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Molecular Characterization and Antimicrobial Susceptibility of *Vibrios* Isolated from Healthy and Diseased Aquacultured Freshwater Fishes

Gaber S. Abdellrazeq and Samy A. Khaliel

Department of Microbiology, Faculty of Veterinary Medicine,
Alexandria University, Edfina, Rosetta-line, Egypt

Abstract: The objective of this study was to investigate the incidence, molecular features, as well as the antimicrobial susceptibilities of *Vibrios* isolated from cultured fish in Egypt. A total of three hundred samples of *Oreochromis niloticus*, *Mugil cephalus* and *Clarias gariepinus* (50 healthy and 50 diseased for each) were collected and analyzed by bacteriological, molecular and antimicrobial methods. Green and yellow bacterial colonies were recovered on thiosulfate citrate bile salt sucrose agar plates. The phenotypic characteristics of the isolates, including morphological, physiological and biochemical traits were determined and confirmed using API 20E system. Following isolation, the 16S rRNA gene specific for the genus *Vibrio* and Iron-cofactored superoxide dismutase (*sod*B) gene were investigated using PCR. Antimicrobial susceptibility to 13 antimicrobial agents was determined by the disc agar-diffusion method. Results revealed 51(17%), 25 (8.33%), 24 (8%) and 10 (3.33%) samples were positive for *V. mimicus*, *V. parahaemolyticus*, *V. fluvialis* and *V. splendidus* respectively. The virulence potentiality of the18 isolates of *V. mimicus* by PCR revealed *sod*B gene were found in 2, 3 and 1 of *V. mimicus* isolated from diseased *Oreochromis niloticus*, *Mugil cephalus* and *Clarias gariepinus* respectively. However, the 2 isolates from healthy *Mugil cephalus* were negative for *sod*B gene. *Vibrio* spp. were sensitive to chloramphenicol and ampicillin and resistant to penicillin, streptomycin and kanamycin. The study showed the presence of multiple *Vibrios* in cultured fish especially *V. mimicus* which constituted the highest prevalence rate recovered from *Oreochromis niloticus*, *Mugil cephalus* and *Clarias gariepinus*. Isolation of this type as well as other *Vibrio spp.* from aquaculture raises the public health concern and the importance to find suitable methods to control the infection transmission.

Key words: *Vibrios* · Fish · Molecular Features · Antimicrobial Susceptibility

INTRODUCTION

The *Vibrionaceae* (*Vibrios*) are a group of strains with the following characteristics: they are Gram-negative rods with a polar flagellum enclosed in a sheath, have facultative anaerobic metabolisms, are capable of fermenting D-glucose and grow at 20°C [1]. The bacteria are primarily aquatic and most species are oxidase positive, can reduce nitrate to nitrite, require Na^+ for growth and ferment D-fructose, maltose and glycerol [2]. In addition, most *Vibrio* spp. ferment a variety of carbohydrates without gas production and grow on thiosulfate citrate bile salts sucrose (TCBS) agar medium [3]. A total of 142 species have been recognized in this family (Association of *Vibrio* Biologists website; http://www.vibriobiology.net/). More than 60 species of *Vibrionaceae* have been described since 2007 [1]. These include a marine invertebrate isolates such as coral associated *Vibrios* [2], introduction of nitrogen-fixing *Vibrios* within an endophyte-like ecological niche [4] and an isolation of new *Vibrio* spp. from the surface of cheese [5] have been reported. In a study of more than 300 *vibrio* genome sequences, [6] concluded that the

Corresponding Author: Gaber Abdellrazeq, Department of Microbiology, Faculty of Veterinary Medicine, Alexandria University, Edfina, Rosetta-line, Egypt.
P.O. Box: 22758, Egypt. Fax: +20452960450.

Vibrio pan-genome comprises 17,000 gene families, differentially present and/or expressed in any given species.

Aquaculture in brackish and marine water is growing worldwide. New cultured species are introduced and types of aquaculture vary from outdoor to indoor and from flow through to recirculated water, at various temperatures. In these types of aquaculture various *Vibrio* spp. play an important role, as causative agents of fish diseases. The *Vibrios* are routinely found in aquatic environments, including estuaries, marine coastal waters and sediments and aquaculture settings worldwide. Moreover, a few *vibrio* spp. have extended their range beyond the marine environment, occurring predominantly in brackish and even freshwater environments [7]. The capacity of *vibrio* spp. to persist in the aquatic environment, their ecology and association with abiotic and biotic factors, as well as environmental surveillance for public health have been described [8-12]. Three species, *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*, are well-documented human pathogens [13-15]. *V. mimicus* [16] is a recognized pathogen with similar characteristics to *V. cholerae*, except an ability to ferment sucrose [17]. Other species within the genus, such as *V. alginolyticus* [18], *V. fluvialis* [19], *V. furnissii* [20], *V. metschnikovii* [21] and *V. hollisae* [22] are occasional human pathogens [23].

The conventional standard microbiological method is based on phenotypic identification, which requires several days to carry out the enrichment step, cultivation and biochemical tests [24]. Some *Vibrio* spp. can cause problems owing to variability in biochemical characteristics within species [7] and can become a "viable but non-culturable" (VBNC) organism resulting in unsuccessful isolation of some *Vibrio* spp. [25, 26]. A molecular biological method, such as polymerase chain reaction (PCR), is more rapid, sensitive and specific than standard culturing methods for detection of low microbial concentrations and detection of VBNC pathogens [25, 27].

Aquaculture in Egypt remains a growing, vibrant and important production sector for high-protein animal food that is easily digestible and of high biological value. However, a major setback in aquaculture is the sudden outbreak of diseases, especially those caused by *Vibrio* spp., which are considered significant economic and public health problems. To address this issue, this study was undertaken to isolate *Vibrios* from aquacultured freshwater fish and characterize their molecular features, as well as the antimicrobial susceptibilities.

MATERIALS AND METHODS

Fish Samples Collection: A total of 300 samples of *Oreochromis niloticus*, *Mugil cephalus* and *Clarias gariepinus* (*n*= 100 for each) were collected and cooled immediately without direct contact with ice (about 7°C to 10°C) and transported immediately to our laboratory, then processed as soon as possible for postmortem examination after disinfection of the skin with 70% ethyl alcohol.

Bacterial Enrichment and Isolation: Bacterial enrichment and isolation were carried out according to Elliot *et al.* [28]. Briefly, surface tissues, gills, gut and meat were pooled into a blender, blended at high speed for 90 sec and 50 g of homogenate was transferred into a new blender. 450 ml PBS dilution water was added (1:10 dilution) and blended for 1 min at 8,000 RPM. One ml portion of the 1:10 dilution inoculated into 10 ml of single-strength alkaline peptone water (APW) and incubated overnight at 35±2°C. A 3-mm loopful from the top 1 cm of APW tubes containing the sample showing growth streaked onto each TCBS plate. All TCBS plates incubated at 35±2°C overnight. Both green and yellow developed colonies from crowded plates streaked to a non-selective agar {Tryptic soy agar (TSA)-2% NaCl agar} for purity, incubated overnight at 35 ±2°C and proceeded with identification using a single isolated colony. For freezer storage of cultures, the isolates were maintained in tryptic soy broth (TSB) with 20% glycerol at -80°C.

Biochemical Identification and Presumptive Isolate Screening: Typical colonies were screened by picking a portion of each isolated suspected colony from the isolation agar and testing as presented in Table 1 [28, 29]. Two or more suspicious colonies were transferred from TCBS agar with a needle to arginine glucose slant (AGS). The slant was streaked, the butt was stabbed and incubated with the cap loose overnight at 35±2°C. Slant and butt colures, gas and H_2S in AGS were observed and recorded. Cell suspensions of the suspected cultures were prepared in 2% NaCl and used in the API 20E diagnostic strip [30] for further confirmation of isolates.

PCR identification of *Vibrionaceae*: Culture templates were prepared by growing of single fresh colonies overnight at 35±2°C in TSB-2% NaCl. One ml of each culture was centrifuged in a microcentrifuge tube for 3 min

Table 1: Biochemical characteristics of human pathogenic *Vibrionaceae* *

		V. alginolyticus	*V. cholerae*	*V. fluvialis*	*V. furnissii*	*V. hollisae*	*V. metschnikovii*	*V. mimicus*	*V. parahaemolyticus*	*V. vulnificus*	*A. hydrophilia* **	*P. shigelloides* **
TCBS agar		Y	Y	Y	Y	NG	Y	G	G	G	Y	G
mCPC agar		NG	P	NG	NG	NG	NG	NG	NG	Y	NG	NG
CC agar		NG	P	NG	NG	NG	NG	NG	NG	Y	NG	NG
AGS		KA	Ka	KK	KK	Ka	KK	KA	KA	KA	KK	nd
Oxidase		+	+	+	+	+	-	+	+	+	+	+
Arginine dihydrolase		-	-	+	+	-	+	-	-	-	+	+
Ornithine decarboxylase		+	+	-	-	-	-	+	+	+	-	+
Lysine decarboxylase		+	+	-	-	-	+	+	+	+	V	+
Growth	0% NaCl	-	+	-	-	-	-	+	-	-	+	+
in (w/v):	3% NaCl	+	+	+	+	+	+	+	+	+	+	+
	6% NaCl	+	-	+	+	+	+	-	+	+	+	-
	8% NaCl	+	-	V	+	-	V	-	+	-	-	-
	10% NaCl	+	-	-	-	-	-	-	-	-	-	-
Growth at 42°C		+	+	V	-	nd	V	+	+	+	V	+
Acid from:	Sucrose	+	+	+	+	-	+	-	-	-	V	-
	D-Cellobiose	-	-	+	-	-	-	-	V	+	+	-
	Lactose	-	-	-	-	-	-	-	-	+	V	-
	Arabinose	-	-	+	+	+	-	-	+	-	V	-
	D-Mannose	+	+	+	+	+	+	+	+	+	V	-
	D-Mannitol	+	+	+	+	-	+	+	+	V	+	-
	ONPG	-	+	+	+	-	+	+	-	+	+	-
	Voges-Proskauer	+	V	-	-	-	+	-	-	-	+	-
Sensitivity												
to:	10 µg O/129	R	S	R	R	nd	S	S	R	S	R	S
	150 µg O/129	S	S	S	S	nd	S	S	S	S	R	S
	Gelatinase	+	+	+	+	-	+	+	+	+	+	-
	Urease	-	-	-	-	-	-	-	V	-	-	-

* [28] ** *Aeromonas hydrophila, Plesiomonas shigelloides*

Abbreviations: TCBS, thiosulfate-citrate-bile salts-sucrose; mCPC, modified cellobiose-polymyxin B-colistin; CC agar, Cellobiose colistin agar; AGS, arginine-glucose slant; Y = yellow NG = no or poor growth S = susceptible nd = not done G = green V = variable among strains R = resistant P = purple, V = variable KK = Slant alkaline / Butt alkaline KA = Slant alkaline /Butt acidic, Ka = Slant alkaline/ Butt slightly acidic

Table 2: Primer pairs used for identification of *Vibrio* spp.

Target species	Primer	sequence 5'–3'	Amplicon size (bp)
All *vibrio* spp.	V.16S-700F	CGG TGA AAT GCG TAG AGA T	663 bp
	V.16S-1325R	TTA CTA GCG ATT CCG AGT TC	
V. mimicus	Vm.sodB-F	CAT TCG GTT CTT TCG CTG AT	121 bp
	Vm.sodB-R2	GAA GTG TTA GTG ATT GCT AGA GAT	

at 15,000 x g. The pellet was washed twice with physiological saline then resuspended in 1.0 ml dH_20 and boiled 10 min. Template was stored at -20°C until use [28]. The primer sets (Amershame Bioscience), their corresponding gene targets and size of expected amplification products are presented in Table 2 [31]. PCR was designed to target the 16S rRNA gene of *Vibrio* spp. and Iron-cofactored superoxide dismutase *(sod*B) gene of *V. mimicus*. PCR amplification for detection of each *Vibrio* spp. and *V. mimicus* was performed in 20 µl reaction mixture containing 4.0 µl of 5X PCR buffer, 2.4 µl 3 mM $MgCL_2$, 0.4 µl of 0.2 mM of deoxynucleoside triphosphate mix, 1.0 µl of 0.5 µM of each primer, 0.1 µl of 0.5 U/µl of taq polymerase and 2.0 µl of DNA template. Reaction mixture for 16S rRNA gene primer was heated at 96°C for 5 min in the initial denaturation step, followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 63°C for 30 s and primer extension at 72°C for 30 s. A final extension was performed at 72°C for 7 min. Thermal cycling profile for *sod*B gene primer was as follows: a 15-min soak at 93°C followed by 35 cycles of 92°C for40 s, 57°C for 1 min and 72°C for 1.5 min and a final soak at 72°C for 7 min. PCR amplicons were electrophoresed in 1.5% agarose for 1 h at 100 V and then visualized by ethidium bromide staining and UV illumination [31].

Antibiotic Susceptibility Test: The agar diffusion tests as qualitative methods to determine whether a bacterium is resistant, intermediately resistant or susceptible were carried out in accordance with the international recommendations given by the National Committee for Clinical Laboratory Standards [32]. Commercial Mueller-Hinton agar (Oxoid™) was prepared according to manufacturer's instructions. After autoclaving, the medium cooled to 50°C. 25 to 30 ml per

plate was measured and poured into 15 x 100 mm plates. Agar should be poured into flat on a level pouring surface to a uniform depth of 4 mm. Five isolates for each of *V. mimicus*, *V. parahaemolyticus* and *V. fluvialis* and two isolates for *V. splendidus* were tested for susceptibility to 13 antibiotics which include Ampicillin, Amoxicillin, Cephalothin, Chloramphenicol, Erythromycin, Gentamicin, Kanamycin, Nalidixic acid, Norfloxacin, Oxytetracycline, Penicillin, Streptomycin and Trimethoprim/ Sulphamethoxazole. Escherichia coli ATCC 25922 was used for quality control. Using the published CLSI guidelines, we determined the susceptibility or resistance of each tested isolate to each drug tested.

RESULTS

In this study, a total of 300 samples of *Oreochromis niloticus*, *Mugil cephalus* and *Clarias gariepinus* (*n*= 100 for each) were collected and subjected to a full bacteriological investigation for *Vibrios*. Samples were identified by conventional microbiological methods using various selective media and specific biochemical reactions. As shown in Table 3, results of *Oreochromis niloticus* revealed 12 and 4 of *V. fluvialis* and *V. splendidus* respectively were isolated from healthy fish. However, 18 and 10 of *V. mimicus* and *V. parahaemolyticus* respectively were isolated from

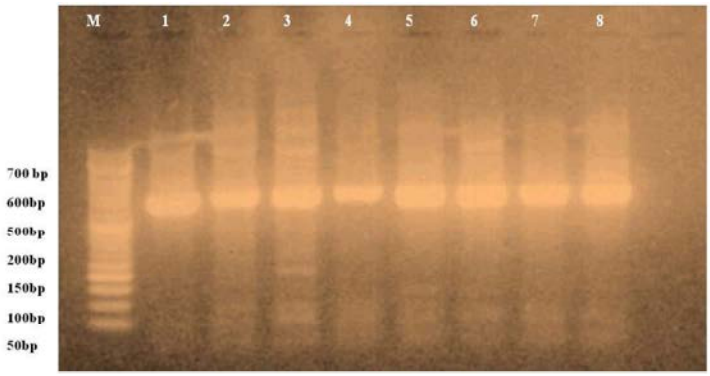
Table 3: Incidence of *Vibrio* spp. isolated from healthy and diseased fish

			Vibrio positive samples		No. of isolates for each species	
	Fish species	Samples No.	No.	%	No.	species
Oreochromis niloticus	Healthy	50	16	32	12	*V. fluvialis*
					4	*V. splendidus*
	Diseased	50	28	56	18	*V. mimicus*
					10	*V. parahaemolyticus*
Mugil cephalus	Healthy	50	18	36	9	*V. mimicus*
					5	*V. parahaemolyticus*
					4	*V. fluvialis*
	Diseased	50	26	52	15	*V. mimicus*
					7	*V. parahaemolyticus*
					4	*V. splendidus*
Clarias gariepinus	Healthy	50	6	12	4	*V. fluvialis*
					2	*V. splendidus*
	Diseased	50	16	32	9	*V. mimicus*
					3	*V. parahaemolyticus*
					4	*V. fluvialis*

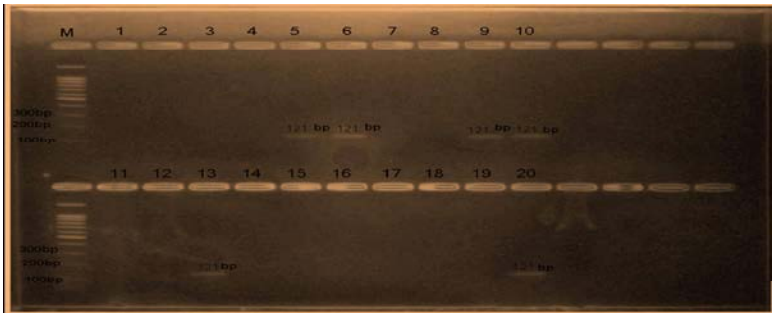
Table 4: Frequency of antimicrobial susceptibility of *Vibrio* spp. isolated from healthy and diseased fish

	V. mimicus (n = 5)						*V. parahaemolyticus* (n = 5)						*V. fluvialis* (n = 5)						*V. splendidus* (n = 3)					
	S		I		R		S		I		R		S		I		R		S		I		R	
vibrio spp.	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Ampicillin	5	100	-	-	-	-	5	100	-	-	-	-	5	100	-	-	-	-	-	-	3	100	-	-
Amoxicillin	2	40	3	60	-	-	-	-	-	-	5	100	-	-	2	40	3	60	2	66.66	-	-	1	33.33
Cephalothin	-	-	2	40	3	60	-	-	3	60	2	40	-	-	-	-	5	100	-	-	1	33.33	2	66.66
Chloramphenicol	5	100	-	-	-	-	5	100	-	-	-	-	5	100	-	-	-	-	3	100	-	-	-	-
Erythromycin	5	100	-	-	-	-	-	-	3	60	2	40	5	100	-	-	-	-	-	-	3	100	-	-
Gentamycin	-	-	5	100	-	-	5	100	-	-	-	-	2	40	3	60	-	-	-	-	3	100	-	-
Kanamycin	-	-	-	-	5	100	-	-	3	60	2	40	-	-	5	100	-	-	-	-	-	-	3	100
Nalidixic acid	2	40	3	60	-	-	-	-	3	60	2	40	1	20	-	-	4	80	3	100	-	-	-	-
Norfloxacin	5	100	-	-	-	-	-	-	-	-	5	100	-	-	-	-	5	100	-	-	3	100	-	-
Oxytetracycline	-	-	5	100	-	-	-	-	-	-	5	100	-	-	4	80	1	20	-	-	3	100	-	-
Penicillin	-	-	-	-	5	100	-	-	-	-	5	100	-	-	5	100	-	-	1	33.33	-	-	2	66.66
Streptomycin	-	-	-	-	5	100	-	-	5	100	-	-	5	100	-	-	-	-	3	100	-	-	-	-
Trimethoprim sulphamethoxazole	2	40	-	-	3	60	-	-	-	-	5	100	-	-	-	-	5	100	-	-	1	33.33	2	66.66

S: Sensitive; I: Intermediate sensitive; R: Resistant



Lane (M): DNA marker of 50 bp; Lane 1 to 8 are positive to *vibrio* spp. with a 663 bp.
Fig. 1: Representative polyacrylamide gel electrophoresis of *vibrio* spp.



Lane (M): DNA marker of 100 bp; Lane 1and 2 were *V. mimicus* from healthy *Mugil cephalus*. Lane 3- 8 were *V. mimicus* from diseased *Oreochromis niloticus*.
Lane 9- 14 were *V. mimicus* from diseased *Mugil cephalus*. Lane 15- 20 were *V. mimicus* from diseased *Clarias gariepinus*. Lane 5, 6, 9, 10, 13 and 20 are positive to *V. mimicus* with a 121 bp.
Fig. 2: Representative polyacrylamide gel electrophoresis of *V. mimicus*

diseased fish. Results of *Mugil cephalus* revealed 9, 5 and 4 of *V. mimicus, V. parahaemolyticus and V. fluvialis* respectively were isolated from healthy group. However, 15, 7 and 4 of *V. mimicus, V. parahaemolyticus and V. splendidus* respectively were isolated from diseased group (Table 3). Results of *Clarias gariepinus* revealed 4 and 2 of *V. fluvialis and V. splendidus* respectively were isolated from healthy fish. However, 9, 3 and 4 of *V. mimicus, V. parahaemolyticus and V. fluvialis* respectively were isolated from diseased group (Table 3).

The isolated *Vibrio* spp. produced unique and clear PCR bands of the 663bp, corresponding to the 16S rRNA gene as shown in Fig. 1. Results of the virulence potentiality of the18 isolates of *V. mimicus* by PCR revealed *sod*B gene were found in 2, 3 and 1 of *V. mimicus* isolated from diseased *Oreochromis niloticus*, *Mugil cephalus* and *Clarias gariepinus* respectively. However, the 2 isolates from healthy *Mugil cephalus* were negative for *sod*B gene (Fig. 2).

As presented in Table 4 and shown in Fig. 3, results of antimicrobial susceptibility of *Vibrio* spp. showed sensitivity to chloramphenicol (100%), ampicillin (83.33%). However, the isolates showed resistance to penicillin (70%) and variable resistance to the other antimicrobial agents.

DISCUSSION

The study of the environmental distribution and dynamics of *Vibrios* has a long history since many species contain potential human and animal pathogens [7]. Although association with animals can be an important part of the life cycle of many *Vibrio* spp., there are others that only loosely associate with animals or not at all. Hence there is considerable public health and economic interest in determining factors correlated to increased abundance of *Vibrios* [33]. *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *V. mimicus* are the most common species of *V. naceae* causing infection to humans. *V. cholera* O1/O139 is the causative agent of cholera which is endemic in less developed and developing countries [34, 35]. *V. parahaemolyticus* is

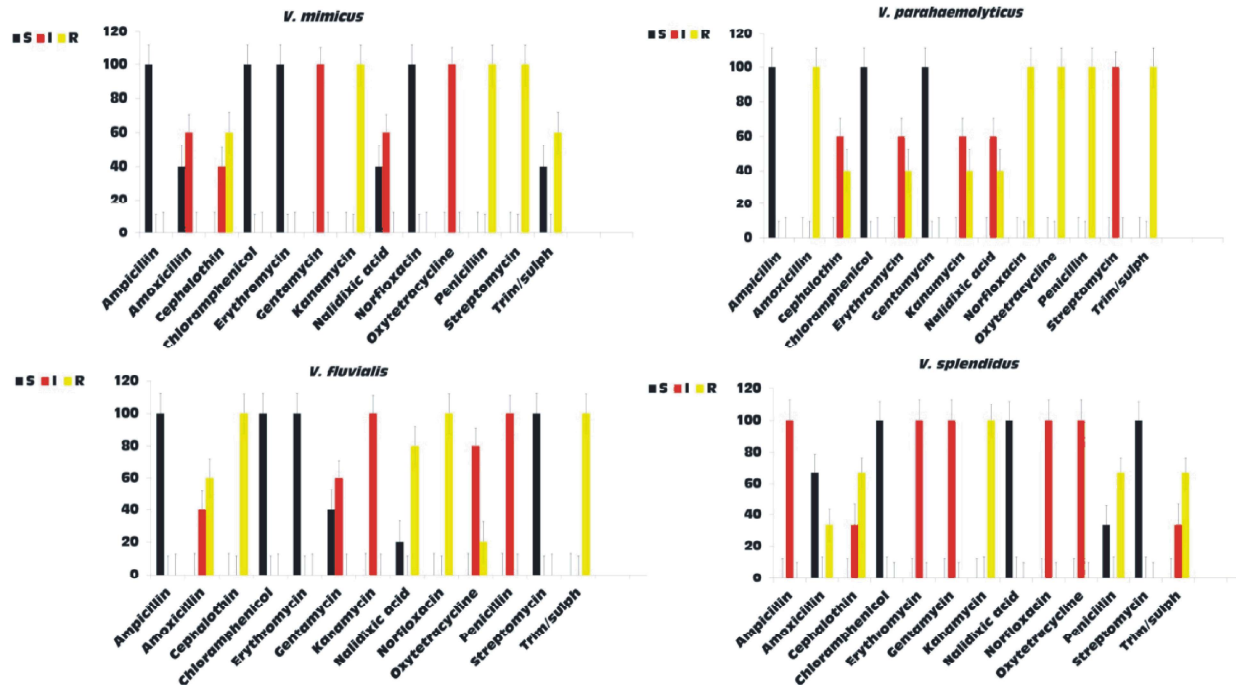


Fig. 3: Comparative analysis of rates of antimicrobial susceptibility of *Vibrio* spp. isolated from healthy and diseased fish samples. Error bars represent the standard error of the mean.

compatible with marine/brackish aquatic environment adjusting well to the broad range of salinities. It is commonly found on shellfishes and all varieties of finfish that are traditionally taken from marine and shore areas [36]. *V. fluvialis* is considered to be an emerging foodborne pathogen and has been implicated in outbreaks and sporadic cases of acute diarrhea [37]. Besides, *V. fluvialis* posed a significant economic threat for aquaculture for being pathogenic for cultured fish and lobsters [38]. According to this knowledge, the present study aimed to assess the occurrence of members of *Vibrios* in Egyptian aquaculture and investigate their susceptibility to antimicrobials. In the present study, we focused on three fish species to be examined (*Oreochromis niloticus*, *Mugil cephalus* and *Clarias gariepinus*).

In this study, a full bacteriological investigation of isolated *Vibrio* spp. was done by morphological, colonial, biochemical characters and API 20E system. Samples were identified by conventional microbiological methods using various selective media, e.g. TCBS, mCPC and CC [29, 28]. As shown in Table 3, in healthy *Oreochromis niloticus*, 32% (16/50) was positive for *Vibrio* spp. (12 and 4 for *V. fluvialis* and *V. splendidus* respectively). The intestine and gills were found to be the highest sites for the recovery of *Vibrio* species with an incidence of 8% of the total number of the examined fish. The obtained results were agreed with the results obtained by Hala [39] who said that the intestine was the prediction site for the recovery of *Vibrio* spp. from healthy *Oreochromis niloticus* fish. These results were nearly similar to results obtained by Anwar *et al*. [40] who discovered 9 isolates of *V. fluvialis* from *Oreochromis niloticus*. In diseased *Oreochromis niloticus,* 56% (28/50) were found to be positive for *Vibrio* spp. (18 and 10 for *V. mimicus* and *V. parahaemolyticus* respectively). These results were nearly similar to results obtained by Radwan [41] who recovered *Vibrio* spp. with an incidence of 25% of diseased *Oreochromis niloticus* and to results obtained by Hala [42] who isolated *Vibrio* spp. from *Oreochromis niloticus* with an incidence of 29%. On the other hand, these results were higher than the results obtained by Abd-El-Gaber *et al.* [43] who isolated *Vibrio* spp. from diseased *Oreochromis niloticus* with a percentage of 42%.

With regard to the incidence of *Vibrio* spp. among the healthy and diseased *Mugil cephalus*, it was found that 36% (18/50) healthy *Mugil cephalus* were positive

for *Vibrio* spp. (9, 5 and 4 for *V. mimicus, V. parahaemolyticus* and *V. fluvialis* respectively). However, in diseased *Mugil cephalus,* 52% (26/50) were positive for *Vibrio spp.* (15, 7 and 4 for *V. mimicus, V. parahaemolyticus* and *V. splendidus* respectively). These results were nearly agreed with the results obtained by Abd-El-Latif *et al*. [44] who isolated *Vibrio* spp. with a percentage of 33.75% from healthy *Mugil cephalus* fish. However, it was higher than the results obtained by Hala [42] who isolated *Vibrio* spp. from diseased *Mugil cephalus* with a percentage of 20%.

With regard to the incidence of *Vibrio* spp. among the healthy and diseased *Clarias gariepinus*, it was found that 12% (6/50) healthy *Clarias gariepinus* were positive for *Vibrio* spp. (4 and 2 for *V. fluvialis and V. splendidus* respectively). However, in diseased *Clarias gariepinus,* 32% (16/50) were positive for *Vibrio* spp. (9, 3 and 4 for *V. mimicus*, *V. parahaemolyticus* and *V. fluvialis* respectively). These results were agreed with the results obtained by Ogbulie and Okpokwassili [45] who reported that the bacteriological assay of diseased and healthy *Clarias gariepinus* revealed a higher colonization of the organs and tissues of the diseased than the healthy fish. As human infection with these isolated *Vibrio* spp. could be associated with gastroenteritis, a rapid and sensitive detection is essential both from food safety and from epidemiologic perspectives.

A simple and rapid identification method of *Vibrio*-related disease to aquaculture settings is essential for taking preventive and curative measures in aquaculture. PCR-based identification is a suitable alternative because it is comparatively easy, less expensive and can be completed within several hours [31, 46, 47, 48]. Regarding the molecular identification of *Vibrio* spp. by PCR, the isolated *Vibrio* spp. produced unique and clear PCR bands of the 663bp, corresponding to the 16S rRNA gene as shown in Fig. 1. After identification of *Vibrio* spp., the next major task of this study was to evaluate the virulence potentiality. We randomly selected 18 isolates of *V. mimicus* (6 from each isolates of diseased *Oreochromis niloticus*, *Mugil cephalus* and *Clarias gariepinus*); in addition to 2 isolates from healthy *Mugil cephalus.* These isolates were examined by PCR for presence of *sod*B gene. As shown in Fig. 2, *sod*B gene were found in 2, 3 and 1 of *V. mimicus* isolated from diseased *Oreochromis niloticus*, *Mugil cephalus* and *Clarias gariepinus* respectively. However, the 2 isolates from Healthy *Mugil cephalus* were negative for *sod*B gene. These results indicated that the PCR could confirm the biochemical identification of the isolated *V. mimicus* and these results concurrent with the results recorded by Clinical Laboratory Standards Institute [31] who diagnosed *V. mimicus* strains from shellfish based on the *sod*B gene. *V. mimicus* is one of the causative agents of gastroenteritis [49] associated with eating raw shellfish [50, 51], causing sporadic diarrhea in many countries including Japan [51] and Thailand [52]. Isolation of this type as well as other *Vibrio* spp. from Egyptian aquaculture raises the public health concern and the importance to find swuitable methods to control the infection transmission.

Methods used to control *Vibrio* bacteria in aquaculture production systems include antibiotics and medicated feeds. Chemicals and antibiotics are widely used to prevent or treat such infections. However, according to Nogueira-Lima *et al.* [53], evaluating the risks associated with the use of chemicals in aquaculture is difficult due to the lack of quantitative data from most countries involved in this activity. Increasing antibiotic resistance poses important risks to human health [54, 55] and can affect the course of infectious diseases [56]. The third and final task of this study was to investigate the antimicrobial susceptibility of our isolates. In the present study, antimicrobial resistance screening (Table 4 and Fig. 3) showed that *Vibrio* species showed sensitivity to chloramphenicol (100%) and ampicillin (83.33%). However, the isolates showed resistance to penicillin (70%) and variable resistance to the other antimicrobial agents. These obtained data were nearly similar to data obtained by Toranzo *et al.* [57] who pointed out that outbreaks of *Vibriosis* was controlled by using a feed additive of Oxytetracycline and chloramphenicol and also similar to results obtained by Zulkifli *et al*. [58] who reported that most isolates of *Vibrio* species were resistant to penicillin but most were susceptible to chloramphenicol. The results was similar to that recorded by Ruangpan and Kitao [59] who found the majority of the *Vibrio* spp. isolates were resistant to streptomycin and disagreed with Shyne Anand *et al.* [60] who discovered that more than 50% of the *V. parahaemolyticus* strains tested were sensitive to streptomycin, Nalidixic acid and amoxicillin and also disagreed with Nilima *et al*. [61] who found that all strains

of *Vibrio* spp. were mostly resistant to ampicillin and gentamicin and sensitive to streptomycin and Oxytetracycline.

In conclusion, the study showed the presence of multiple *Vibrios* in aquacultured freshwater fish especially *V. mimicus* which constituted the highest prevalence rate recovered from *Oreochromis niloticus*, *Mugil cephalus* and *Clarias gariepinus*. Isolation of this type as well as other *Vibrio* spp. from aquaculture raises the public health concern and the importance to find suitable methods to control the infection transmission. Good hygienic measurements and administration of an effective drug in fish farms may help minimize the risk of foodborne illnesses caused by *Vibrios.*

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