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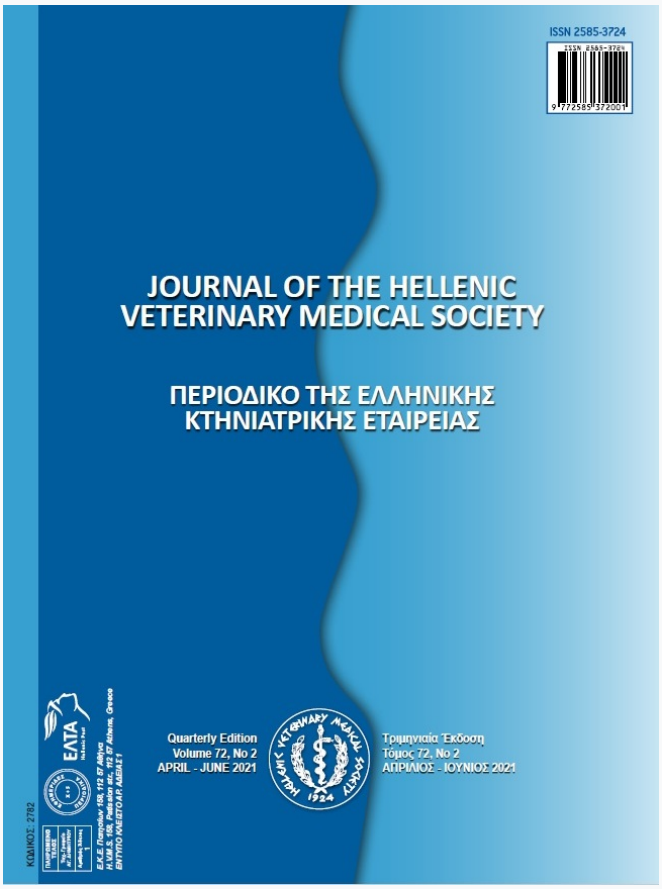
Journal of the Hellenic Veterinary Medical Society

Vol 72, No 2 (2021)



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D.E.A. GOBARAH, S.M. HELMY, N.B. MAHFOUZ, H.A. FAHMY, M.A.M. ABOU ZEID, E.M. MOUSTAFA

doi: 10.12681/jhvms.27517

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To cite this article:

GOBARAH, D., HELMY, S., MAHFOUZ, N., FAHMY, H., ABOU ZEID, M., & MOUSTAFA, E. (2021). Phenotypic and Molecular Characterization of Vibrio Species Isolated from Fish markets in Egypt. *Journal of the Hellenic Veterinary Medical Society*, *72*(2), 2817–2824. https://doi.org/10.12681/jhvms.27517

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J HELLENIC VET MED SOC 2021, 72(2): 2817-2824
ΠΕΚΕ 2021, 72(2): 2817-2824

Research article
Ερευνητικό άρθρο

Phenotypic and Molecular Characterization of *Vibrio* Species Isolated from Fish markets in Egypt

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ABSTRACT: Vibriosis is considered a worldwide, threatening bacterial disease that affects mariculture, with high mortalities and severe economic losses**.** Some *Vibrio* species have been frequently involved in outbreaks of food-borne diseases worldwide. The Genus *Vibrio* includes over eighty-five species present in marine and natural habitats of seawater, and the species are widely distributed throughout the world. This work aimed to isolate *Vibrio* species from different markets in Kafr EL-Sheikh Governorate, representing 40% of Egypt's fish production. Samples of Nile tilapia, (*Oreochromis niloticus*), grey mullet (*Mugil cephalus*) and African sharptooth catfish (*Clarias gariepinus*) were collected alive and examined for *Vibrio* species. Isolation and identification of *Vibrio* species were made using colonial morphology and biochemical characteristics, then confirmed using 16S rRNA gene-specific for the genus *Vibrio* and multiplex PCR using species-specific primers. 52 (34. 6%) *Vibrio* isolates were obtained from examined fishes. The highest incidence of *Vibrio* species was detected in *C. gariepinus* (64%), followed by *M. cephalus* (36%) and then *O. niloticus* (24%). In the case of *C. gariepinus*, *V. alginolyticus* was the most predominant species (32%), followed by *V. fluvialis* (12%), *V. cholerae*, *V. parahaemolyticus* (8%), and *V. splendidus* (4%). In the case of *O. niloticus*, the predominant *Vibrio* species were *V. alginolyticus* (12%), followed by *V. parahaemolyticus* (5. 33%), *V. cholerae* (4%), and then *V. splendidus* and *anguillarum* (1. 33%). In *M. cephalus*, *V. alginolyticus* also wasthe predominant species (14%), followed by *V. cholerae* (12%), *V. parahaemolyticus, V. fluvialis* (2%), and *V. splendidus* (2 %). *V. alginolyticus*, *V. cholerae,* and *V. parahaemolyticus* were found to produce PCR products of 737, 304, and 897 bp, respectively. This study highlights the incidence of *Vibrio* species in fish in Egypt.

Keywords: fish, PCR, *Vibrio* species

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Date of initial submission: 24-12-2021
Date of revised submission: 11-01-2021
Date of acceptance: 22-04-2021

INTRODUCTION

Mariculture represents a significant investment for fishers in Egypt; however, the diseases and the high cost of feeding are the main obstacles affectingthis sector's sustainability and profitability (Abdelaziz *et al.,* 2017). Aquaculture considers the fastest growing sector of high protein resources and contributes to economic development and social stability worldwide, promoting nutritional standards and relieving poverty in some developing countries (Béné *et al.,* 2015; FAO 2015). The genus *Vibrio* comprises aquatic microbes that usually live in coastal and estuarine water bodies (Ghenem *et al*., 2017). *Vibrios* are Gram-negative, halophilic, pathogenic bacteria that are a straight or curved rod shape, motile via a single polar flagellum, and negatively impact aquatic ecosystems and human health (Morris and Black 2015; Luan *et al.,* 2007; Lee *et al.,* 2015). *Vibrio* is strongly correlated with high salinity (30-35 ppt), parasitic infestation, high temperature, and mechanical injuries. These factors suppress the immunity and increase fish's susceptibility to vibriosis (Nagasawa and Cruz-Lacierda 2004; Haenen *et al.,* 2014; El-Bouhy *et al.,* 2016). These bacteria are ubiquitous in these environments as they have been isolated from seawater, fish, and shellfish (Alonzo *et al.,* 2017). *Vibrio* species in the aquaculture industry are serious opportunistic pathogens to cultured hosts such as finfish, shrimp, and shellfish (Liu *et al.,* 2016). Most of the aquatic diseases are caused by *Vibrio* species, which leads to a significant problem for the development of aquaculture with great economic losses worldwide because of its high morbidity and mortality rates (mortality ≥ 50%) (Al-Taee1 *et al.,* 2017). Several *Vibrio* species have been concerned with the health problems in marine animals. A recent report showed that *V. alginolyticus, V. harveyi, V. parahaemolyticus, V. owensii,* and *V. campbelli* are the most common species infecting farmed aquatic animals (Nor-Amalina *et al.,* 2017). Among *Vibrio* species, *V. anguillarum*, *V. parahaemolyticus,* and *V. vulnificus* are the main pathogenic species found in saltwater, while *V. cholerae* and *V. mimicus* are the main ones found in freshwater culture (Fouz*et al.,* 2002). Identification of *Vibrio* species is based mainly on their morphological, physiological, and biochemical characteristics (Alsina and Blanch 1994a, b). Traditional food analysis for microorganism's presence relies on the growth of bacteria in artificial media; also, culture techniques are often time-consuming and unreliable. Polymerase chain reaction (PCR) procedures are rapid and highly specific for detecting many pathogens (Anzar and Alarcon 2008).

In Egypt, fish are popular seafood; therefore, they are consumed in high quantities. This study was carried out to investigate *Vibrio* species 's presence in *Oreochromis niloticus, Mugil cephalus,* and *Clarias gariepinus* during the winter and spring seasons. It causes high economic losses, may threaten seafood safety, and increase the risk of illness in people who consume raw fish. This study also shows how to differentiate between different *Vibrio* species using conventional (cultivation and biochemical identification) and molecular methods. 16SrRNA gene was used as a housekeeping gene for the detection of genus *Vibrio*. Multiplex PCR using species-specific primers adopted further molecular identification.

MATERIALS AND METHODS

Samples

Samples of seventy-five Nile tilapia (*Oreochromis niloticus*), fifty grey mullet (*Mugil cephalus*) and twenty-five African sharptooth catfish (*Clarias gariepinus*) were collected from different markets in the Kafr El-Sheikh Governorate, Egypt, from January to May 2018 and transported immediately to the Department of Microbiology, Animal Health Research Institute in Kafr El-Sheikh Governorate. Fish were examined clinically for any abnormalities, including hemorrhages, skin ulceration, fin erosion, and abdominal distention. Bacteriological isolation of *Vibrio* species was performed from samples of the kidney, heart, liver, gills, and skin (Noga, 2010).

Ethical approval

All fish handling was conducted under the guidelines for the care and use of animals for scientific purposes established by the Ethics Committee of the Faculty of Veterinary Medicine, Kafr El-Sheikh University, Egypt.

Isolation and identification of *Vibrio* species

Primary isolation was made from internal organs (Alapide-Tendencia *et al.,* 1997) on trypticase soya broth with 3% NaCL that was incubated at 30°C for 24 hours. Then a loopful streaked on the TCBS media. Plates were incubated at 30°C for 24 hours and examined for the presence of typical colonies of *Vibrio* species. The colonies were examined for morphological characterizations, such as shape, Gram stain, and motility. Biochemical characterization was carried out us-

ing the following tests; oxidase, string test, triple sugar iron, arginine hydrolysis, indole production, methyl red, Voges-Proskauer, citrate utilization, urease, hydrogen sulfide production, nitrate reduction, gelatin liquefaction, ornithine decarboxylase, L-lysine decarboxylase, arginine decarboxylase, β-galactosidase (ONPG), salt tolerance and sensitivity to vibriostatic agent O/129 (Elliot *et al.,* 1995; Austin and Austin 1987).

Molecular identification

Molecular identification was applied to confirm 10 *Vibrio* isolates previously identified by phenotypic and biochemical characteristics.

The material used for DNA extraction

1. QIAamp DNA Mini Kit Catalog no. 51304

The QIAamp DNA Mini Kit provides silica-membrane-based nucleic acid purification for different types of samples. The spin-column procedure does not require mechanical homogenization; therefore, the total hands-on preparation time is only 20 minutes.

2. Ethanol 96% (Applichem)

Equipment and apparatuses used for the extraction of nucleic acids

Extraction of nucleic acids was performed using 1. 5 ml Eppendorf tubes, 20-200 µl mono- channel micropipettes, 100--1000 µl (Biohit), 200 µl and 1000 µl sterile filter tips, a centrifuge (Sigma sartorius), and a type II-A biosafety cabinet (Thermo).

PCR Master Mix used for cPCR

Emerald Amp GT PCR mastermix (Takara) Code No. RR310A

It contains Emerald Amp GT PCR mastermix (2x premix) and PCR grade water.

DNA extraction

DNA extraction from samples was performed using the QIAamp DNA Mini Kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of a lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. Then the sample was washed and centrifuged, as suggested by the manufacturer. Nucleic acid was eluted with 100 µl of the elution buffer provided in the kit.

PCR amplification

Primers were used in a 25-µl reaction containing:

12. 5 µl of EmeraldAmp Max PCR Master Mix **(Takara, Japan)**

1 µl of each primer of 20 pmol

4. 5 µl of water

6 µl of the DNA template

The reaction was carried out in an applied biosystem 2720 thermal cycler.

Detection of *Vibrio* 16srRNA gene (universal primers) and species-specific primers

Ten isolates suspected to be *Vibrio* species were molecularly confirmed, using primers targeting 663 bp of the 16S rRNA gene-specific for genus *Vibrio*. Then, multiplex PCR targeting 737 bp of *Collagenase* gene-specific for *V. alginolyticus*, 304 bp of the *omp*W gene-specific for *V. cholerae*, 410 bp of the *Hsp60* gene-specific for *V. vulnificus*, 121 bp of the *sodB* gene-specific for *V. mimicus*, and 897 bp of the *flaE* gene-specific for *V. parahaemolyticus* as shown in Table (1).

The amplification conditions were; 5 min of primary denaturation at 94ºC, 35 cycles of secondary denaturation for 30 sec at 94ºC, annealing (16S rRNA PCR: 40 sec at 56ºC; typing PCR: 1 min at 57ºC), and extension at 72ºC for 45 sec (16S rRNA and 1 min for typing PCR. A final extension was adjusted for 10 min. The Biotechnology Unit supplied the positive controls, Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Dokki, Giza, Egypt. The amplified products were resolved by electrophoresis in 1. 5% agarose gel (Applichem, Germany, GmbH), and a gene ruler 1 Kb plus DNA Ladder (Fermentas, Thermo Scientific, Germany) determined the sizes of the amplified product. Photographing was done bythe gel documentation system (Alpha Innotech, Biomedia).

Table 1: Oligonucleotide primers sequences
Source: Metabion (Germany).

Target	Gene	Sequence	Amplified product	Reference
Vibrio spp.	*16SrRNA*	CGGTGAAATGCGTAGAGAT TTACTAGCGATTCCGAGTTC	663 bp	**Tarr*et al*., 2007**
V. mimicus	*sodB*	CAT TCG GTT CTT TCG CTG AT GAA GTG TTA GTG ATT GCT AGA GAT	121 bp	
V. parahaemolyticus	*flaE*	GCA GCT GAT CAA AAC GTT GAG T ATT ATC GAT CGT GCC ACT CAC	897 bp	
V. vulnificus	*Hsp60*	GTC TTA AAG CGG TTG CTG C CGC TTC AAG TGC TGG TAG AAG	410 bp	
V. alginolyticus	*Collagenase*	CGAGTACAGTCACTTGAAAGCC CACAACAGAACTCGCGTTACC	737 bp	**Abu-Elala*et al*., 2016**
V. cholerae	*ompW*	caccaagaaggtgactttattgtg ggtttgtcgaat tag cttcac c	304 bp	**De Menezes et al., 2014**

Table 2: Prevalence of *Vibrio* species isolated from the examined fishes.

Recovered isolates	***Oreochromis niloticus* (75)**		***Mugil cephalus* (50)**		***Clarias gariepinus* (25)**		**Total (150)**	
	NO	%	NO	%	NO	%	NO	%
V. alginolyticus	9	12	7	14	8	32	24	16
V. cholerae	3	4	6	12	2	8	11	7.33
V. parahaemolyticus	4	5.33	2	4	2	8	8	5.33
V. fluvialis	0	0	2	4	3	12	5	3.33
V. splendidus	1	1.33	1	2	1	4	3	2
V. anguillarum	1	1.33	0	0	0	0	1	0.66
Total	18	24	18	36	16	64	52	34.6

Analysis of the PCR Products.

The PCR products were separated by electrophoresis on 1% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using 5V/cm. For gel analysis, 40 µl of the products were loaded in each gel slot. A generuler 100 bp ladder (Fermentas, Thermo, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra), and the data were analyzed through computer software.

RESULTS

In this study, one hundred and fifty samples of fish (75 *O. niloticus*, 50 *M. cephalus*, and 25 *C. gariepinus*) were collected and subjected to a full bacteriological investigation. Samples were identified by conventional microbiological methods using selective media and specific biochemical reactions. As shown in Table 2, the highest incidence of *Vibrio* species was detected in *Clarias gariepinus* (64%), followed by *Mugil cephalus* (36%) and then *Oreochromis niloticus* (24%). *V. alginolyticus* was the most predominant species in *Oreochromis niloticus, Mugil cephalus, and Clarias gariepinus*. The result of *Clarias gariepinus* revealed that *V. alginolyticus* was the most predominant species (32%), followed by *V. fluvialis* (12%), *V. cholera*, *V. parahaemolyticus* (8%), and *V. splendidus* (4%). In the case of *Oreochromis niloticus*, the predominant *Vibrio* species were *V. alginolyticus* (12%), followed by V. parahaemolyticus (5. 33%), *V. cholerae* (4%), and then *V. splendidus* and *anguillarum* (1. 33%). In *Mugil cephalus*, *V. alginolyticus* also was the predominant species (14%), followed by *V. cholerae* (12%), *V. parahaemolyticus*, *V. fluvialis* (2%), and *V. splendidus* (2%).

There were two typical morphologies for colonies of *Vibrio* species on TCBS agar. Typical colonies of *V. alginolyticus, V. cholerae, V. anguillarum,* and *V. fluvialis* were smooth, yellow (sucrose positive), and 2-3mm in diameter, while *V. parahaemolyticus* and *V. splendidus* were smooth, green (sucrose negative). On the microscopic examination, all the selected colonies revealed Gram-negative comma-shaped (curved) bacilli, motile by single polar flagella, non-spore former, and non-capsulated.

Table 3: Biochemical characteristics of different *Vibrio* strains

	V. alginolyticus	*V. cholerae*	*V. parahaemolyticus*	*V. fluvialis*	*V. splendidus*	*V. anguillarum*
TCBS agar	Y	Y	G	Y	G	Y
Growth in						
0% Nacl	-	+	-	-	-	-
3% Nacl	+	+	+	+	+	+
6% Nacl	+	-	+	+	+	+
8% Nacl	+	-	+	v	+	-
10% Nacl	+	-	-	-	-	-
Arginine dihydrolase	-	-	-	+	+	+
Ornithine decarboxylase	+	+	+	-	-	-
Lysine decarboxylase	+	+	+	-	-	-
Catalase	+	+	+	+	+	+
Citrate	+	+	+	+	+	+
Indole	+	+	+	+	+	+
Urease	-	-	-	-	-	-
Gelatine liquefaction	+	+	+	+	+	+
Voges proskauer	+	v	-	-	-	+
Methyl red	-	+	+	+	+	-
ONPG	-	+	-	+	V	+

TCBS, thiosulfate-citrate-bile salts, + =positive, - =negative, v =variable, Y=Yellow, G=Green

The biochemical characters of different isolated *Vibrio* strains were shown in Table (3). All the isolated *Vibrio* species were oxidase positive, catalase positive, gelatin liquefaction positive, citrate positive, indole positive, urease negative, H_2S negative, sensitive to O/129 (150 mg), and string test positive to most of *Vibrio* species (figure 1). At the same time, the other biochemical characters differ from one strain to another.

Figure 1:. positive string test for *Vibrio***.**

Vibrio specie's genotypic characters were assessed by examining ten isolates of *Vibrio* species for the genus gene and the species genes. All the isolates were positive for 16s rRNA gene. *Vibrio alginolyticus* (7 isolates) were positive to the species primer (*Collagenase*), *Vibrio cholerae* (2 isolates) were positive to the species primer (*ompW*), and *Vibrio parahaemolyticus* (1 isolate) was positive to the species primer (*flaE*). None of the isolates were positive to *sodB* and *Hsp60* primers of *Vibrio mimicus*and *Vibrio vulnificus,* respectively, as shown in Table 4 and figures2 and 3.

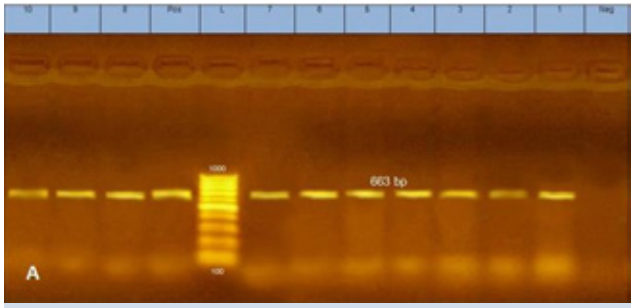

Figure 2: Agarose gel electrophoresis of 16S rRNA gene amplification for the molecular identification of *Vibrio* isolates with amplicon size of 663 bp, Ladder: 100 bp. .

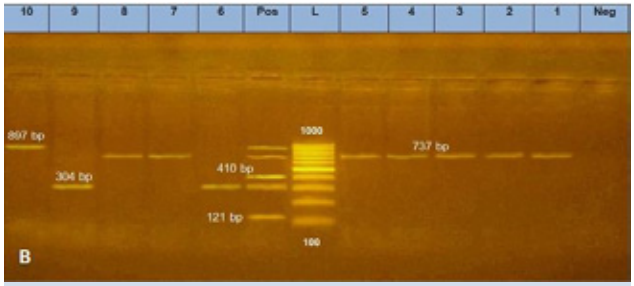

Figure 3: Agarose gel electrophoresis of DNA fragments generated by multiplex PCR with *Vibrio* species. Lanes 1, 2, 3, 4, 5, 7, 8 were *Vibrio alginolyticus* (at 737 bp), lanes 6, 9 were *Vibrio cholerae* (at 304 bp) and lane 10 was *Vibrio parahaemolyticus* (at 897 bp).

Table 4: Molecular identification of the 10 *Vibrio* isolates

Sample	Vibrio 16S rRNA	V. cholerae	V. mimicus	V. parahaemolyticus	V. vulnificus	V. alginolyticus
1	+	-	-	-	-	+
2	+	-	-	-	-	+
3	+	-	-	-	-	+
4	+	-	-	-	-	+
5	+	-	-	-	-	+
6	+	+	-	-	-	-
7	+	-	-	-	-	+
8	+	-	-	-	-	+
9	+	+	-	-	-	-
10	+	-	-	+	-	-

+ =positive, - =negative

DISCUSSION

Bacterial diseases represent one of the most significant problems currently affecting the productivity, development, and expansion of the aquaculture sector. Control of these diseases is difficult because fish are often farmed in systems in which production is dependent on natural environmental conditions. Most bacterial diseases are associated with changes or deterioration of the aquatic environment (Shyne *et al.,* 2008). *Vibrio* spp. have attracted the global interest of the microbiology community and zoonotic diseases experts for being a pathogen of public health concern (Austin and Austin, 2016).

Vibriosis is an economically dangerous infectious disease of cultured freshwater fish, and it is one of the significant diseases occurring in marine and brackish water fishes. *Vibrio* infections are characterized by hemorrhagic septicemia, red necrotic lesions in the abdominal musculature, exophthalmia, as well as erythema of the fin's bases and around the vent (Shahat and Mehana 2000).

Several factors have been recommended to influence *Vibrio*'s survival, persistence, and ability to cause infection. These factors include water temperature, ultraviolet (UV), and salinity (Lipp *et al*., 2002).

This study's limitation is the need to convince the fish farm owners and the fish vendors to take the fish samples necessary for our investigation.

V. alginolyticus causes human illness at significant morbidity and mortality rates, and it was the principal causative agent of marine vibriosis. It was frequently isolated from many outbreaks in Gilthead Sea bream and European Sea bass populations (Zorrilla*et al.,* 2003). *V. cholerae* was first identified as the causative agent of cholera, while *V. parahaemolyticus* is now the predominant etiology of human seafood-borne infections in developing countries (Percival *et al*., 2014).

In most cases, vibriosis outbreaks were attributed to immune suppression because of stress factors. For this particular disease, high water temperature and sudden water temperature fluctuation were among the main triggering factors for *Vibrio* invasion and outbreaks. Historically, this problem was related to spring syndrome (a fall syndrome) (Winfield, 2018).

This study was done during the winter and spring seasons where the most predominant *Vibrio* species isolated from *O. niloticus, M. cephalus, C. gariepinus* was *V. Alginolyticus* followed by *V. cholerae* and *V. parahaemolyticus,* respectively. The high isolation rate of *V. alginolyticus* could be attributed to that; it was present year-round, while the other *Vibrio* species were periodically detectable in summer but less common in winter (Di *et al.,* 2016). Moreover, Chen *et al*. (2010) and Yang *et al.,* (2008) considered that water temperature is the most critical factor affecting *Vibrio* distribution.

The present investigation indicates the total prevalence of vibriosis among examined fish was (34. 6%). This result is similar to that obtained by Moustafa *et al.,* (2010), who found that the prevalence of vibriosis in fish samples from Qarun Lake and Suez Gulf was (34%) and Eissa *et al.,* (2013), who detected vibriosis among naturally infected marine fishes (36%). Likewise, this result is lower than that obtained by Abd El-Gaber *et al.,* (1997), who isolated *Vibrio* species from 40 % of the examined *O. niloticus* and*M. cephalus* fish in both Qarun and Manzala Lakes. Also, Adebayo-Tayo *et al*., (2011) recorded that vibriosis was found in about (44. 2%) of examined seafood samples

obtained from Oron creek infected with *Vibrio* bacteria. The high isolation rate *Vibrio* could be attributed to environmental stresses, significantly organically polluted water, high salinity, and poor hygiene. In addition, Moustafa *et al*. (1990) supported these findings as he found that water pollution and high salinity were the major stress factors for vibriosis among fish.

This high incidence reflects the nature of *Vibrio* spp., known as a halophilic waterborne bacterium, which commonly inhabits worldwide environmental water sources.

On the other hand, this result is higher than the result obtained by Radwan(1995), who detected *Vibrio* species *in O. niloticus* with an incidence of 25%. Also, Levican *et al.,* (2020) isolated *Vibrio* species from farmed *Genypterus chilensis* (21. 6%).

This difference in prevalence percentages may be related to the differences in area, fish species, change in the fish immune system and time, methods of sampling, salinity level, sample sizes, different climate, and water quality characters.

In this study, the highest incidence of *Vibrio* species was detected *in Clarias gariepinus* (64%) followed by *Mugil cephalus* (36%) and then *Oreochromis niloticus* (24%).

The presence of *Vibrio* spp. in the freshwater fish samples suggests increasing in food-borne illness if these fish are consumed in undercooked form. They also could cross-contaminate ready-to-eat foods that are in the same environment.

The causative agent of vibriosis is the genus *Vibrio*. Traditional detection methods, based on cultivation using selective media and characterization of suspected colonies by biochemical reactions, are time-consuming, as they can take 3-4 days. Therefore, in this study, we used a simple, more rapid, sensitive, specific, and reliable method for detecting and characterizing bacteria. Such rapid methods include the polymerase chain reaction (PCR) technique, an in vitro technique used to amplify specific DNA fragments using two specific oligonucleotide primers (Sambrook *et al.,* 1989).

In all the *Vibrio* species, the 16S rRNA gene fragment (663bp) was amplified, confirming the genus in all the isolates while the species-specific genes could differentiate the species of *Vibrio* from each other. PCR confirmed *V. alginolyticus* at a 737 bp chromosomal locus-specific to this species, PCR confirmed *V. cholerae* at a 304 bp chromosomal locus-specific to this species, and PCR confirmed *V. parahaemolyticus* atan 897 bp chromosomal locus-specific to this species. Neither *V. vulnificus* or *V. mimicus* was detected in all examined fish samples using the culture and PCR methods.

CONCLUSION

The results show that the incidence of *Vibrio* spp. *in Oreochromis niloticus*, *Mugil cephalus, a*nd *Clarias gariepinus* collected in Kafr EL-Sheikh Governorate was34. 6%. The highest incidence of *Vibrio* species was detected in *C. gariepinus (*64%), followed by *M. cephalus* (36%) and then *O. niloticus*(24%).

In *C. gariepinus* n=16 (64%) with frequencies of n=8 (32%), n=2 (8%), n=2 (8%), n=3 (12%), n=1 (4%), n=0 (0%) for *V. alginolyticus, V. cholerae, V. parahaemolyticus, V. fluvialis*, *V. splendidus,* and *V. anguillarum* respectively. Likewise, the incidence of *Vibrio* spp. in *M. cephalus*, was n=18 (36%) with frequencies of n=7 (14%), n=6 (12%), n=2 (4%), n=2 (4%), n=1 (2%), n=0 (0%) for *V. alginolyticus, V. cholerae, V. parahaemolyticus, V. fluvialis*, *V. splendidus,* and *V. anguillarum* respectively. On the contrary, the incidence of *Vibrio* spp. In *O. niloticus* was n=18 (24%), with frequencies of n=9 (12%), n=3 (4%), n=4 (5. 33%), n=0 (0%), n=1 (1. 33%), n=1 (1. 33%) for *V. alginolyticus, V. cholerae, V. parahaemolyticus, V. fluvialis*, *V. splendidus,* and *V. anguillarum* respectively. In this study, the bacterial identifications described were based on culturing the strain on TCBS agar media, followed by morphological and biochemical identification. The confirmation was made by using PCR. The molecular identification showed that the most predominant strains were *V. alginolyticus, V. cholerae,* and *V. parahaemolyticus*. The early detection of the bacterial pathogens using the PCR technique before the onset of clinical symptoms offers the possibility of early action and treatment.

ACKNOWLEDGEMENT

We would like to sincerely thank Prof. Ashraf M. Ahmed, Department of Microbiology, Faculty of Veterinary Medicine, Kafrelsheikh University for his help and support in this research.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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