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# Multidrug Resistance, V16S rRNA Gene Sequencing, High Frequency of Toxins Encoding Genes (tox, tdh, trh) in Parahaemolyticus and Non - parahaemolyticus Vibrios Isolated from Shellfish

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author AMZ designed the study, carried out isolation and characterization of bacteria from different sources, wrote the protocol, managed the analyses of the study and wrote the first draft of the manuscript. Author DMS participated with author AMZ in molecular typing and sequences analysis. Both authors read and approved the final manuscript.*

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## ABSTRACT

PCR amplification of V16S-rRNA, tox, tdh, trh was applied as a molecular typing approach to identify potentially toxigenic vibrio species. Thirty vibrio strains were isolated from several types of shellfish collected from Ismailia district, Egypt. Isolates were recognized by conventional biochemical features and PCR amplification then tested for their sensitivity against twelve antibiotics. Multiple sequences alignment of V16S-rRNA indicated the high incidence of *V.parahaemolyticus* (n= 6) *V. cholerae* (n=5) and *V. fluvialis* (n=5) among the investigated groups. *V. harveyi* (n=3), *V. alginolyticus* ( n=2 )*V. vulnificus* (n=1) and (n=8) *vibrio sp* were reported as well. Toxins encoding genes (tox, tdh, trh) shown high incidence frequency in the majority of the identified vibrio species. Identified isolates were reported as potential pathogenic and multiple

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antibiotic resistant with noticeably high resistant indexes ranged from (0.5-1). The results significantly emphasized that the isolates have been originated from potential risk sources of infection.

*Keywords: Vibrios; Virulence genes; multi drug resistance; Egypt.*

## 1. INTRODUCTION

Vibrio infections are considerably spread worldwide. Consideration was given to this genus due to the occurrence of various strains that are conceivably pathogenic to humans and other organisms [1]. Pathogenic vibrios incorporate in the incidence of several frequent syndromes of clinical problems: gastroenteritis ,wound infection, and even septicemia [2,3]. Various incidents of Vibrio-associated gastroenteritis are noticeably undetected since vibrios are not effectively examined in typical stool cultures. Epidemiologic data demonstrate that the majority of these infections are foodborne and frequently induced by consumption of raw or undercooked shellfish. Vibrio species are a natural portion of the bacterial flora in aquatic environments and initially reported as opportunistic pathogens [4]. *Vibrio cholerae* is a common human pathogen inducing cholerae epidemics globally. In addition to V. cholerae, various other Vibrio species were reported as significant human pathogens and implicated in food-borne illnesses. *Vibrio parahaemolyticus*, *V. vulnificus* and non-O1/non-O139 V. cholerae have been recovered from human cases experiencing non-cholerae Vibrio diseases (vibriosis), frequently linked to the ingestion of real or undercooked shellfish and seafood or direct contact of skin wounds to sea water [5,6]. However the majority of Vibrio species are nonpathogenic, they are assumed to generate a substantial reservoir of the common virulence and antibiotic resistance genes. The mobility of these genes and the adequate transfer may induce the revolution of a susceptible-nonpathogenic strain to resistant-pathogenic one [7,8,9]. Sequences information obtained by many authors signify the frequent incidence of pathogenicity markers such as tox, tdh,trh, in Vibrios species rather than V. Parahaemolyticus [10,11].

Gastroenteritis induced by *Vibrio parahaemolyticus* has been experienced globally, however only sporadic instances [7]. The bacterium is naturally found in seafood, but pathogenic isolates can certainly induce gastroenteritis in humans are uncommon in environmental samples (2 to 3%) [7] and may often not identified [9]. The virulence of V. parahaemolyticus depends on the occurrence of a thermostable direct hemolysin (tdh) and/or the thermostable direct hemolysin-related gene (trh) [6]. These two are involved in gastrointestinal infections [9]. In this study, we investigated the prevalence of multidrug vibrios comprising toxins encoding genes (tox, trh, tdh) obtained from several different shellfish and seawater at Ismailia coastal district, Egypt where seafood is the main food of most inhabitants.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

A total of two hundred eighty four (n=284) samples of shellfishes, shrimps (n=100), Carpet shell clams (n=100), Sea snail Murex sp (n=40) ,Sea snail *Murex forskoehlii* (n=4) and seawater (n=4) were received by random sampling from the regional suppliers in Ismailia City, Egypt in the period extending from April to September 2015 to survey the incidence and distribution of Vibrio species. Virtually all specimens were delivered to the laboratories of Biotechnology Research Institute at Suez Canal University, refrigerated at 4°C or placed on an ice pack and processed within a short while after arrival.

### 2.2 Enrichment and Isolation

Five grams of individual shellfish flesh and muscles was incised with the help of a sterile scalpel after removing the carapace. Each of these 5g flesh samples was homogenized in 45 ml of 3% NaCl containing 1% alkaline peptone water (APW, pH: 8.6) by a sterile blender. The shellfish homogenates were incubated at 37°C for 18 hours [12,13]. As described in [14] water samples were enriched by introducing 100 ml of each sample aseptically to a similar volume of 1% alkaline peptone water possessing 3% NaCl. Consequently were incubated at 37°C for 18h. Following incubation, the shellfish homogenate and enriched samples of water were introduced to thiosulphate citrate bile salts sucrose agar medium (TCBS Oxoid) using an inoculating loop and kept at 37°C for 18h as mentioned in [15, 16]. Both yellow and green color colonies with blue centers were considered for further confirmation by biochemical tests including Gram staining, oxidase, and catalase tests, culturing on SIM and TSI media as described by [17].

### 2.3 Antibiotic Susceptibility Test

Thirty (n=30) suspected Vibrio isolates were examined for their susceptibility to twelve antimicrobial agents ampicillin, amoxicillin, cephalothin, chloramphenicol, erythromycin, gentamicin, kanamycin, nalidixic acid, norfloxacin, oxytetracycline, streptomycin, and sulphamethoxazole (Oxoid) (Table 3). The evaluation was carried out on Mueller–Hinton Agar (Oxoid) and the modified Kirby–Bauer disk diffusion method was applied [18]. The antibiogram of every Vibrio colony was determined according to the breakpoints of the inhibition zone diameters for each antibiotic agents and as demonstrated by the disk manufacturer. The test results were interpreted as reported by the guidelines of the National Committee for Clinical Laboratory Standards [19] for antimicrobial susceptibility assessment. Resistance to greater than four antibiotics was considered as multidrug resistant MDR.

Multidrug resistance index (MDRI) of the individual isolate was calculated by division of the number of antibiotics to which the isolate was resistant to the whole number of antibiotics to which the isolate was exposed:

$$\text{MDRI (\%)} = \frac{\text{number of antibiotics resisted}}{\text{total number of antibiotics used}} \times 100$$

### 2.4 Genomic DNA Isolation

Thirty suspected Vibrio isolates were randomly selected for DNA isolation. Spin column based kit was used for genomic DNA purification from bacteria (Jena Bioscience, Germany). DNA quality was evaluated using 1% Agarose gel electrophoresis (1X TAE). While DNA concentration and purity were measured with a NanoDrop ND-1000 spectrophotometer at 260 and 280 nm.

### 2.5 PCR Amplification and DNA Sequencing Analysis

PCR reactions Table 1. were amplified in a 25 µl total volume, using 12.5 µl OnePCR master mix (GeneDireX,USA), 40ng DNA template, 20 pmole of each primer pair and MilliQ water. PCRs were performed in a lab cycle (SensoQuest, Germany). PCR products were separated on 1.5% agarose gel (1X TAE), visualized and photographed under UV light with G:BOX gel documentation system (SYNGENE, England). Amplicon size was determined using GeneSys image capture software in presence of 1000bp DNA size ladder (Solisdyne).

Semi-nested PCR has been applied on PCR products comprising non-specific bands. 40µg from the first round amplicon were taken as a template for the second PCR cycle and undergone further amplification under typical cycles conditions [20]. Nucleotide sequences were determined at Macrogen sequencing facility (Macrogen Inc., Seoul, Korea). Purification of PCR products was performed using plate MSNU030 (Millipore SAS, Molsheim, France). Then, PCR products were subjected to Sanger-sequencing with the Big-Dye terminator V3.1 sequencing kit using ABI PRISM 3730xl automated sequencer (Applied Biosystems), USA. Mega 5.0 software has been used for creating neighbor-joining phylogenetic tree to examine genetic relatedness among identified strains.

## 3. RESULTS

### 3.1 Identification of Vibrios

Thirty isolates were recovered from TCBS medium and successfully suspected as Vibrio sp through biochemical testing which then identified by PCR Fig. (1-A) and multiple sequences alignments analysis for V16S-rRNA gene. Potential pathogenic Vibrios have been characterized as (20% n=6) *Vibrio parahaemolyticus* (16.6% n=5) *Vibrio cholerae* (16.6% n=5) *Vibrio fluvialis* (10% n=3) *Vibrio harveyi* in addition to (6.6% n=2) *Vibrio alginolyticus* (3.3% n=1) *Vibrio vulnificus* and (26.6% n=8) not identified vibrio species. Toxigenic characters markers were recorded in noticabley high frequencies, tox genes (Fig. 1-B) were recorded in all isolates, tdh and trh (not shown) were found in (90% n=27) from the identified isolates.

### 3.2 Antibiotic Resistance Pattern

The antibiogram pattern of the 30 Vibrio isolates shown in Table 2. indicates that antibiotic resistance is common among the isolated vibrios. 99% of strains had a significantly high multi drug resistant index MDRI, demonstrating resistance to all tested antibiotics belonging to different classes. Among the isolated species, V. cholerae and V. parahaemolyticus members have comprised considerably high (MDRI) to approximately 100% of the tested antibiotics. In the same context the results demonstrated that (n=27 /90%) of identified vibrios were resistant to Amoxicillin followed by (n=25/83%) resistant to Erythromycin then (n=24/80%) resistant to Ampicillin, Gentamycin, Cephalothin and Streptomycin, (n=23/76.6%) resistant to ChloramphenicolNorfloxacin Oxytetracycline (n=22/73%) resistant to Kanamycin, Sulfamethoxazole while less resistant was to Sulfamethoxazole resistance (n=18/60%).

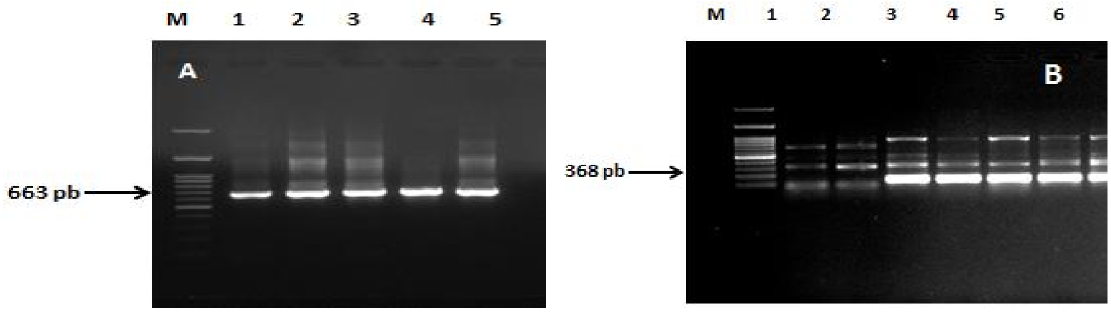


**Fig. 1 (A-B). Characterization of Vibrio strains by PCR, Lane 1(M): DNA ladder (1000 bp) marker Fig. 1A. Positive pattern to V16sRNA gene (663 pb), Fig. 1B. Positive pattern to tox R gene (386 pb)**

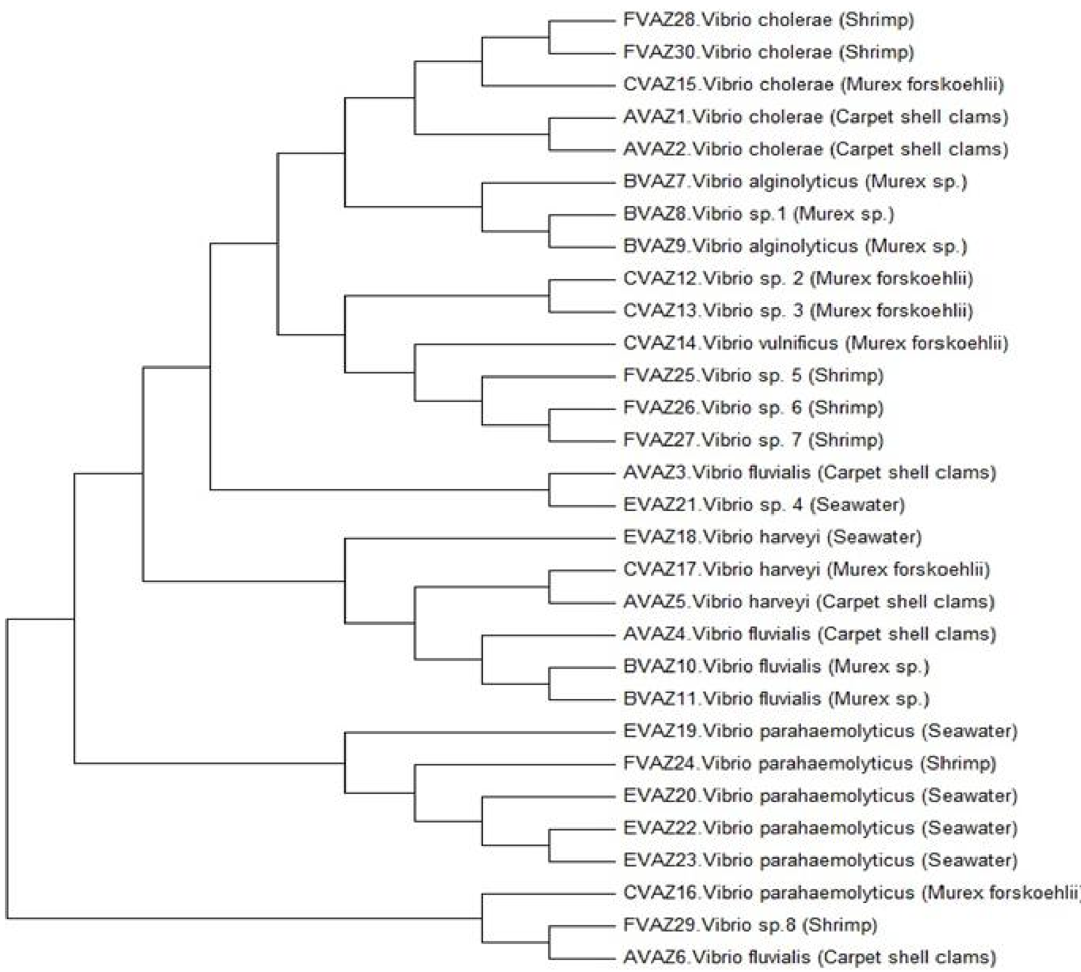


**Fig. 2. Mega 5.0 Neighbor-joining phylogenetic tree of *V16rRNA* gene sequences from *Vibrio* strains isolated from different sources in Ismailia district, Egypt**

**Table 1. Primer sequences of examined markers and predicted length of PCR amplified products**

| Target genes | Oligo sequences (5'-3') | Product length (bp) | Cycle conditions |
|---|---|---|---|
| V.16S-rRNA_F | 5'-CGG TGA AAT GCG TAG AGA T-3' | | 94°C 5 min, 95 °C 1 min, 52 °C 1 min, |
| V.16S-rRNA_R | 5'-TTA CTA GCG ATT CCG AGT TC-3' | 663 | 72 °C 1 min for 35 cycles, 72 °C 7 min |
| toxR_F | 5'-GTC TTC TGA CGC AAT CGT TG-3' | | 94°C 5 min, 95 °C 1 min, 56 °C 1 min, |
| toxR_R | 5'-ATA CGA GTG GTT GCT GTC ATG-3' | 368 | 72 °C 1 min for 35 cycles, 72 °C 7 min |
| tdh F | 5'CGT TGA TTA TTC TTT TAC GA3' | | 94°C 5 min, 95 °C 1 min, 44 °C 1 min, |
| tdh R | 5'TTT GTT GGA TAT ACA CAT3' | 623 | 72 °C 1 min for 35 cycles, 72 °C 7 min |
| trh F | 5'CTC TAC TTT GCT TTC AGT3' | | 94°C 5 min, 95 °C 1 min, 44 °C 1 min, |
| trh R | 5'AAT ATT CTG GAG TTT CAT3' | 460 | 72 °C 1 min for 35 cycles, 72 °C 7 min |

**Table 2. Antibiogram of identified *Vibrio* species regarding origin and MDRI**

| Number | Isolate | Origin | Resistance | Susceptibility | MDRI |
|---|---|---|---|---|---|
| AVAZ1 | *Vibrio cholerae* | Carpet shell clams | Amp, Aml, Kf, C, E, Cn, K, Na, Nor, Ot, S. Sxt. | 0 | 1 |
| AVAZ2 | *Vibrio cholerae* | Carpet shell clams | Amp, Aml, Kf, C, E, Cn, K, Na, Nor, Ot, S. Sxt. | 0 | 1 |
| AVAZ3 | *Vibrio fluvialis* | Carpet shell clams | Amp, Aml, Kf, E, Cn, K, Na,Nor, Ot, S. | C,Nor,Sxt | 0.75 |
| AVAZ4 | *Vibrio fluvialis* | Carpet shell clams | S | Cn | 0.08 |
| AVAZ5 | *Vibrio harveyi* | Carpet shell clams | Amp, Aml, Kf, C, E, Cn, K, Na, Nor, Ot, S. Sxt. | 0 | 1 |
| AVAZ6 | *Vibrio fluvialis* | Carpet shell clams | Amp, Aml, Kf, C, E, Cn, K, Na, Nor, Ot, S, Sxt. | 0 | 1 |
| BVAZ7 | *Vibrio alginolyticus* | *Murex* sp | Amp, Aml, Cn, Na, Nor, S. Sxt. | 0 | 0.63 |
| BVAZ8 | *Vibrio* sp1 | *Murex* sp | Amp, Aml, Kf, C, E, Cn, K, Na, Nor, Ot | 0 | 0.83 |
| BVAZ9 | *Vibrio alginolyticus* | *Murex* sp | Amp, Aml, Kf, C, E, Cn, Nor, Ot, S. | 0 | 0.75 |
| BVAZ10 | *Vibrio fluvialis* | *Murex* sp | Amp, Kf, E, Cn, Nor, Ot, S, Sxt. | 0 | 0.66 |
| BVAZ11 | *Vibrio fluvialis* | *Murex* sp | Amp, Aml, Kf, C, E,Na, Nor, Ot, S, Sxt. | 0 | 0.75 |
| CVAZ12 | *Vibrio* sp. 2 | *Murex forskoehlii* | Amp, Aml, Kf, C, E, Cn, K, Na, Ot. | 0 | 0.75 |
| CVAZ13 | *Vibrio* sp. 3 | *Murex forskoehlii* | Cn, K, Na,Nor, Ot, S. Sxt. | C | 0.58 |
| CVAZ14 | *Vibrio vulnificus* | *Murex forskoehlii* | Amp, Aml, Kf, C, E, Cn, K, Nor. | 0 | 0.58 |
| CVAZ15 | *Vibrio cholerae* | *Murex forskoehlii* | Amp, Aml, Kf, C, E, Cn, K, Ot, Sxt. | Na,Nor | 0.75 |
| CVAZ16 | *Vibrio parahaemolyticus* | *Murex forskoehlii* | Amp, Aml, Kf, C, E, Cn, K, Nor, Ot, S. Sxt. | 0 | 0.91 |
| CVAZ17 | *Vibrio harveyi* | *Murex forskoehlii* | Amp, Aml, Kf, C, E, Cn, K, Na, Nor, Ot, S. Sxt. | 0 | 1 |
| EVAZ18 | *Vibrio harveyi* | Seawater | Amp, Aml, Kf, C, E, Cn, K, Na, Nor, Ot, S. Sxt. | 0 | 1 |
| EVAZ19 | *Vibrio parahaemolyticus* | Seawater | Amp, Aml, Kf, C, E, K, Na, Nor, Ot, S. Sxt. | 0 | 0.91 |
| EVAZ20 | *Vibrio parahaemolyticus* | Seawater | Amp, Aml, Kf, C, E, Cn, K, Ot, S. Sxt. | 0 | 0.83 |
| EVAZ21 | *Vibrio* sp. 4 | Seawater | Amp, Aml, Kf, C, Na, Nor, Ot, S, Sxt. | 0 | 0.75 |
| EVAZ22 | *Vibrio parahaemolyticus* | Seawater | Amp, Aml, Kf, C, Nor, Ot, S. Sxt. | 0 | 0.66 |

| Number | Isolate | Origin | Resistance | Susceptibility | MDRI |
|---|---|---|---|---|---|
| EVAZ23 | *Vibrio parahaemolyticus* | Seawater | Amp, Aml, Kf, C, E, Cn, K, Na, Nor, Ot, S. Sxt. | 0 | 1 |
| FVAZ24 | *Vibrio parahaemolyticus* | Shrimp | Amp, Aml, Kf, C, E, Cn, K, Na, Nor, Ot, S. Sxt. | 0 | 1 |
| FVAZ25 | *Vibrio* sp.5 | Shrimp | Aml, Kf, C, E, Cn, K. | 0 | 0.5 |
| FVAZ26 | *Vibrio* sp.6 | Shrimp | Aml,C, E, Cn, K, Na, Ot, S. Sxt. | 0 | 0.75 |
| FVAZ27 | *Vibrio* sp. 7 | Shrimp | Amp, Aml, Kf, C, E, Cn, K, Na, Nor, S. Sxt. | Ot | 0.91 |
| FVAZ28 | Vibrio choleraee | Shrimp | Amp, Aml, Kf, E, K, Nor, S. | Sxt | 0.58 |
| FVAZ29 | *Vibrio* sp.8 | Shrimp | Amp, Aml, Kf, E, Cn, K, Nor, Sxt. | 0 | 0.66 |
| FVAZ30 | Vibrio choleraee | Shrimp | Amp, Aml, C, E, Cn, K, Na, Nor, Ot, S, Sxt. | 0 | 0.91 |

**Table 3. Prevalence of virulence factors coding genes in Vibrio strains**

| Number | Isolate | Origin | V16S RNA gene | tox gene | tdh gene | trh gene |
|---|---|---|---|---|---|---|
| AVAZ1 | *Vibrio cholerae* | Carpet shell clams | + | + | - | + |
| AVAZ2 | *Vibrio cholerae* | Carpet shell clams | + | + | - | - |
| AVAZ3 | *Vibrio fluvialis* | Carpet shell clams | + | + | + | - |
| AVAZ4 | *Vibrio fluvialis* | Carpet shell clams | + | + | - | + |
| AVAZ5 | *Vibrio harveyi* | Carpet shell clams | + | + | + | + |
| AVAZ6 | *Vibrio fluvialis* | Carpet shell clams | + | + | + | + |
| BVAZ7 | *Vibrio alginolyticus* | *Murex* sp | + | + | + | + |
| BVAZ8 | *Vibrio* sp1 | *Murex* sp | + | + | + | + |
| BVAZ9 | *Vibrio alginolyticus* | *Murex* sp | + | + | + | + |
| BVAZ10 | *Vibrio fluvialis* | *Murex* sp | + | + | + | + |
| BVAZ11 | *Vibrio fluvialis* | *Murex* sp | + | + | + | + |
| CVAZ12 | *Vibrio* sp. 2 | *Murex forskoehlii* | + | + | + | + |
| CVAZ13 | *Vibrio* sp. 3 | *Murex forskoehlii* | + | + | + | + |
| CVAZ14 | *Vibrio vulnificus* | *Murex forskoehlii* | + | + | + | + |
| CVAZ15 | *Vibrio cholerae* | *Murex forskoehlii* | + | + | + | - |
| CVAZ16 | *Vibrio parahaemolyticus* | *Murex forskoehlii* | + | + | + | + |
| CVAZ17 | *Vibrio harveyi* | *Murex forskoehlii* | + | + | + | + |
| EVAZ18 | *Vibrio harveyi* | Seawater | + | + | + | + |
| EVAZ19 | *Vibrio parahaemolyticus* | Seawater | + | + | + | + |
| EVAZ20 | *Vibrio parahaemolyticus* | Seawater | + | + | + | + |
| EVAZ21 | *Vibrio* sp. 4 | Seawater | + | + | + | + |
| EVAZ22 | *Vibrio parahaemolyticus* | Seawater | + | + | + | + |
| EVAZ23 | *Vibrio parahaemolyticus* | Seawater | + | + | + | + |
| FVAZ24 | *Vibrio parahaemolyticus* | Shrimp | + | + | + | + |

| Number | Isolate | Origin | V16S RNA gene | tox gene | tdh gene | trh gene |
|---|---|---|---|---|---|---|
| FVAZ25 | *Vibrio* sp.5 | Shrimp | + | + | + | + |
| FVAZ26 | *Vibrio* sp.6 | Shrimp | + | + | + | + |
| FVAZ27 | *Vibrio* sp. 7 | Shrimp | + | + | + | + |
| FVAZ28 | Vibrio choleraee | Shrimp | + | + | + | + |
| FVAZ29 | *Vibrio* sp.8 | Shrimp | + | + | + | + |
| FVAZ30 | Vibrio choleraee | Shrimp | + | + | + | + |

**Table 4. Antibiotic resistance pattern of *Vibrio species* (n = 30) isolates from different sources**

| Antibiotics agents | Percentage of resistance within *Vibrios* species | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***V. choleraee*** **( 16.6% n=5)** | ***V. parahaemolyticus*** **( 20% n=6)** | ***V. alginolyticus*** **( 6.6% n=2)** | ***V. vulnificus*** **(3.3% n=1)** | ***V. fluvialis*** **(n=5)** | ***V. harveyi*** **(10% n=3)** | ***V sp.*** **(26.6% n=8)** | **Resistant N/%** |
| Ampicillin (AMP10 µg) | 5 | 6 | 2 | 1 | 4 | 3 | 3 | 24 (80%) |
| Amoxicillin (AML10 µg | 5 | 6 | 2 | 1 | 4 | 3 | 6 | 27( 90%) |
| Kanamycin (K30 µg) | 5 | 5 | 0 | 1 | 2 | 3 | 6 | 22 (73%) |
| Chloramphenicol (C30 µg) | 5 | 6 | 1 | 1 | 2 | 3 | 5 | 23 (76.6%) |
| Erythromycin (E15 µg) | 5 | 5 | 1 | 1 | 4 | 3 | 6 | 25(83.3%) |
| Gentamycin (CN10 µg) | 5 | 4 | 2 | 1 | 3 | 3 | 6 | 24(80%) |
| Cephalothin (Kf 30) | 4 | 6 | 1 | 1 | 4 | 3 | 5 | 24(80%) |
| Nalidixic acid (NA30 µg) | 4 | 3 | 1 | 0 | 3 | 3 | 4 | 18 (60%) |
| Norfloxin (Nor10µg) | 5 | 5 | 2 | 1 | 4 | 3 | 3 | 23 (76.6%) |
| Oxytetracycline (OT30 µg) | 5 | 6 | 1 | 0 | 4 | 3 | 4 | 23 (76.6%) |
| Streptomycin (S10 µg) | 5 | 6 | 2 | 0 | 5 | 3 | 3 | 24(80%) |
| Sulphamethoxazole(Sxt 25 µg) | 5 | 6 | 1 | 0 | 3 | 3 | 4 | 22 (73%) |

## 4. DISCUSSION

Vibrio species are the primary agents of bacteria-associated illness and mortality from seafood consumption globally [21,22]. Vibrio infections have been the leading cause of seafood-borne bacterial illness since 2001, with incidence increasing over the past decade [22]. The conducted PCR assay indicated that a total of 100% (30/30) vibrio isolates were positive for tox gene and 90% (27/30) were positive for toxins tdh and trh genes which trigger the fact of the high incidence of pathogenic markers in a wide variety of vibrios originated from different sources. This study manifested the high incidence of Vibrio species of potential medical significance such as *Vibrio parahaemolyticus* (n= 6) and *Vibrio cholerae* (n=5) *Vibrio fluvialis* (n=5) *Vibrio harveyi* (n=3), *Vibrio alginolyticus* (n=2), *Vibrio vulnificus* (n=1) as a seafood linked pathogens which is evolved as a public health concern. Although *Vibrio parahaemolyticus* was well known to carry specific toxigenic markers such as tox, tdh and trh our findings indicated the acquisition of these genes by other vibrio species which support the possibility of gene transfer from toxigenic strains to nontoxigenic ones originated from the same source. Sequences analysis and neighbor join alignment indicated that isolates originated from the same source show more genetic relatedness. Prevention of Vibrio infections needs raising awareness of these infections by clinicians, laboratory professions, and epidemiologists.

Although antibiotic resistance was primarily thought to have developed in the clinical settings, considerably more attention is now being directed towards understanding the ecological and environmental factors associated with level of resistance genes acquisition among Vibrios [23,24]. While some studies have attempted to emphasize the dissemination of level of resistance genes between environmental and pathogenic isolates [25], the complexity of the processes and the relative information shortage on this subject indicate a dearth of enough knowledge in this field.

## 5. CONCLUSIONS

Antibiotic resistance is progressively identified as a serious threat to global health, with few recent antimicrobial alternatives in innovation. Sensitivity assays against 12 antimicrobial drugs have been implemented in order to characterize the evolution of drug resistance and susceptibility trends in vibrio species. Thirty vibrio isolates were tested for their susceptibility to a common panel of antimicrobial agents. The overall resistance of vibrios to antimicrobials was significantly high. The conclusions alarm to a significant impact in restricting the choice of treatment option. This is a reason for consideration because of their potential to induce human infections. We have indicated the presence of virulance factors encoding genes among non parahaemolyticus vibrio strains isolated from non-clinical (environmental) samples.

Like several developing countries around the world, uncooked food hygiene and antimicrobial resistance epidemiology continue to be at the beginning phase in the area of investigation. Our results convey current baseline platform of antimicrobial resistant pathogenic Vibrios from shellfish in Ismailia, Egypt.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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