Khayat, M. E. and Refaey, M. A. (2021). Prevalence and virulence genes of vibrio and Aeromonas species isolated from Nile tilapia and mugil fish farms in Egypt. Adv. Anim. Vet. Sci., 9(10): 1625-1631.

Abdelsalam, M.; Elgendy, M. Y.; El fadadny, M. R.; Ali, S. S.; Sherif, A. H. and Abolghait, S. K. (2022). A review of molecular diagnoses of bacterial fish diseases. Aquac. Int., 1-18.

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.

El-Adawy, M.M.(2010). Studies on bacterial pathogens affecting *Mugil capito* fish.Thesis, M. V. Sc. (bacteriology) Fac. Vet.Med., Suez canal Universit y.Eleonor, V.A.; L.A. Tendencia and Dureza, 1997.Isolation of Vibrio species from Penaeusmonod on (fabricious) with red disease syndrome. Aquac., 154: 107-114.

Al-Gammal, A. M.; Mohamed, M. F.; Basma, T. A.; Hossein, W. N.; El Kazzaz, W. M. and Mabrok, M. (2020). Molecular typing, antibiogram, and PCR-RFLP based detection of *Aeromonas hydrophila* complex isolated from *Oreochromis niloticus*. Pathogens, 9(3): 238.

Al-Maleky, G.M.; Karim, R. M. and Al-Abresm, A. N. (2011). Survey of *Aeromonas hydrophila* in Three Marine Fish Species from North West Arabian Gulf, Iraq. Bas.J.Vet.Res., 10(2):72-77.

Aly, S.; Eisa, A. and Noha, El Banna (2019). Characterization of Vib*rio Alginolyticus* Infection in Gilthead Seabream (*Sparus Auratus*, L) Cultured in Egypt. Suez Canal Vet. Med. J., 24(2): 278-301.

Austin, B. and Austin, D. A. (2012). Aeromonadaceae representatives (motile aeromonads). In Bacterial Fish Pathogens, (pp.119–146). Springer.

Boran, H.; Terzi, E.; Altinok, I.; Capkin, E. and Bascinar, N. (2013). Bacterial diseases of cultured Mediterranean horse mackerel (*Trachurus mediterraneus*) in sea cages. Aquac., 396(3): 8–13.

Camargo, M.M. and Martinez, C.B. (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotrop. Ichthyol., 5: 327-336.

Dalia, E. A. G.; Salwa, M. H.; Nadia, B. M.; Hanan, A. F. and Mayada, A.M.A.(2022). Virulence genes and antibiotic resistance profile of Vibrio species isolated from fish in Egypt. Vet. Res. Forum, 13(3): 315 – 321.

Deng, Y.; Xu, L.; Chen, H.;Liu, S.; Guo, Z.; Cheng, C.; Ma, H. and Feng, J. (2020). Prevalence, virulence genes, and antimicrobial resistance of Vibrio species isolated from diseased marine fish in South China. Sci. Rep., 10(1): 14329.

Dissasa, G.; Lemma, B. and Mamo, H. (2022). Isolation and identifcation of major bacteria from three Ethiopian rift valley lakes live and processed fish and water samples. implications in sanitary system of fish products. BMC Vet. Res., 18(1): 439.

Edris, A. M.; Fatin, S. H.; Hassan, M. A. and Abd El Latif, Z. A. (2013). Demonstration of vibrio species in marine fish with special Reference to *Vibrio parahaemolyticus.* Benha Vet. Med. J., 25(2): 271-275.

Eid, H. M.; El-Mahallawy, H. S.; Shalaby, A. M.; Elsheshtawy, H. M.; Shetewy, M. M. and Eidaroos N. H. (2022). Emergence of extensively drug-resistant *Aeromonas hydrophila* complex isolated from wild *Mugil cephalus* (stripped mullet) and Mediterranean seawater. Vet. World, 15(1): 55-64.

Eissa, I. A. M.; Maather, El-Lamei; El-Gamal, A.; Somaya, S. El-Shafii and Inas, El-Saeed (2015). Isolation, biochemical and molecular detection of *Aeromonas hydrophila* from cultured *Oncorhynchus mykiss.* Suez Canal Vet. Med. J., 23(2): 261-269.

Eissa, I. A. M.; Derwa, H. I.; Mona, I.; Maather, El Lamie; Amina, A. D.; Hassnaa, Elsheshtawy and Bayoumy, E. M. (2018). Molecular and phenotypic

characterization of Photobacterium damsael among some marine fishes in Lake Temsah. Microb. Pathog., 114: 315- 322.

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.

El-Dakroury, M. F.; El-Gohary, M. S. and El-Gamal, A. M. (2020). Bacterial causes for mortality syndrome in some marine fish farms with treatment trials. Pak. J. Biol. Sci., 23(12): 1513-1522.

Ellis, A. E. (1999). Immunity to bacteria in fish. Fish and Shellfish. Immunol., 9(4): 291–308.

Er, A.; Ipek, Z. Z.; Arslan, M. N. and Kayis, S. (2021). *Vibrio Vulnificus* Infection in Horse Mackerel (*Trachurus Mediterraneus*) and Its Characteristic Symptoms: An Experimental Study. Bull. Eur. Assoc. Fish Pathol., 41 (4): 169–72.

Escobar, L. E.; Escobar-Dodero, J. and Phelps, N. B. D. (2018). Infectious disease in fish: global risk of viral hemorrhagic septicemia virus. virus. Rev. Fish Biol. Fish., 28: 637-655.

Ezzat, M.; Essawy, A. E.; El-Atta, A. and El-banna, N. (2014). Prevalence of common bacterial pathogens in marine fishes. Suez Canal Vet. Med. J., 19(1): 153-165.

Ezzat, M.; Mohaeed, G.; Abd El-Hak, M. and Wahdan, A. (2018). Prevalence and Genotypic Characterization of *Vibrio Alginolyticu*s in Somemarine Fishes. Suez Canal Vet. Med. J., 23(2): 81-90.

FAO. The state of world fsheries and aquaculture. (2020). Food and Agriculture Organization, Rome, Italy.

Gordon, L.; Giraud, E.; Ganiere, J. P.; Armand, F.; Bouju-Albert. A.; De la Cotte, N.; Mangion, C. and Le Bri, H. (2007). Antimicrobial resistance survey in a river receiving effluents from freshwater fish farms. J. Appl. Microbiol.,102(4): 1167–1176.

Hamouda, A. H.; Moustafa, E. M. and Zayed, M. M. (2019). Overview on the most prevailing bacterial diseases infecting Oreochromis niloticus at Aswan fish hatchery, Egypt. Adv. Anim. Vet. Sci., 7(11): 950-961.

Hala, F.A.; Eman, Y. T.; Hediat, M.S. and Sara, S.M. (2021). Isolation, identification and antimicrobial profile of Aeromonas spp., Pseudomonas spp. and Vibrio spp. from the Nile tilapia, *Oreochromis niloticus* in fish farms. Egypt. Aquat. Biol. Fish., 25(3): 171–85.

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.

Maather El-Lamie, M.M. and Heba Abdel-Mawla, I. (2015). Isopods infestation in relation to vibriosis of some marine fishes. Egy. J. Aquac., l 5(2): 13-30.

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

Mzula, A.; Wambura, P.N.; Mdegela, R. H. and Shirima, G.M. (2019). Phenotypic and molecular detection of Aeromonads infection in farmed Nile tilapia in Southern highland and Northern Tanzania. Heliyon, 5(8).

Narvaez, P.; Vaughan, D. B.; Grutter, A. S. and Hutson, K. S. (2021). New perspectives on the role of cleaning symbiosis in the possible transmission of fish diseases. Rev. Fish Biol. Fish., 31(2): 233-251.

Noha, M. S.; Sahar, T. M. and Abdel-Gawad, F. K.H. (2018). Monitoring some pathogenic bacteria in water and fish of Lake Qaroun. Egypt. Aquat. Biol. Fish., 22(5): 73-85.

Noga, E. J. (2010). Fish Disease, Diagnosis and Treatment, John Wiley and Sons, Hoboken, NJ, USA.

Noor El-Deen, A. E.; Abu-Bryka, A. Z. and Abou Zaid, A. A. (2023). Filed Studies on the most Prevailing Bacterial Diseases Affecting Some Cultured Marine Fishes. Egypt. J. Vet. Sci., 54 (2):173-181.

Quinn, P. J.; Markey, B. K.; Carter, M. E.; Donnelly, W. J. C. and Leonard, F. C. (2002). Veterinary microbiology and microbial disease. Blackwell science. PP. 113-116.

Rozi, R.; Rahayu, K. and Daruti, D. N. (2017). Detection and analysis of hemolysin genes in *Aeromonas hydrophila* isolated from Gouramy (Osphronemus gouramy) by polymerase chain reaction (PCR). IOP Conference Series Earth and Environmental Science, 137(1): 012001.

Sahar, F. M.; Soliman, B.A.; Sayed, M.A. and Rana, Abdel Aliem (2018). Seasonal growth of Arabian scad Trachurus indicus from the Gulf of Suez, Red Sea, Egypt. Egypt. Aquat. Biol. Fish., 22(1): 65- 73.

Shawna, L. S. and Brian, D. (2020). Salmonid antibacterial immunity: an aquaculture perspective. Biology, 9(10): 331.

Singh, V.; Rathore, G.; Kapoor, D.; Mishra, B. N. and Lakra, W. S. (2008). Detection of aerolysin gene in *Aeromonas hydrophila* isolated from fish and pond water. Indian J. Microbiol., 48:453–458.

Sonkol, R.A.; Torky, H.A. and Khalil, S.A. (2020). Molecular characterization of some virulence genes and antibiotic susceptibility of *Aeromonas Hydrophila* isolated from fish and water. Alex. J. Vet. Sci., 64(2): 34-42.

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.

Toranzo, A. E.; Magarinos, B. and Romalde, J. L. (2005). A review of the main bacterial fish diseases in mariculture systems. Aquac., 246(1-4): 37– 61.

Yen, P. T. H.; Tram, N. D. Q. and Linh, N. Q. (2022). Isolation and determination of Vibrio spp. pathogen from Sciaenops ocellatus suffering from hemorrhagic disease. JEBAS, 10(2): 405 – 415.

Zorriehzahra, M. J.; Yazdanpanah-Goharrizi, L.; Rokhbakhsh-Zamin, F.; Kazemipour, N. and Kheirkhah, B. (2020). Isolation, biochemical and molecular detection of *Aeromonas hydrophila* from cultured *Oncorhynchus mykiss.* Iranian J.Fish. Sci., 19(5): 2422 -2436.

دراسات عن الأمراض البكتيرية الأكثر انتشارا في أسماك الباغة

شيماء منصور – ولاء الشاعر

مركز البحوث الزراعية - معهد بحوث الصحة الحيوانية – فرع الأسماعيلية

تم تجميع عدد 60 سمكة من أسماك الباغة خلال موسم صيف 2022 من قناة السويس بمحافظة الأسماعيلية بطريقه عشوائيه وقد تم تسجيل معظم العلامات المرضية و الصفة التشريحية التي ظهرت علي هذه الأسماك و قد وجد أن معظم البكتريا المعزولة و المسببه للأمراض البكتيرية الأكثر انتشارا في أسماك الباغة تنتمي لميكروب الايريوموناس هيدروفيلا والفيبريو الجينوليتيكس والتي تم التعرف عليهم بالطرق التقليدية . كما تم التأكيد علي تصنيف المعزولات البكتيرية عن طريق تحديد جين 16إس و الكشف عن ضراوة المعزولات البكتيرية وذلك عن طريق تحديد بعض جينات الضراوة باستخدام تفاعل البلمرة المتسلسل. كانت نسبة الاصابة الكلية لبكتيريا الايريوموناس هيدروفيلا والفيبريو الجينوليتيكس في أسماك الباغة 40% و30% علي التوالي . كما تم عزل البكتيريا من الاعضاء الداخلية للأسماك المصابة بحيث كانت النسبة الأكثر إنتشارا في الكبد يليها الكلي ثم الطحال و النسبة الأقل كانت في الخياشيم.