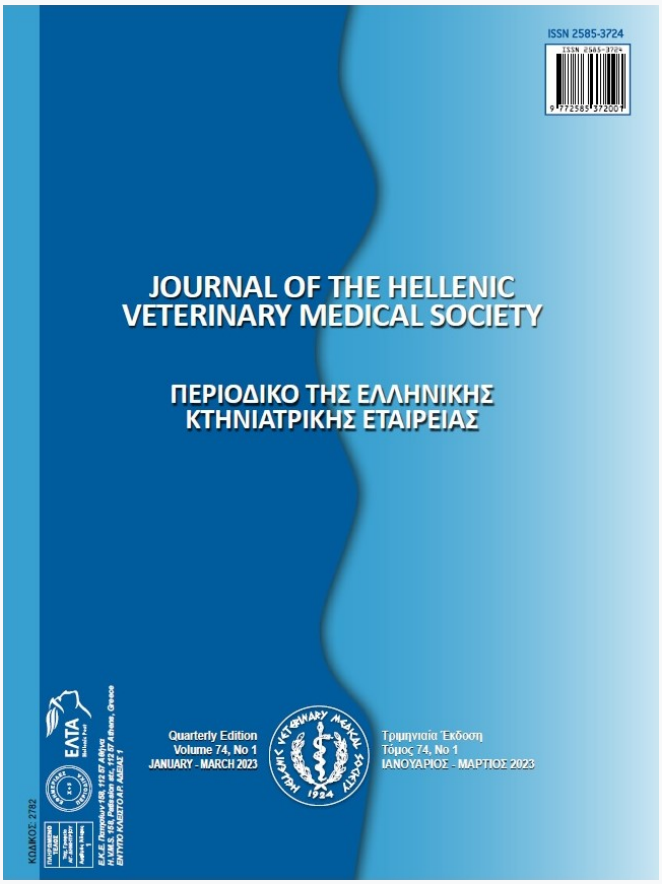
Journal of the Hellenic Veterinary Medical Society

Vol 74, No 1 (2023)



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doi: 10.12681/jhvms.24078

To cite this article:

Gobarah, D., Helmy, S., Mahfouz, N., Fahmy, H., Abou Zeid, M., & Moustafa , E. (2023). Molecular Characterization of Antimicrobial Resistance of Vibrio Species Isolated from Fish in Egypt. *Journal of the Hellenic Veterinary Medical Society*, *74*(1), 5101–5110. https://doi.org/10.12681/jhvms.24078 (Original work published April 11, 2023)

J HELLENIC VET MED SOC 2023, 74 (1): 5101-5110
ΠΕΚΕ 2023, 74 (1): 5101-5110

Research article
Ερευνητικό άρθρο

Molecular Characterization of Antimicrobial Resistance of *Vibrio* Species Isolated from Fish in Egypt

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ABSTRACT: *Vibrio sp*. is the most widely recognized and serious pathogen in fish and shellfish marine aquaculture around the world generated disease, not only to fish but also to human causing gastro-enteritis. *Vibriosis* is a common disease caused by several *Vibrio spp*.;*V. harveyi, V. parahaemolyticus, V. alginolyticus,V. anguillarum, V. vulnificus* etc. *Vibrio sp.*is Gram negative, straight or curved short rods, non-sporulating, non-capsulated, arranged singley or in chains, motile, oxidase positive, catalase positive, citrate positive, string test positive, indol positive, urease negative, H_2S negative.The current study was conducted to spot light on identification, molecular characterization and antimicrobial resistance of *Vibrio sp.* isolated from fish in Egypt and to detect the presence of some of β- lactams resistance genes and class 1 integron. About 52*Vibrio* isolates were isolated from 150 fish (75 *Oreochromis niloticus, 50 Mugil cephalus, 25 Clarias gariepinus*) in Kafr El-Sheikh Governorate in Egypt. Twenty isolates of *Vibrio* species were tested against 11 antimicrobials for antimicrobial resistance. All the isolates were highly sensitive (100%) to ciprofloxacin and norfloxacin. *V. alginolyticus* showed high resistance to Ampicillin and intermediate resistance to streptomycin, cefotaxime and erythromycin. *V. cholerae* showed high resistance to Ampicillin and intermediate resistance to erythromycin. *V. parahaemolyticus* showed high resistance to gentamycin and intermediate resistance to Ampicillin. *V. fluvialis* showed high and intermediate resistance to Ampicillin. *V. splendidus* showed high resistance to Ampicillin and intermediate resistance to streptomycin and cefotaxime. Five isolates showing multidrug resistance were tested for bla_{TEM}, bla_{CMY2}, bla_{CTX} genes and class 1 integron. The gene bla_{TEM} was detected in 100% of the isolates, while bla_{CMY2} and bla_{CTX} genes were detected in 0% of the isolates. Class 1 integron was detected with a percentage of (100%) in the 5 examined isolates. A class 1 integrons bearing streptomycin/ spectinomycin resistant gene cassette of *aadA2* were discovered on *V. parahaemolyticus* isolate. As there is few reports about antibiotic resistance in *Vibrio,* this study highlight the incidence of some of the β-lactams resistance genes and class 1 integrons in *Vibrio species*.

Keywords: β-lactam resistance genes, Class 1 integron, fish, PCR, *Vibrio* species

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Date of initial submission: 22-11-2021
Date of acceptance: 12-12-2022

INTRODUCTION

Vibrio species are the most predominant heterotrophic microscopic bacteria in the marine environment and are widely distributed in the coastal seawaters and/or brackish waters and have been isolated from seawater, fish, and shellfish (Alonzo *et al.,* 2017 and Drais *et al*., 2018). They are likewise found on the surface and/or in the gastrointestinal tract of marine animals or other organisms (Colweli and Grimes, 1984; Austin and Austin,1993 and Jun and Woo, 2003). Because of the rapid expansion of intensive mariculture and the consequent deterioration of culture conditions, the disease; vibriosis; caused by the genus Vibrio, occurs frequently worldwide, which influences a large number of fish species (Austin and Austin, 1993). Members of this family *Vibrionaceae* are Gram-negative, facultative, non-spore forming mostly characterized with comma-shaped rod.

Some of *Vibrio sp.* are referred to as significant human pathogens such as *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* (Moriarty, 1997), and others cause major financial losses in the aquaculture sector, such as *V. harveyi and V. anguillarum* that cause vibriosis with high mortality rates (Rippey, 1994).

Antimicrobial resistance is one of the most challenging public medical issues (Llor *et al*., 2014) that are directly associated with the management and control of diseases (Ansari *and* Raissy 2010) and it has been seriously studied in traditional fish farming systems in temperate waters (Alderman *et al*., 1998). Intensive fish farming in the world has resulted in growing issues of bacterial diseases, which prompted subsequent heavy antimicrobial use (Cabello, 2006). Resistance to the antimicrobials is viewed as another significant contributor to the virulence of the fish pathogen. Recently, antimicrobial resistance has been risen and evolved in *Vibrio* spp. resulting from the excessive utilization of antibiotics in agriculture, aquaculture systems and human medicine (Cabello *et al*., 2013). Most of the common antibiotics which are frequently used are no longer effective. The wide use and misuse of antibiotics in agriculture, aquaculture, and livestock production are considered one of the key factors that impact the spread and development of antimicrobial resistance. Multidrug-resistant bacteria are another emerging challenge when a bacterial cell gets resistant to multiple antibiotics (Faja *et al.,* 2019).

The majority of hereditary determinants which give resistance to antibiotics are found in the plasmid. Plasmids and portable hereditary elements such as integrons are considered contributors in the dissemination of antibiotic resistance genes and regarded as promotors of multi-drug resistance (Gonzalez-Plazaa *et al.,* 2019). Antibiotic contamination from facilities of manufacturing advanced the horizontal mobility of plasmids carrying antibiotic resistance genes among aquatic bacteria (Flach *et al*., 2015). Integrons are portable hereditary elements that have a significant job in the spread and acquisition of antimicrobial resistance genes. Class 1 integron is composed of two conserved regions (CS): 5`CS contains the integrase gene, and 3`CS that normally contain qacEΔ1 and sul1 resistance genes to quaternary ammonium compounds and sulfonamide, respectively. Between these CSs, there is a variable region where gene cassettes can be embedded (Li and Zhao 2018). These cassettes present protection from most classes of antibiotics (Deng *et al*., 2015).

Class 1 integrons are the most frequently found in clinical isolates of gram-negative bacteria (Mala *et al.*, 2016). Class 1 integrons are found in environmental and clinical isolates of *V. cholerae*, while very few resistance integrons have been found in *Vibrio* species other than the *V. cholerae* (Taviani *et al*., 2008).

Just little reports have revealed antibiotic resistance determinants in the *Vibrio* spp. that are a reservoir of resistance genes.

The current study was carried out to spotlight on the incidence of some of the β-lactams resistance genes and class 1 integrons in *Vibrio species* isolated from fishes in Egypt.

MATERIAL AND METHODS

A- Materials

1. Fish Samples

A total of 150 clinically diseased fish samples (75 *Oreochromis niloticus*, 50 *Mugil cephalus* and 25 *Clarias gariepinus*) were collected alive from private fish farms at KafrelSheikh governorate (Gobarah *et al*., 2021). The collected fish were transported immediately to the Department of Microbiology, Animal Health Research Institute in KafrelSheikh governorate and held in well-prepared glass aquaria supplied with sufficient amounts of dechlorinated tap water with continuous aeration using an electric air pump (Eissa, 2016) till investigation.

B- Methods:

1. Clinical and postmortem examinations:

The gathered fish were clinically examined to identify any external changes or clinical abnormalities (McVicar, 1982). Postmortem examination of the internal organs was carried out on sacrificed and freshly dead fish according to Austin and Austin (2012) and Eissa (2016).

2. Bacterial isolation

Tissue samples were aseptically collected from the skin, liver, kidney, heart and gills of the examined fish, pre-enriched on Tryptic Soy Broth (TSA) with 3% NaCl and incubated for at 30˚C for 18-24 hrs. After that, they were streaked on tryptic soy agar and incubated at 30˚C for 48 hrs. Pure single colonies on TSA were further streaked on selective media; Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS -Himedia) media and incubated for 18-24 hrs at 30 °C.

3. Phenotypic identification of bacterial isolates

Phenotypic identification of the bacterial isolates was identified according to Bergey's, (1994) and Madigan and Martinko (2005). Bacterial colonies that were suspected to be *Vibrio* has shown yellow or green color on TCBS agar. Pure cultures were subjected to Gram staining and motility test and then viewed microscopically (Cruickshank *et al.,* 1975). Further conventional biochemical tests following the rules proposed by Kreig and Holt (1984), MacFaddin (2000), and the FDA manual (Jayasinghe *et al*., 2010) were performed.

4. Antimicrobial susceptibility test:

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method (Stratev *et al.,* 2015) utilizing Mueller-Hinton agar (Difco), as indicated by the National Committee for Clinical Laboratory Standards "NCCLS" (2001) guidelines. A total of 11 different antimicrobial sensitivity discs were utilized to investigate the resistance of the isolates. Sensitivity discs with variable concentrations were used to determine the susceptibility of the isolated bacterial strains to different antimicrobial agents (Oxoid Limited,Basingstoke, Hampshire, UK). The tested antimicrobial agents and their corresponding concentrations were as follows: gentamycin (10 µg/disk), ampicillin (10 µg/disk), streptomycin (10 µg/disk), nitrofurantoin (300 µg/disk), ciprofloxacin (5 µg/disk), sulpha-trimethoprime (25 µg/disk), nalidixic acid (30 µg/disk), cefotaxime (30 µg/disk), erythromycin (15µg/disk), amikacin (30µg/disk) and norfloxacin (10µg/disk). After incubating the inoculated plates at 30 °C for 18-24 hrs, the interpretations of the zones of inhibition were estimated as the maximal inhibition zone for the growth of microbe is said to that antibiotic had greatest impact on the microbe growth according to the guidelines stipulated by National Committee for Clinical Laboratory Standards "(CLSI, 2010)".

5. Molecular identification by Polymerase Chain Reaction (PCR) :

DNA extraction

The Genomic DNA was extracted from a pure culture of bacterial isolates using the DNA extraction kit (QIAamp DNA Mini Kit - Catalogue no. 51304 - Hilden, Germany) following the manufacturer's instructions.

Detection of β- lactams resistance genes and class 1 integron.

Five isolates of *Vibrio sp.,* showing multidrug resistance, were screened for the presence of some of β- lactams resistance genes and class 1 integron. PCR assays were applied to target bla_{TEM}, bla_{CMY2}, bla_{CTX} genes and class 1 integron. Details of primers used are listed in (Table 1). Initial optimization experiments for

Table 1. Primers used to detect β- lactams resistance genes, class 1 integron and virulence genes

Target gene	Sequence	Amplified product	Reference
bla_{TEM}	5'-ATCAGCAATAAACCAGC 3'-CCCCGAAGAACGTTTTC	516 bp	Colom *et al*., 2003
bla_{CMY2}	5'-TGG CCA GAA CTG ACA GGC AAA 3'-TTT CTC CTG AAC GTG GCT GGC	462 bp	Pérez-Pérez and Hanson, 2002
bla_{CTX}	5'-ATG TGC AGY ACC AGT AAR GTK ATG GC 3'-TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593 bp	Archambault *et al*., 2006
class 1 integron cassettes	5'-CSGGC ATC CAA GCA GCA AG 3'-CSAAG CAG ACT TGA CCT GA	Variable	Sow *et al*., 2007

Table 2. Cycling conditions of PCR

Target gene	Primary denaturation	Amplification				Final extension	Reference
		Secondary denaturation	Annealing	Extension	No. of cycles		
bla_{TEM}	94˚C/ 5 min.	94˚C/ 30 sec	54˚C/ 40 sec	72˚C/ 45 sec	35	72˚C / 10 min.	Colom *et al*. (2003)
bla $_{CMY2}$	94˚C/ 5 min.	94˚C/ 30 sec	55˚C/ 40 sec	72˚C/ 45 sec	35	72˚C/ 10 min.	Pérez-Pérez and Hanson, (2002)
bla $_{CTX}$	94˚C/ 5 min.	94˚C/ 30 sec	54˚C/ 40 sec	72˚C/ 45 sec	35	72˚C/ 10 min.	Archambault *et al*. (2006)
class 1 integron cassettes	94˚C/ 5 min.	94˚C/ 1 min.	50˚C/ 1 min.	72˚C/ 2 min.	35	72˚C/ 10 min.	Sow *et al*. (2007)

Table 3. Prevalence of *Vibrio* species isolated from the examined fishes (Gobarah *et al*., 2021)

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

each primer were conducted to ascertain optimal PCR conditions for $MgCl_2$ and annealing temperatures and the reaction mixture of the total volume of 25 µL was prepared. The PCR conditions used are presented in (Table 2).

RESULTS

1. Clinical and postmortem examination

The external gross lesions of the examined fish revealed scale detachment, fins erosion, corneal opacity, pale gills, skin darkening, ulcers, redness at base of anal fin and hemorrhagic areas around the mouth. Meanwhile, postmortem examination revealed congestion, swelling of the spleen, liver and kidney and hemorrhage in abdominal cavity.

2. Bacterial isolation and Incidence of *Vibrio species*

Bacterial isolation in the current data revealed that *Vibrio sp.* could be isolated from 18 out of 75 *Oreochromis niloticus* samples; with an incidence of 24% and the isolated *Vibrio species* were identified as *V. alginolyticus* (9), *V. cholerae* (3), *V. parahaemolyticus* (4), *V. fluvialis* (0), *V. splendidus* (1) and *V. anguillarum* (1). Moreover, *Vibrio sp.* could be isolated from 18 out of 50 *Mugil cephalus* with an incidence of 36%. The isolated *Vibrio species* were identified as *V. alginlyticus* (7), *V. cholerae* (6), *V. parahaemolyticus* (2), *V. fluvialis* (2), *V. splendidus* (1) and *V. anguillarum* (0) However, in the case *Clarias gariepinus,* it could be isolated from 16 out of 25 fish; with an incidence of 64%. The isolated *Vibrio species* were identified as *V. alginlyticus* (8), *V. cholerae* (2), *V. parahaemolyticus* (2), *V. fluvialis* (3), *V. splendidus* (1) and *V. anguillarum* (0) (Table 3) (Gobarah *et al*., 2021**).**

3. Phenotypic identification of bacterial isolates

Phenotypic analysis of *Vibrio sp*. colonies on TCBS agar media revealed that the bacterial colonies of *V. alginolyticus* were large (2-4 mm) and mucoid yellow; colonies of *V. cholerae* and *V. fluvialis* were large (2-3mm) and smooth yellow, while the colonies of *V. parahaemolyticus* were large (2-5mm) and green. By Gram staining, all colonies revealed Gram-negative, straight or curved short rods, non-sporulating, non-capsulated, arranged singly or in chains, motile, oxidase positive, catalase positive, citrate positive, string test positive, indole positive, urease negative, H_2S negative while the other biochemical tests differed from species to another (Gobarah *et al*., 2021**).**

4. Antimicrobial susceptibility test:

Antimicrobial susceptibility test was done for 20

isolates (8 *V. alginolyticus,* 5 *V. cholerae,* 3 *V. fluvialis,* 2 *V. parahaemolyticus,* 2 *V. splendidus)* against 11 antimicrobials. All the isolates were highly sensitive (100%) to ciprofloxacin and norfloxacin. *V. alginolyticus* showed high resistance to Ampicillin and intermediate resistance to streptomycin, cefotaxime and erythromycin. *V. cholerae* showed high resistance to Ampicillin and intermediate resistance to erythromycin. *V. parahaemolyticus* showed high resistance to gentamycin and intermediate resistance to Ampicillin. *V. fluvialis* showed high and intermediate resistance to Ampicillin. *V. splendidus* showed high resistance to Ampicillin and intermediate resistance to streptomycin and cefotaxime.

5. Molecular identification by Polymerase Chain Reaction (PCR) and β- lactamase resistance genes and class 1 integron

Results of the current study revealed that all the 5 isolates *(V. alginolyticus, V. parahaemolyticus, V. fluvialis, V. cholerae and V. cholerae)* held bla_{TEM} gene however they don·t hold bla_{CMY2} and bla_{CTX} resistance genes (Figure 1, 2, 3). All the isolates were positive to class 1 integron. Class 1 integrons sequencing indicated thatthe gene cassette containing aminoglycoside adenyltransferase (*aadA2*) gene encoding resistance to streptomycin and spectinomycin at 900 bp in *V. cholerae* isolate (Table 4).

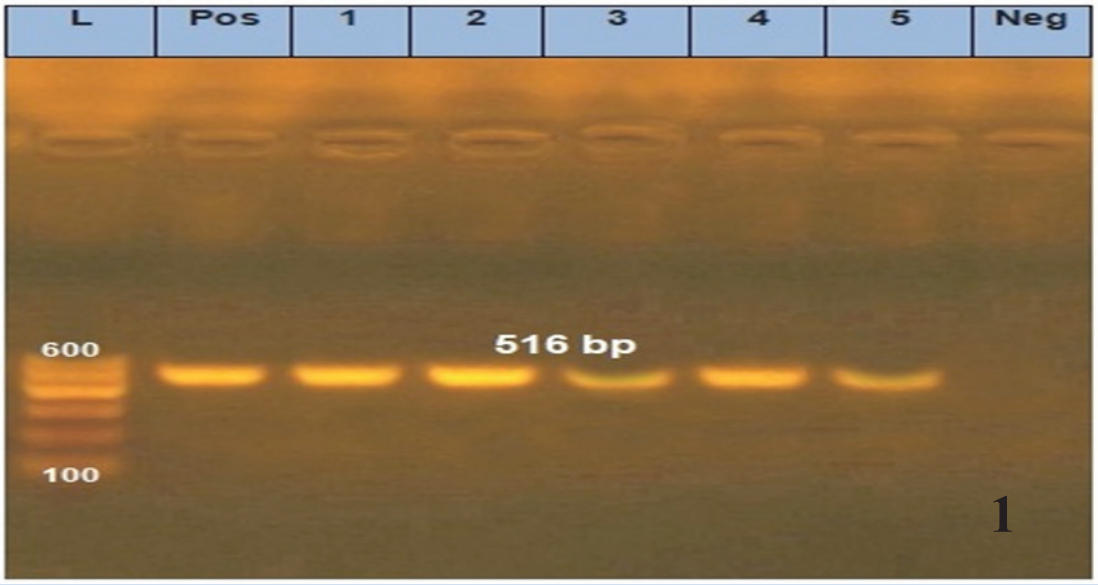

Figure 1. Agarose gel electrophoresis of PCR products after amplification of bla_{TEM} gene at 516 bp. MWM-molecular weight marker (100-600 bp DNA ladder), + control (Positive, Negative) +lane (1,2,3,4,5) different strains of *Vibrio* **(***V.. alginolyticus, V. parahaemolyticus, V. fluvialis, V. cholerae and V. cholerae respectively)*

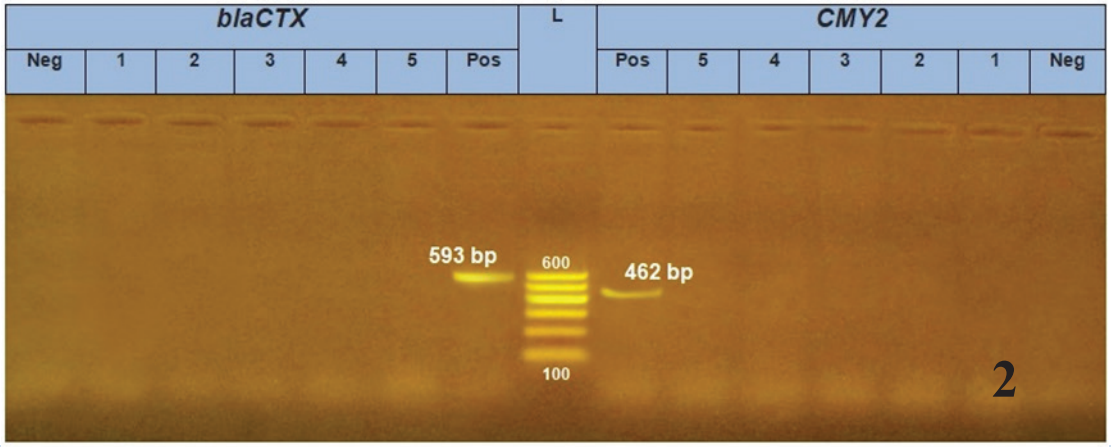

Figure 2. Agarose gel electrophoresis of PCR products after amplification of bla_{CTX} gene at 593 bp and bla_{CMY2} gene at 462 bp. MWM-molecular weight marker (100-600 bp DNA ladder), + control (Positive, Negative) +lane (1, 2, 3, 4, 5) different strains of *vibrio (V. alginolyticus, V. parahaemolyticus, V. fluvialis, V. cholerae and V. cholerae respectively)*

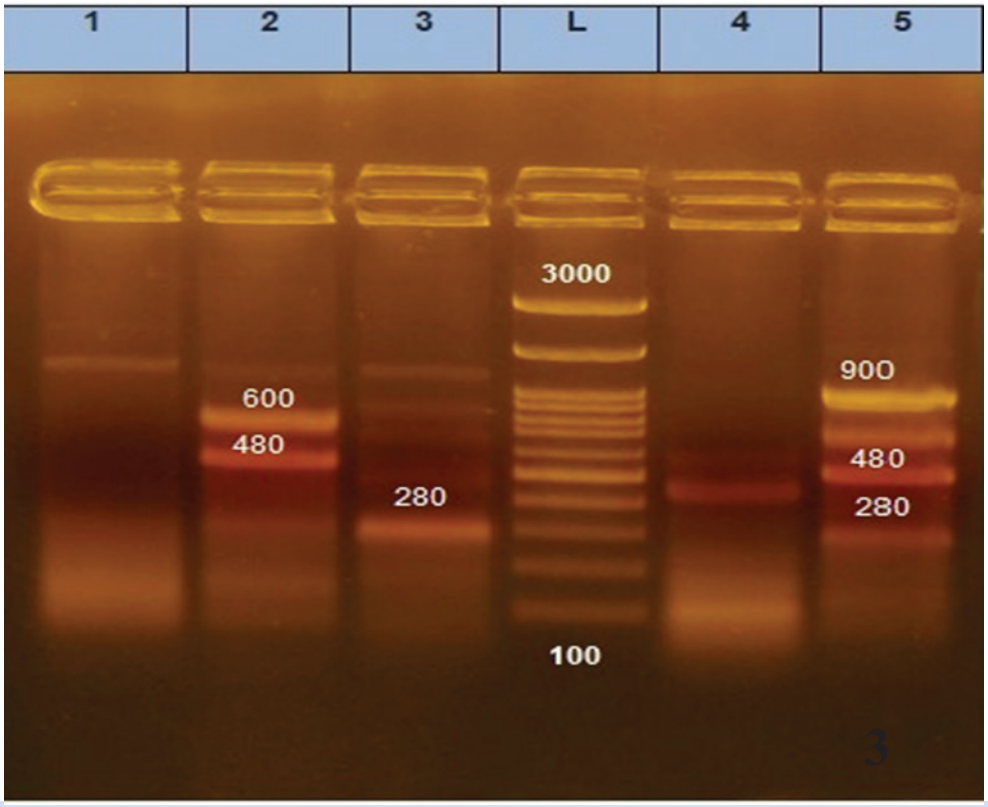

Figure 3. Agarose gel electrophoresis of PCR products of class 1 integrons in *Vibrio* isolates. lane (1,2,3,4,5) different strains of *vibrio* (*V.. alginolyticus, V. parahaemolyticus, V. fluvialis, V. cholerae and V. cholerae respectively).*
Isolates 1, 2, 3, 4 and 5 are positive for class 1 integron..
Isolate no. 2: an empty integron (480 bp, 600 bp).
Isolate no. 3: an empty integron (280 bp).
Isolate no. 5: *aadA2* (900 bp) an empty integron (280 bp, 480 bp)

Table 4. Resistance phenotypes and incidence of resistance genes in some *Vibrio sp*. isolated from fishes:

NO	Isolate	Resistance pattern	MDR	Resistance Genes	Class 1 integron
1	*V. alginolyticus*	AMP, SXT, STR	+	bla_{TEM}	
2	*V. parahaemolyticus*	AMP, STR, ERY, NIT	+	bla_{TEM}	
3	*V. fluvialis*	AMP, GEN, ERY	+	bla_{TEM}	
4	*V. cholerae*	AMP, NIT, ERY	+	bla_{TEM}	
4	*V. cholerae*	AMP, GEN, ERY	+	bla_{TEM}	*aadA2*

DISCUSSION

Bacterial diseases are the major serious problems in aquaculture (Ibrahim *et al.,* 2013). Bacterial fish pathogens are normally present in the fish surrounding environment; nonetheless, under certain stress conditions, they may cause extraordinary financial mortalities with 80% mortalities in fish farms. That is why protecting the aquaculture industry from bacterial diseases is a priority (Ljubojevic Pelic, 2018; Hamouda *et al.,* 2019; Hamouda and Moustafa 2020).

The infected fish showed scale detachment, fins erosion, this may be due to the lytic activity of the bacterial infection. The hemorrhages all over the body may be due to the hemolysin secreted by the isolated bacteria which make hemolysis of the RBCs as well as, elastase enzyme which contribute significantly to vascular damage because the blood vessels are mainly composed of elastic and collagenous fibers (Hamouda *et al.,* 2019 and Hamouda and Moustafa 2020). The post mortem changes may be attributed to the isolated septicemic bacteria and their virulence genes.The clinical signs displayed in the current study nearly corresponded with that recorded by previous authors (Eissa, 2016; El-Gamal *et al.,*2018 and Hardi *et al.,* 2018)

Regarding the incidence of *Vibrio sp.*; 18 out of

75 *Oreochromis niloticus* samples; (24%) ; and the isolated *Vibrio sp*. were identified as *V. alginolyticus* (9), *V. cholerae* (3), *V. parahaemolyticus* (4), *V. fluvialis* (0), *V. splendidus* (1) *and V. anguillarum* (1). Moreover, *Vibrio sp.* was 18 out of 50 *Mugil cephalus* with an incidence of 36% and the isolated *Vibrio sp.* were identified as*V. alginlyticus* (7), *V. cholerae* (6), *V. parahaemolyticus* (2), *V. fluvialis* (2), *V. splendidus* (1) and *V. anguillarum* (0). However, in case *Clarias gariepinus,* 16 out of 25 fish could be isolated; with an incidence of 64%; and the isolated *Vibriosp.* were identified as *V. alginlyticus* (8), *V. cholerae* (2), *V. parahaemolyticus* (2), *V. fluvialis* (3), *V. splendidus* (1) *and V. anguillarum* (0). These findings may differ completely or partially with that recorded by many authors and this could be due to abiotic and biotic conditions of the environments where the studies were performed (El-Gamal *et al.,* 2018; Hardi *et al*., 2018; Hamouda *et al.,* 2019; Hamouda and Moustafa 2020).

The colonies morphology, Gram staining and the biochemical profile of the detected bacteria are nearly identical to those reported by many other previous authors (Ahmed and Shoreit, 2001; Noga, 2010; Abd El-Kader and Mousa-Balabel, 2017; El-Gamal *et al.,* 2018 and Hardi *et al*., 2018)

The antimicrobial susceptibility testing method is of major time-wise; was applied to distinguish antibiotic resistance and to decide the best treatment for a particular bacteria. Clinical microbiology relies on these techniques to select the agent of choice for a bacterial infection, and to know the local and the global epidemiology of antimicrobial sensitivity. The information on measure of antimicrobials that utilized in aquaculture are not available in many nations, there is evidence recommends that the amount of antimicrobials utilized in aquaculture in developing countries is limited in certain countries including Egypt, huge amounts of antimicrobials are normally utilized in aquaculture without consultation or supervision (WHO, 2006). The spread and development of antimicrobial resistance as a result of exposure to antimicrobial agents is recorded widely in both human and veterinary medicine. It is also well known that aquatic bacteria and pathogens of fish can develop resistance due to exposure to antimicrobials (Sørum, 2006).

One of the main findings in the current study was the wide spread of resistance to antimicrobials in *Vibrio* spp. isolated from fishes. The resistance to ampicillin and erythromycin was prevalent, the results were in concurrence with the previous studies (Kitiyodom *et al*., 2010). This is not surprising as both antibiotics are naturally created and scattered in the environment (Rosser and Young, 1999; Bani *et al.,* 2007). Resistance to cefotaxime and gentamycin, although detected in only a few strains, it could be of noteworthiness, because the presence of such resistance is an indication of the potential to spread.

The polymerase chain reaction has been proven to be a more accurate and rapid method for the identification of bacterial pathogens.The 16S ribosomal RNA gene is a highly conserved region present in bacteria which plays a major role in gene coding. It is considered as a standard marker for bacterial phylogenetic analysis to differentiate the species (Nagpal *et al.,* 1998).

In the present work, the β-lactam resistant genes were identified by the use of the specific primers of these genes (bla_{TEM} gene had molecular weight 516 bp, bla_{CMY2}gene had molecular weight 462 bp and bla_{CTX} gene had molecular weight 593 bp). It was observed that (100%) of the tested isolates had bla_{TEM} gene, while $bla_{CTX}$and bla_{CMY2} genes were not detected in the tested isolates of *Vibrio sp*. (0%). These results are in accordance to many authors; Rojas *et al.* (2011) found that all isolates of *V. parahaemolyticus* isolated from oysters and mussels possessed the $bla_{TEM\text{-}116}$gene; Silvester *et al.* (2019) investigated that all the *Vibrios* isolated from Cochin Estuary, seafood and shrimp farms harbored the bla_{TEM} gene and detected the $bla_{CTX\text{-}M}$gene in only 1.1% of the *Vibrio sp*. And Li *et al.,* (2015) who found bla_{TEM} gene in 1 of 2 strian of *V. parahaemolyticus* that screened for β-Lactamase genes and first detected bla_{CMY2} gene in 1 isolate of 2 isolates of *V. parahaemolyticus*. On other hand, Ceccarelli *et al.* (2016) screened the *V. cholerae* isolates showing reduced susceptibility in Bangladesh to detect the ESBL *(bla_{CTX}, bla_{TEM}* and *bla_{SHV})* and found that all the isolates were negative.

The PCR result showed the presence of a class 1 integron in all (100%) of the five isolates of *Vibrio* species. DNA sequencing results of the purified PCR fragments showed the presence of one antibiotic resistance gene cassettes inside the class 1 integron. The *aadA2* gene cassettes were found in the *V. cholerae* isolate at 900 bp while the other isolates of *Vibrio* were devoid of resistance genes. The *aad*A2 allele is widespread among bacteria species around the world. This is similar to the result obtained by Jiang *et al.* (2014) who found that all isolates harbored class1 in-

tegrons but he found that only one carried gene cassette without any resistance genes while Canto de Sá *et al*. (2010) found *aadA2* gene cassettes at 918bp in class 1 integrons in 1 genotypes of environmental *V. cholerae* non-O1/non-O139 and Dalsgaard *et al.* (2000) also detect *aadA2* gene cassettes at 1009 bp in *V. cholerae*.

CONCLUSION

The results of the current study concluded that *Vibrio sp.*is Gram-negative, straight or curved short rods, non-sporulating, non-capsulated, arranged singly or in chains, motile pathogen, not only to fish but also to human causing gastroenteritis. Moreover, the presence of multidrug-resistant *Vibrio sp*. harboring mobile genetic elements in fishes.The presence of antimicrobial resistance determinants with transferable genetic elements can promote the rapid dissemination of antimicrobial resistance among *Vibrio sp*. All the isolates were highly sensitive (100%) to ciprofloxacin and norfloxacin. Five isolates showing multidrug resistance were tested for bla_{TEM}, bla_{CMY2}, bla_{CTX} genes and class 1 integron. The genes bla_{TEM} was detected in 100% of the isolates, while and bla_{CTX} genes were detected in 0% of the isolates. Class 1 integron was detected with a percentage of (100%) in the 5 examined isolates. A class 1 integrons bearing streptomycin/spectinomycin resistant gene cassette of *aadA2* were discovered on *V. cholerae* isolate. Further studies in Egypt and elsewhere are required to investigate the usage level of antibiotics in aquatic farms, to determine antimicrobial resistance, the dissemination and acquisition of resistance determinants and to evaluate the dangerof the transfer of resistant bacteria or genes to humans through the food chain.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Abd El-Kader M, Mousa-Balabel T (2017). Isolation and molecular characterization of some bacteria implicated in the seasonal summer mortalities of farm-raised *Oreochromis niloticus* at Kafr El-Sheikh and Dakahlia governorates. Alex. J. Vet. Sci.53 (2) 107-113.

Ahmed Sh M, Shoreit AM (2001). Bacterial hemorrhagic septicemia in *Oreochromis niloticus* at Aswan fish hatcheries. Assiut Vet. Med. J. 45 (89) : 190-206.

Alderman DJ and Hastings TS, (1998). Antibiotic use in aquaculture: development of antibiotic resistance potential for consumer health risks. Int. J. Food Sci. Technol. 33:139–155.

Alonzo KHF., Cadiz RE, Traifalgar RFM and Corre, VLJr, (2017). Immune responses and susceptibility to *Vibrio parahaemolyticus* colonization of juvenile *Penaeus vannamei* at increased water temperature. *Aquac. Aquar. Conserv. Legis.* (*Bioflux*), 10 (5) : 1238-1247..

Ansari M, Raissy M, (2010). In vitro susceptibility of commonly used antibiotics against *Vibrio spp*. isolated from Lobster (*Panulirus homarus*). African Journal of Microbiology Research, 4: 2629-2631.

Archambault M, Petrov P, Hendriksen RS et al., (2006). Molecular characterization and occurrence of extended-spectrum beta-lactamase resistance genes among Salmonella entericaserovar Corvallis from Thailand, Bulgaria, and Denmark. Microb Drug Resist. 12 (3) :192-8.

Austin B. and Austin DA. (2012). Bacterial fish pathogens; diseases of farmed and wild fish. Springer,New York, London).

Austin, B. and Austin, D.A. (1993) : Bacteriological fish pathogens: Disease in farmed and wild fish, 2nd ed. Ellis Harwood, London. pp. 265-30.

Bani S, Mastromarino PN, Ceccarelli D et al. (2007). Molecular characterization of ICEVchVie0 and its disappearance in *Vibrio cholerae* O1 strains isolated in 2003 in Vietnam. FEMS Microbiol Lett 266: 42–48.

Bergey DH (1994). Bergey's Manual of Determinative Bacteriology, ed. R. E. Buchaman and N. E. Gibbons. 9th ed. Baltimore, Williams and Wilkins.

Cabello FC, Godfrey HP, Tomova A, et al., (2013). Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. Environ Microbiol. 15:1917–42..

Cabello FC, (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environ. Microbiol.* 8: 1137–1144.

Canto de Sá1, L.L.; Lourenço da Fonseca, E.; Pellegrini, M.; Freitas, F.; Loureiro, E. C. B. and Vicente, A. P. (2010): Occurrence and composition of class 1 and class 2 integrons in clinical and environmental O1 and non-O1/non-O139 *Vibrio cholerae* strains from the Brazilian Amazon. Mem Inst Oswaldo Cruz, Rio de Janeiro, 105 (2) : 229-232.

Ceccarelli D, Alam M, Huq A and Colwell RR, (2016). Reduced Susceptibility to Extended-Spectrum β-Lactams in *Vibrio cholerae* Isolated in Bangladesh. Front Public Health. 4:231.

CLSI (2010). Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline, 3rdEdn. Austin, TX.

Colom K, PèrezJ, Alonso R, et al., (2003). Simple and reliable multiplex PCR assay for detection of bla_{TEM}, bla_{SHV} and bla_{OXA-1} genes in Enterobacteriaceae. FEMS Microbiology Letters. 223: 147-151.

*Colwell, R.R. and Grimes, D.J. (1984) :*Vibrio diseases of marine fish populations, Helgolander Meeresunter Suchungen., 37:265-287.

Cruickshank R, Duguid JP, Marmion BP, Swain RH (1975). Medical Microbiology. The practical of Microbiology. Chucohill Livingstone.6 12th edition. (11), Edinburgh, London and New York.

Dalsgaard A, Forslund A, Serichantalergs O et al., (2000). Distribution and Content of Class 1 Integrons in Different *Vibrio cholerae* O-Serotype Strains Isolated in Thailand. Antimicrobial Agents And Chemotherapy, 44 (5) :1315–1321.

Deng Y, Bao X, Ji L, et al., (2015). Resistance integrons: class 1, 2 and 3 integrons. Ann Clin Microbiol Antimicrob, 14:45..

Drais AA, Ahmad A, Alwan, MG. et al., (2018). Antimicrobial resistance and Plasmid profile of *Vibrio alginolyticus* isolated from Malaysian seawater. International Journal of ChemTech Research, 11 (10) : 375-383.

Eissa AE (2016). Clinical and Laboratory Manual of Fish diseases. LAP LAMBERT Academic Publishing, Germany.

El-Gamal AM, El-Gohary MS, Gaafar AY (2018). Detection and Molecular Characterization of Some Bacteria Causing Skin Ulceration in Cultured Nile Tilapia (*Oreochromis niloticus*) in Kafr El-Sheikh Governorate. Int. J. Zool. Res. 14: 14-20.

Faja OM, Abd Sharad A, Younis KM, et al., (2019). Isolation, detection of virulence genes, antibiotic resistance genes, plasmid profile, and molecular typing among *Vibrio parahaemolyticus* isolated in Malaysian seawater from recreational beaches and fish. Veterinary World, 12 (7) : 1140-1149.

Flach CF, Johnning A, Nilsson I, et al., (2015). Isolation of novel IncA/C and IncN fluoroquinolone resistance plasmids from an antibiotic-polluted lake. J. Antimicrob. Chemother. 70, 2709–2717.

Gobarah DEA, Helmy S, Mahfouz N, Fahmy H, Abou Zeid M, and 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.

Gonzalez-Plazaa JJ, Blaub K, Milakovića M, et al., (2019). Antibiotic-manufacturing sites are hot-spots for the release and spread of antibiotic resistance genes and mobile genetic elements in receiving aquatic environments. Environment International 130 (2019) 104735.

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 Advances in Animal and Veterinary Sciences 7 (11) : 950-961.

*Hamouda, A. H. and Moustafa, E. M. (2020) : I*nsight on Prevailing Bacterial Diseases Affecting Grass carp (*Ctenopharyngodon idella*) at Aswan Fish Hatchery, Egypt. Alexandria Journal of Veterinary Sciences. 64 (1) :5-16.

Hardi E H, Nugroho RA, Saptiani G, Sarinah R, Agriandini M, Mawardi M (2018). Identification of potentially pathogenic bacteria from tilapia (*Oreochromis niloticus*) and channel catfish (*Clarias batrachus*) culture in Samarinda, East Kalimantan, Indonesia. Biodiversitas. 19 (2) : 480-488.

Ibrahim I, Abdullah M, Abdelrahman H, Abdelsalam A (2013). Necrotizing *Klebsiella pneumonia*. Chest 144, 216A-216A.

Jayasinghe C, Ahmed S, Kariyawasam M, (2010). The Isolation and Identification of *Vibrio* Species in Marine Shrimps of Sri Lanka. Journal of Food and Agriculture, 1, 36-44..

Jiang Y, Yao L, Li F, et al., (2014). Characterization of antimicrobial resistance of *Vibrio parahaemolyticus* from cultured sea cucumbers *(Apostichopus japonicas).* Letters in Applied Microbiology 59: 147-154.

Jun, L. and Woo, N. Y. S. (2003) : Pathogenicity of vibriosis in fish: An overview. Journal of Ocean University of China, 2 (2) :117-128.

Kitiyodom S, Khemtong S, Wongtavatchai J, et al., (2010). Characterization ofantibiotic resistance in *Vibrio* spp. isolated from farmed marine shrimps *(Penaeus monodon).* FEMS Microbiol Ecol. 72 (2) : 219–227.

Kreig N, Holt J (1984). Bergey's Manual of systemic bacteriology Vol.1.William and Wilkins, Baltimore, M.D.21202, USA.

Li L and Zhao X (2018). Characterization of the resistance class 1 integrons in *Staphylococcus aureus* isolates from milk of lactating dairy cattle in Northwestern China. BMC Veterinary Research, 14:59.

Li R, Lin D, Chen K, et al., (2015). First Detection of AmpC β-Lactamase bla_{CMY-2} on a Conjugative IncA/C Plasmid in a *Vibrio parahaemolyticus* Isolate of Food Origin. Antimicrob Agents Chemother. 59 (7) : 4106–4111.

Ljubojevic Pelic D (2018). Accession of carcass quality of common carp (*Cyprinus carpio* L.). *Journal of Agronomy, Technology and Engineering Management,* 1 (1) : 119-123.

Llor C. and Bjerrum L (2014). Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. Ther

Adv Drug Saf. 5 (6) :229-41.

MacFaddin JF (2000). Biochemical tests for identification medical bacteria. Warery Press Inc, Baltimore, Md. 21202 USA.

Madigan M. and Martinko J. (2005). Brock Biology of Microorganisms, 11thed. Prentice Hall.

Mala W, Kaewkes W, Tattawasart U, et al., (2016). Sxt Element, Class 1 Integron And Multidrug-Resistance Genes Of *Vibrio Cholerae* Isolated From Clinical And Environmental Sources In Northeast Thailand. J Trop Med Public Health, 47 (5) :957-966.

McVicar AH. (1982). Ichthyophonus infections of fish. In: Roberts RJ (ed) Microbial diseases of fish. Academic Press. London,: 243–69.

Moriarty DJW, (1997). The role of microorganisms in aquaculture ponds. *Aquaculture.* 151, 333-349.

Nagpal M.L, Fox KF, Fox A (1998). Utility of 16S–23S rRNA spacer region methodology: how similar are interspace regions within a genome and between strains for closely related organisms. J. Microbiol. Methods. 33, 211-219.

National Committee for Clinical Laboratory Standards "NCCLS" (2001). Performance standards for antimicrobial susceptibility testing. Supplement M100-S11. Villanova, PA, USA.

Noga EJ (2010). Fish Diseases, Diagnosis and Treatment. 2nd Edition, Iwa State University, Press, Ames.

Pérez-Pérez FJ and Hanson ND, (2002). Detection of Plasmid-Mediated AmpC ß-Lactamase Genes in Clinical Isolates by Using Multiplex PCR. Journal Of Clinical Microbiology. 2153–2162.

Rippey SR, (1994). Infectious diseases associated with molluscan shellfish consumption. *Clin. Microbiol.Rev.* 7, 419-425.

Rojas MVR, Matté, MH, Dropa, M, et al., (2011). Characterization Of *Vibrio Parahaemolyticus* Isolated From Oysters And Mussels In São Paulo, Brazil. Rev. Inst. Med. Trop. Sao Paulo, 53 (4) :201-205.

Rosser SJ and Young HK (1999). Identification and characterization of class 1 integrons in bacteria from an aquatic environment. J Antimicrob Chemoth 44: 11–18.

Silvester R, Pires J, Van Boeckel TP, et al., (2019). Occurrence of *β-Lactam* Resistance Genes and Plasmid-Mediated Resistance Among *Vibrios* Isolated from Southwest Coast of India. Microb. Drug Resist., 25 (9) :1306-1315.

Sørum H, (2006). Antimicrobial Drug Resistance in Fish Pathogens. Chapter 13. DOI: 10.1128/9781555817534.ch13.

Stratev D, Daskalov H, Vashin I (2015). Characterization and determination of antimicrobial resistance of haemolytic *Aeromonas* species isolated from common carp (*Cyprinus carpio*). Rev. Med. Vet. 166 (1-2) : 54-61.

*Sow, A.G.; Wane, A.; Diallo, M.H.; Boye, C.S. and Aïdara-Kane, A. (2007) :*Genotypic characterization of antibiotic-resistant *Salmonella enteritidis*isolates in Dakar, Senegal. J Infect Developing Countries, 1 (3) : 284-288.

Taviani E, Ceccarelli D, Lazaro N, et al., (2008). Environmental *Vibrio* spp., isolated contain a polymorphic group of integrative conjugative elements and class1integrons. FEMS Microbiol Ecol 64:45–54.

World Health Organization (WHO) (2006). Antimicrobial Use in Aquaculture and Antimicrobial Resistance-Report of a Joint FAO/OIE/ WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance, Seoul, Republic of Korea, 13-16 June 2006, WHO Document Production services, Geneva.