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

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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 singly or in chains, motile, oxidase positive, catalase positive, citrate positive, string test positive, indol positive, urease negative, H2S 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 *blaTEM*, *blaCTX*, *blaCMY* genes and class 1 integron. The gene *blaTEM* was detected in 100% of the isolates, while *blaCMY* and *blaCTX* 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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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 promoters of multi-drug resistance (Gonzalez-Plaza 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-trimethoprim (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 **blaTEM**, **blaCMY2**, **blaCTX** 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

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Sequence</th>
<th>Amplified product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>blaTEM</strong></td>
<td>5'-ATCAGCAATAAACCAGC 3'-CCCCGAAGAACGTTTTC</td>
<td>516 bp</td>
<td>Colom <em>et al.</em>, 2003</td>
</tr>
<tr>
<td><strong>blaCMY2</strong></td>
<td>5'-TGG CCA GAA CTG ACA GGC AAA 3'-TTT CTC CTG AAC GTG GCT GCC</td>
<td>462 bp</td>
<td>Pérez-Pérez and Hanson, 2002</td>
</tr>
<tr>
<td><strong>blaCTX</strong></td>
<td>5'-ATG TGC AGY ACC AGT AAR GTK ATG GC 3'-TGG GTR AAR TAR GTS ACC AGA AYC AGC GG</td>
<td>593 bp</td>
<td>Archambault <em>et al.</em>, 2006</td>
</tr>
<tr>
<td>class 1 integron cassettes</td>
<td>5'-CSGGC ATC CAA GCA GCA AG 3'-CSAAG CAG ACT TGA CCT GA</td>
<td>Variable</td>
<td>Sow <em>et al.</em>, 2007</td>
</tr>
</tbody>
</table>
each primer were conducted to ascertain optimal PCR conditions for MgCl₂ 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* (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. alginolyticus* (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, H₂S 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

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**Table 2. Cycling conditions of PCR**

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primary denaturation</th>
<th>Secondary denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>No. of cycles</th>
<th>Final extension</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt;</td>
<td>94˚C/ 5 min.</td>
<td>94˚C/ 30 sec</td>
<td>54˚C/ 40 sec</td>
<td>72˚C/ 45 sec</td>
<td>35</td>
<td>72˚C/ 10 min.</td>
<td>Colom et al. (2003)</td>
</tr>
<tr>
<td>bla&lt;sub&gt;CMY2&lt;/sub&gt;</td>
<td>94˚C/ 5 min.</td>
<td>94˚C/ 30 sec</td>
<td>55˚C/ 40 sec</td>
<td>72˚C/ 45 sec</td>
<td>35</td>
<td>72˚C/ 10 min.</td>
<td>Pérez-Pérez and Hanson, (2002)</td>
</tr>
<tr>
<td>bla&lt;sub&gt;CTX&lt;/sub&gt;</td>
<td>94˚C/ 5 min.</td>
<td>94˚C/ 30 sec</td>
<td>54˚C/ 40 sec</td>
<td>72˚C/ 45 sec</td>
<td>35</td>
<td>72˚C/ 10 min.</td>
<td>Archambault et al. (2006)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Recovered isolates</th>
<th>Oreochromis niloticus (75)</th>
<th>Mugil cephalus (50)</th>
<th>Clarias gariepinus (25)</th>
<th>Total (150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. alginolyticus</td>
<td>9</td>
<td>7</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>V. cholerae</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>V. parahaemolyticus</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>V. fluvialis</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>V. splendidus</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>V. anguillarum</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>18</td>
<td>16</td>
<td>52</td>
</tr>
</tbody>
</table>

The isolated *Vibrio* species were identified as *V. alginolyticus* (7), *V. cholerae* (6), *V. parahaemolyticus* (2), *V. fluvialis* (2), *V. splendidus* (1) and *V. anguillarum* (0)
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 blaTEM gene however they don’t hold blaCMY2 and blaCTX resistance genes (Figure 1, 2, 3). All the isolates were positive to class 1 integron. Class 1 integrons sequencing indicated that the 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 blaTEM 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)](image1)

![Figure 2. Agarose gel electrophoresis of PCR products after amplification of blaCTX gene at 593 bp and blaCMY2 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)](image2)
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. alginolyticus (7), V. cholerae (6), V. parahaemolyticus (2), V. fluvialis (2), V. splendidus (1) and V. anguillarum (0). However, in case Clar- ias gariepinus, 16 out of 25 fish could be isolated; with an incidence of 64%; and the isolated Vibriosp. were identified as V. alginolyticus (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 (Sørrum, 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 notewor-thiness, 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 (blaTEM gene had molecular weight 516 bp, blaCMY2 gene had molecular weight 462 bp and blaCTX gene had molecular weight 593 bp). It was observed that (100%) of the tested isolates had blaTEM gene, while blaCTX and blaCMY2 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 blaTEM gene; Silvester et al. (2019) investigated that all the Vibrios isolated from Cochin Estuary, seafood and shrimp farms harbored the blaTEM gene and detected the blaCTX gene in only 1.1% of the Vibrio sp. And Li et al., (2015) who found blaTEM gene in 1 of 2 strains of V. parahaemolyticus that screened for β-Lactamase genes and first detected blaCMY2 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 (blaCTX, blaTEM and blaSHV) 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 aadA2 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 blaTEM, blaCMY2, blaCTX genes and class 1 integron. The genes blaTEM was detected in 100% of the isolates, while and blaCTX 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.
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