Received: 01 April 2023, Accepted: 30 May 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 3, 443-448

Antibiotic Resistance Profile and Molecular Characterization of *Vibrio parahaemolyticus* and *Vibrio cholerae* Isolated From Fish

Alaa Eldin M.A. Morshdy, Nehal S.A. Abdelhameed, Karima Abdallah, Ahmed S. El-tahlawy, Rasha M. El Bayomi*

Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt.

***Correspondence**
Corresponding author: Rasha M. El Bayomi
E-mail address: rmazab_2010@yahoo.com

Abstract

The current study was planned for the isolation and identification of *Vibrio* spp. from fish samples (tilapia, mugil, tuna, saurus, pagrus, and shrimp) retailed in Zagazig city, Sharkia Governorate, Egypt. In addition to determine the antimicrobial susceptibility of the isolates. The PCR screening for virulence genes of *V. parahaemolyticus* and *V. cholerae* was also determined. The results in the present study revealed that the most contaminated samples with *Vibrio* spp. were pagrus (56%), followed by tilapia (52 %), then mugil (44 %), saurus (40 %), tuna (36 %), and shrimp (36 %). The most prevalent *Vibrio* spp. were *V. parahaemolyticus* (18.6%) followed by *V. mimicus* (11.3%), and *V. alginolyticus* (10%). *V. cholerae* was isolated in a percentage of 0.7%. *Vibrio* species in the current study were highly resistant to erythromycin (100%), ampicillin (75%), cephalothin (66.7%), sulphamethoxazole (66.7%), and amikacin (50%). PCR screening of virulence genes among various *Vibrio* spp. revealed that *V. parahaemolyticus* harbored *tlh* (100 %), *tdh* (80 %), and *trh* (80 %). While, *V. cholerae* was positive for *rtxC*, *hylA*, and *ompU* genes. As a result, the current data proves that *Vibrio* spp. contamination of fish, which displayed various degrees of antibiotic resistance. In addition, the isolated *Vibrio* spp. had virulent genes that could be dangerous to the consumer's health.

KEYWORDS
Fish, *V. parahaemolyticus*, *V. cholerae*, Antimicrobial susceptibility, Virulence genes.

INTRODUCTION

Fish is a high-protein food for healthy diets all over the world that usually has all the required amino acids, minerals, a lot of vitamins, and Omega-3 fatty acids that are necessary for human life (Morshdy *et al*., 2021). Although seafood is a nutrient-rich diet, undercooked or raw fish is blamed for several foodborne illnesses, which are serious diseases of human public health (Morshdy *et al*., 2022a). One of the most prevalent and pervasive foodborne pathogens linked to food poisoning is *Vibrio* spp. (Trinanes and Martinez-Urtaza, 2021). They exist in nature alongside aquatic organisms in freshwater, estuarine, and marine settings all over the world. The most common *Vibrio* species linked to consuming raw or partially cooked fish are *V. parahaemolyticus* and *V. vulnificus*, whereas *V. cholerae* infection is primarily linked to waterborne outbreaks and can, to a lesser extent, be spread through fish consumption (Caburlotto *et al*., 2016; Ahmed *et al*., 2018). Ingestion of food contaminated with *V. parahaemolyticus* can lead to gastrointestinal illness, including symptoms such as watery diarrhea, abdominal cramps, nausea, vomiting, fever, headache, and/or bloody diarrhea (CDC, 2013). Since antibiotics are mainly used in aquaculture farms to treat, prevent illnesses, and promote growth, the overuse and non-detection of the withdrawal times of these antibiotics increase the development of antibiotic-resistant bacteria and the presence of antibiotic residues in aquatic species like fish (Morshdy *et al*., 2022b). Consumption of contaminated fish tissues may expose humans to such residues (Morshdy *et al*., 2013). The virulence of *V. parahaemolyticus* species is determined by the presence of *Tdh*-thermostable hemolysin (*Trh*) and/or thermostable toxin (*Tdh*), which are encoded by the *trh* and *tdh* genes, respectively (Dileep *et al*., 2003). These two genes are linked to *V. parahaemolyticus*' cytotoxic and hemolytic effects on the host cell (Ahmed *et al*. 2018). The majority of epidemics among the *V. cholerae* serogroups are associated with O1 and O139 serotypes (Nishibuchi and DePaola, 2005). The *HylA* gene for hemolysin in *V. cholerae* is an enterotoxin that damages cells by acting as a pore-forming toxin (Moore *et al*., 2014). Outer membrane proteins, which include *OmpU*, OmpT, *OmpS*, *OmpV*, and others, are important cell-envelope proteins. *OmpU* is thought to contribute to bile resistance and acts as a colonizing factor (Provenzano *et al*., 2000).

Therefore, this study was performed to determine the frequency of *Vibrio* spp. in fish samples sold at fish markets in Sharkia Governorate, Egypt. In addition, the antibiotic resistance profile and virulence screening of *V. parahaemolyticus* and *V. cholerae* were also evaluated.

MATERIALS AND METHODS

Samples collection

A total of 150 samples of tilapia, mugil, tuna, saurus, pagrus,

ISSN: 2090-6277/2090-6269/

and shrimp (25, of each) were selected at random from various places and fish markets in Zagazig city, Sharkia Governorate, Egypt.

Isolation and Identification of Vibrio spp.

Vibrio spp. isolation was carried out in an accordance with FDA guidelines (2004). Fish flesh weighing ten grams was aseptically homogenized in 90 mL of sterile alkaline peptone water (APW, Micro Master - India) and incubated for 24–48 hours at 35±2°C (ISO/TS 21872-1, 2007). A loopful of each inoculated APW broth was streaked onto thiosulfate citrate bile salts sucrose agar plates (TCBS, Hi-Media - India), and the plates were then incubated for 24 hours at 37°C. According to ISO/TS 21872-1 (2007) and ISO/TS 21872-2 (2007), the presumed *Vibrio* colonies were obtained, purified, and then identified biochemically.

Sensitivity to antibiotics

The Kirby-Bauer disc diffusion method was used to assess the antibiotic susceptibility of 24 *Vibrio* isolates against 14 different antibiotics according to Adeyemi *et al.* (2008). The antimicrobial discs used were; cephalothin (CN, 30 µg), ampicillin (AM, 10 µg), imipenem (IPM, 10 µg), tetracycline (T, 30 µg), gentamicin (G, 10 µg), amoxicillin (AMX, 30 µg), amikacin (AK, 30 µg), ciprofloxacin (CP, 5 µg), cefotaxime (CF, 30 µg), levofloxacin (L, 5 µg), meropenem (M, 10 µg), erythromycin (E, 15 µg), ceftazidime (CZ, 30 µg), and sulfamethoxazole (SXT, 25 µg) (Oxoid Limited, Basingstoke, Hampshire, UK). According to NCCLS (2001), inhibitory zones were determined. Multiple antibiotic resistance (MAR) indexes were identified. The MAR index is calculated as follows: MAR index = a/b, where a and b denote the antibiotics number to which the isolates are resistant and the sum of tested antibiotics, respectively.

Molecular recognition

The biochemically identified colonies were genetically confirmed using primers from Metabion, Germany. The manufacturer's instructions for the QIAamp DNA Mini kit were followed (QIAGEN GmbH, Hilden, Germany, Catalogue no.51304). The molecular characterization of virulence factors including thermolabile hemolysin (*tlh*), thermostable direct hemolysin (*tdh*), and *tdh*-related hemolysin (*trh*) genes for *V. parahaemolyticus* and repeat toxin (*rtxC*), hemolysin (*hylA*), and outer membrane protein (*ompU*) as virulence factors of *Vibrio cholerae* were illustrated in Table 1.

RESULTS

Following a bacterial analysis of fish samples in the current study, it was found that 44 % of samples contained *Vibrio* species. pagrus fish had the highest isolation rate of 14 (56%), whereas tuna had the lowest isolation percentage of 9 (36 %) (Table 2). The isolated *Vibrio* species with the highest prevalence were *V. parahaemolyticus* (18.6%) and *V. mimicus* (11.3%), while *V. cholerae* had the lowest percentage (0.7%) (Table 2). Regarding antibiotic sensitivity, *Vibrio* strains were resistant to erythromycin (100%), ampicillin (75 %), cephalothin (66.7 %), sulphamethoxazole (66.7 %), and amikacin (50 %) (Table 3). The majority of *V. parahaemolyticus* isolates demonstrated resistance to at least 4 antibiotics. The average MAR for *Vibrio* isolates was 0.412, with values ranging from 0.071 to 1 (Table 4). PCR screening of virulence genes among various *Vibrio* spp. revealed that *V. parahaemolyticus* isolates possessed *tlh* (100 %), *tdh* (80 %), and *trh* (80 %) virulence genes. While, *V. cholerae* harbored *rtxC*, *hylA*, and *ompU* virulence genes (Figs. 1 and 2).

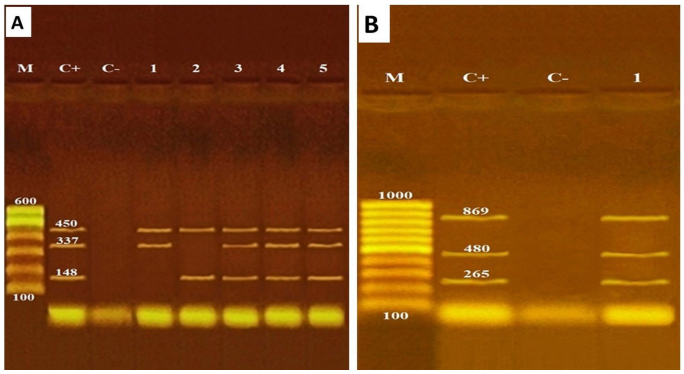

Fig 1. Agarose gel electrophoresis of multiplex PCR. (A) virulence genes for characterization of *V. parahaemolyticus*; *tlh* (450 bp), *tdh* (337 bp), and *trh* (148 bp). M: 100 bp ladder, C+: Control positive, C-: Control negative, 1: positive strains for *tlh* and *tdh* genes, 2: positive strain for *tlh* and *trh* genes, 3, 4 & 5: positive strains for *tlh*, *tdh*, and *trh* genes. (B): virulence genes for characterization of *V. cholerae* of *rtxC* (265 bp), *hylA* (480 bp), and *ompU* (869 bp). C+: Control positive, C-: Control negative; 1: positive strains for *rtx*, *hylA*, and *ompU* genes.

DISCUSSION

Vibrio spp. are microbiological water-borne diseases that are mostly found in various types of seafood and make people more susceptible to health risks. In the current study, *Vibrio* species were noticed in 66 (44%) of the examined fish samples. *V. parahaemolyticus* was found in 28 (18.6%) and *V. cholerae* was detected in 1(0.7%). Nearly similar results of 39% (78/200) of *Vibrio* species were detected in freshwater fish in Egypt (El-Sharaby *et al.*, 2018). *Vibrio* species were recovered from 52% of marketed

Table 1. Molecular characterization of virulence factors for *V. parahaemolyticus* and *Vibrio cholerae*.

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
tlh (F) *tlh* (R)	5′ AAAGCGGATTATGCAGAAGCACTG ′3 5′ GCTACTTTCTAGCATTTTCTCTGC ′3	450	Bej *et al*. (1999)
tdh (F) *tdh* (R)	5′ CCCCGGTTCTGATGAGATATT ′3 5′ TGGAATAGAACCTTCATCTTCACC ′3	337	
trh (F) *trh* (R)	5′ ACTAATGGACAAACCGAATCA ′3 5′ CCAGAAAGAGCAGCCATTG ′3	148	
rtx C (F) *rtxC* (R)	5′ CGACGAAGATCATTGACGAC ′3 5′ CATCGTCGTTATGTGGTTGC ′3	265	Chow *et al*. (2001)
hylA (F) *hylA* (R)	5′ GAGCCGGCATTCATCTGAAT ′3 5′ CTCAGCGGGCTAATACGGTTTA ′3	480	Kumar *et al*. (2009)
ompU (F) *ompU* (R)	5′ ACGCTGACGGAATCAACCAAAG ′3 5′ GCGGAAGTTTGGCTTGAAGTAG ′3	869	

fish samples in Egypt, of which 42.3% were *V. parahaemolyticus*, 26.92% were *V. mimicus*, 19.23% were *V. alginolyticus*, 9.62% were *V. vulnificus*, and 1.92% were *V. cholera* (Morshdy *et al*. 2022a). The highest isolation percentage of 82.85% in freshwater fish was investigated in India, with 6 (6.9%) *V. parahaemolyticus*, 2 (2.3%) *V. vulnificus*, 4 (4.6%) *V. alginolyticus*, and 3 (3.45%) *V. cholera* (Suresh *et al*., 2018). However, 36 (16%) of *Vibrio* isolates were found in crustaceans from fish markets in Sharkia Governorate, Egypt, from which 15.1% were *V. parahaemolyticus* and 0.9% were *V. cholerae* (Ahmed *et al*., 2018). In China, Yan *et al*. (2019) reported 10.33% of *V. cholerae*, 3.89% of *V. parahaemolyticus*, 1.24% of *V. alginolyticus*, and 0.76% of *V. vulnificus*, from freshwater fish. In Croatia, Jakšić *et al*. (2002) reported lower *V. parahaemolyticus*, *V. alginolyticus*, and *V. vulnificus* of 7.14 %, 3.57 %, and 10.71 %, respectively in shrimp samples. Higher percentage results of 88.57% *V. parahaemolyticus* were reported by Tan *et al*. (2020) in shrimp samples in Selangor, Malaysia. In Thailand, *V. parahaemolyticus* was reported in 38% of cultured shrimp samples examined by Yano *et al*. (2014), and in 26.67 % of different kinds of seafood and shrimp in China (Jiang *et al*., 2019). Furthermore, in Turkey, Yücel and Balci (2010) isolated *V. parahaemolyticus*, *V. vulnificus*, and *V. mimicus* from marine fish by a percentage of 40 %, 18%, and 6%, respectively. The occurrence of different *Vibrio* species may vary due to salinity, geography, seasonal variations, and isolation procedures used (Deepanjali *et al*., 2005).

The higher prevalence of *Vibrio* spp. may be attributed to the link between the *Vibrio* spp. and parameters such as temperature and salinity. Higher temperatures in warmer months enhanced the prevalence of *V. parahaemolyticus* (DePaola *et al*., 2000). In addition, the microorganism can stagnate in bottom sediments and enter the water column again when warm temperatures return (Pfeffer *et al*., 2003).

Antimicrobial residues in aquaculture products pose considerable risks to the public's health. The results obtained in the current study were similar to the findings reported by Letchumanan *et al*. (2015), who found the resistance of *V. parahaemolyticus* to several antibiotics including ampicillin (82 %), amikacin (51 %), cefotaxime (37 %), tetracycline (17 %), ceftazidime (15 %), gentamicin (11 %), levofloxacin (9 %), and imipenem (2 %) in Malaysia. In addition, Jiang *et al*. (2019) revealed 95.46 % resistance for ampicillin, 30 % for amikacin, 17.78 % for tetracycline, 17.78% for sulphamethoxazole/trimethoprim, and 2.22 % for ciprofloxacin in different kinds of seafood in China. Moreover, Tan *et al*. (2020) in Selangor, Malaysia found that *V. parahaemolyticus* strains isolated from seafood samples including shrimp were resistant to ampicillin (84.17 %), cephalothin (54.17 %), amikacin (37.50%), ciprofloxacin (13.33 %), gentamicin (6.67 %), cefotaxime (5 %), ceftazidime (5 %), and levofloxacin (1.67%). Our results were following (Jiang *et al*., 2019) who detected that all *V. parahaemolyticus* isolates from different seafood samples in China were sensitive to imipenem and meropenem. Also, Tan *et al*. (2020) observed the susceptibility of *V. parahaemolyticus* isolates obtained from seafood samples in Selangor, Malaysia to imipenem and meropenem (98.33 % of each), tetracycline (94.17 %), levofloxacin (73.33 %), ceftazidime (70.83%), and gentamicin (64.17 %).

The majority of the *Vibrio* isolates in the current study declared multiple MAR indices ranging from 1 to 0.071, with a mean of 0.412, which is more than 0.2, indicating contamination from high-hazard sources, and demonstrating an acquired genetic resistance that poses a public health risk to consumers (Tanil *et*

Table 2. Prevalence and serological identification of *Vibrio* species in the examined fish samples.

Samples	*Vibrio* spp.	*V. parahaemolyticus*	*V. cholerae*	*V. mimicus*	*V. alginolyticus*	*V. vulnificus*
Tilapia	13 (52%)	-	-	6 (46%)	7 (54%)	-
Mugel	11 (44%)	-	-	11 (100%)		-
Tuna	9 (36%)	5 (55.6%) O2: K28	-	-	4 (44.4%)	-
Saurus	10 (40%)	5 (50 %) O5: K17	-	-	-	5 (50 %)
Pagrus	14 (56%)	14 (100%) O10: K52 O2: K28	-	-	-	-
Shrimp	9 (36%)	4 (44.4%) O3: K7	1 (11.1%) O1	-	4 (44.4%) -	-
Total	66 (44%)	28 (18.6)	1 (0.7%)	17 (11.3%)	15 (10%)	5 (3.3%)

Table 3. Antimicrobial susceptibility of *Vibrio* species (n. =24).

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	NO.	%	NO.	%	NO.	%
Erythromycin (E)	0	0	0	0	24	100
Ampicillin (AM)	0	0	6	25	18	75
Cephalothin (CN)	4	16.7	4	16.7	16	66.7
Sulfamethoxazole (SXT)	8	33.3	0	0	16	66.7
Amikacin (AK)	10	41.7	2	8.3	12	50
Gentamicin (G)	12	50	2	8.3	10	41.7
Tetracycline (T)	10	41.7	6	25	8	33.3
Amoxicillin (AMX)	14	58.3	4	16.7	6	25
Ciprofloxacin (CP)	14	58.3	4	16.7	6	25
Cefotaxime (CF)	16	66.7	2	8.3	6	25
Levofloxacin (L)	18	75	2	8.3	4	16.7
Ceftazidime (CZ)	20	83.3	0	0	4	16.7
Imipenem (IPM)	20	83.3	2	8.3	2	8.3
Meropenem (M)	22	91.7	0	0	2	8.3

Table 4. *Vibrio* species' antimicrobial resistance profile (n. =24).

Vibrio spp.	number of Isolates	Profile of antimicrobial resistance	MAR
V. parahaemolyticus	2	E, AM, CN, SXT, AK, G, T, AMX, CP, CF, L, CZ, IPM, M	1
V. parahaemolyticus	2	E, AM, CN, SXT, AK, G, T, AMX, CP, CF	0.71
V. parahaemolyticus	2	E, AM, CN, SXT, AK	0.36
V. parahaemolyticus	2	E, AM, CN, SXT	0.29
V. parahaemolyticus	2	E	0.07
V. cholerae	1	E, AM, CN, SXT, T, CP, CF, CZ	0.57
V. mimicus	2	E, AM, CN, SXT, AK, G, T, AMX, CP, CF, L, CZ	0.86
V. mimicus	2	E, AM, CN, SXT, AK, G	0.43
V. mimicus	2	E	0.07
V. alginolyticus	1	E, AM, CN, SXT, AK, G, T	0.5
V. alginolyticus	2	E, AM	0.14
V. alginolyticus	2	E, AM, CN, SXT	0.29
V. vulnificus	2	E	0.07
Average			0.412

E: Erythromycin; AM: Ampicillin; CN: Cephalothin; SXT: Sulfamethoxazole; AK: Amikacin; G: Gentamicin; T: Tetracycline; AMX: Amoxicillin; CP: Ciprofloxacin; CF: Cefotaxime; L: Levofloxacin; CZ: Ceftazidime; IMP: Imipenem; M: Meropenem.
MAR: Multiple Antibiotic Resistance index (a/b), where (a) is the number of antibiotics to which the isolates are resistant. (b): is the total number of tested antibiotics (n.=14).

al., 2005; Ahmed *et al*., 2018). Similarly, Morshdy *et al*. (2022a) showed that more than 50% of the *Vibrio* strains isolated from marketed fish in Egypt were resistant to more than four antibiotics. The MAR index in the present study was nearly similar to Letchumanan *et al*. (2015) who detected MAR index ranged from 0.07 to 0.79 from shrimp samples in Malaysia and Tan *et al*. (2020) who cited that the MAR indices varied from 0.04 to 0.71 from different types of seafood in Selangor, Malaysia. These results highlight the significance of routine screening of numerous environmental *Vibrio* strains against a wide range of antimicrobial agents and clinically relevant antibiotics to provide useful in vitro baseline data for clinical treatment purposes (Baker-Austin *et al*., 2008). The variation in the MAR index may be because of the variety in the sample sources, geographic location, and methodology.

The PCR-based assay is a well-liked molecular method for detecting and identifying *V. parahaemolyticus* in seafood samples. According to studies conducted in several countries, the pathogenicity of *V. parahaemolyticus* isolates is related to the presence of *tdh* and/or *trh* genes (Nordstrom *et al*., 2007; Ahmed *et al*., 2018). Most environmental strains of *V. parahaemolyticus* are non-pathogenic, whereas 99% of clinical isolates are identified to be pathogenic because they carry *tdh* and/or *trh* genes (Tsai *et al*., 2013). However, only 0-6% of the environmental isolates were carrying the *tdh* and/or *trh* genes which are classified as pathogenic (Letchumanan *et al*., 2014). The presence of *tdh* and/or *trh V. parahaemolyticus* in freshwater and marine fish samples is alarming for several reasons. First, the possibility that these pathogenic isolates could result in gastroenteritis (Jun *et al*., 2012). Second, pathogenic *V. parahaemolyticus* not only contaminates seafood and spreads pathogenesis, but it also causes significant economic losses in the aquaculture industry (Thongjun *et al*., 2013). *V. parahaemolyticus* strains in the present research exhibited virulent genes (*trh* and *tdh*) higher than that was recorded by Letchumanan *et al*. (2015) who detected *V. parahaemolyticus* isolates virulent genes with a percentage of 10% for *trh* and 0 % for *tdh* from retailed shrimp in Malaysia. Moreover, Jiang *et al*. (2019) demonstrated *V. parahaemolyticus* virulent genes with a percentage of 1.11 % for *tdh* and 5.56 % for *trh* from seafood samples in China. In contrast, Tan *et al*. (2020) found that *V. parahaemolyticus* strains isolated from the seafood samples in Selangor, Malaysia including shrimp were negative for pathogenic *tdh* and *trh* genes. While, Lee *et al*. (2018) found that only 4 (2.4%) out of 165 *V. parahaemolyticus* obtained from marine and freshwater fish in Selangor, Malaysia were positive for the *trh* gene, and none of the isolates yielded the *tdh* gene. In Egypt, 14.7% of *V. parahaemolyticus* isolates from crustaceans were positive for the *tdh* and/or *trh* genes (Ahmed *et al*., 2018). The *trh* and *tdh* genes were present in 28.4% and 2.1%, respectively, of *V. parahaemolyticus* isolates from seafood in China, however, none of the isolates carried both genes concurrently (Xie *et al*., 2017). None of *V. parahaemolyticus* isolates from shrimp samples was positive for the *tdh* gene in another investigation conducted in China, although 45.9% harbored the *trh* gene (Xie *et al*., 2015).

The genes encoding virulence-associated factors that contribute to the pathogenicity of *V. cholerae* include the *Rtx* toxin gene cluster (*rtx*A-D) (Lin *et al*., 1999), hemolysin (*hlyA*) (Ruenchit *et al*., 2017), and *OmpU* gene (Rivera *et al*., 2001). In the current study, the *rtx*, *hylA*, and *ompU* genes were present in only isolated *V. cholerae*. Although the majority of ambient *V. cholerae* isolates are not toxic, the *hlyA* gene makes them capable of causing mild gastroenteritis (Saravanan *et al*., 2007). In Egypt, The *ctx* and *hlyA* genes were present in two *V. cholerae* isolates from crustaceans that were serogroups as non-O1 and non-O139 (Ahmed *et al*., 2018). Moreover, Xu *et al*. (2019) detected a high occurrence of *rtxC* and *hylA* virulent genes of *V. cholerae* recovered from freshwater fish in Shanghai, China with a percentage of 95.8% and 87.8%, respectively. Furthermore, the *rtx*A and *hlyA* genes were found in 31.5% and 18.5%, respectively, of *V. cholerae* strains isolated from ornamental fish species (Zago *et al*., 2017).

CONCLUSION

The current investigation proved that several fish and shrimp samples in Egyptian markets have potentially harmful *Vibrio* species. A higher percentage of isolated *Vibrio parahaemolyticus* harbored virulent genes that can pose a risk to the public health. To increase seafood safety, it is essential to monitor the presence and antibiotic resistance profile of *Vibrio* spp. in retail seafood. It is necessary to adopt appropriate food safety controls to check the quality of the fish being produced and consumed and to allow the abuse of antibiotics in aquaculture only under veterinary supervision.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Adeyemi, A., Enyinnia, V., Nwanze, R., Smith, S., Omonigbehin, E., 2008. Antimicrobial susceptibility of potentially pathogenic halophilic

Vibrio species isolated from seafoods in Lagos, Nigeria. Afr. J. Biotechnol. 7, 3791-3794.

Ahmed, H.A., El Bayomi, R.M., Hussein, M.A., Khedr, M.H., Remela, E.M.A., El-Ashram, A.M., 2018. Molecular characterization, antibiotic resistance pattern and biofilm formation of *Vibrio parahaemolyticus* and *V. cholerae* isolated from crustaceans and humans. Int. J. Food Microbiol. 274, 31-37.

Baker-Austin, C., McArthur, J.V., Tuckfield, R.C., Najarro, M., Lindell, A.H., Gooch, J.A.N., Stepanauskas, R., 2008. Antibiotic resistance in the shellfish pathogen *Vibrio parahaemolyticus* isolated from the coastal water and sediment of Georgia and South Carolina, USA. J. Food Prot. 71, 2552-2558.

Bej, A.K., Patterson, D.P., Brasher, C.W., Vickery, M.C., Jones, D.D., Kaysner, C.A, 1999. Detection of total and hemolysin-producing *Vibrio parahaemolyticus* in shellfish using multiplex PCR amplification of tl, *tdh*, and *trh*. J. Microbiol. Methods 36, 215-25.

Caburlotto, G., Suffredini, E., Toson, M., Fasolato, L., Antonetti, P., Zambon, M., Manfrin, A., 2016. Occurrence and molecular characterization of *Vibrio parahaemolyticus* in crustaceans commercialized in Venice area, Italy. Int. J. Food Microbiol. 220, 39–49.

CDC, 2013. Signs & Symptoms: *Vibrio parahaemolyticus* illnesses associated with consumption of shellfish, United States. accessed 20 October 2019.

Chow, K., Ng, T, Yuen, K., Yam, W., 2001. Detection of *Rtx* toxin gene in *Vibrio cholerae* by PCR. J. Clin. Microbiol. 39, 2594-2597.

Deepanjali, A., Kumar, H.S., Karunasagar, I., Karunasagar, I., 2005. Seasonal variation in abundance of total and pathogenic *V. parahemolyticus* in oyster along the southwest coast of India. Appl. Environ. Microbiol. 71, 3575-3580.

DePaola, A., Kaysner, C.A., Bowers, J., Cook, D.W., 2000. Environmental investigations of *Vibrio parahaemolyticus* in oysters after outbreaks in Washington, Texas, and New York (1997 and 1998). Appl. Environ. Microbiol. 66, 4649-4654.

Dileep, V., Kumar, H.S., Kumar, Y., Nishibuchi, M., Karunasagar, I., Karunasagar, I., 2003. Application of polymerase chain reaction for detection of *Vibrio parahaemolyticus* associated with tropical seafoods and coastal environment. Lett. Appl. Microbiol. 36, 423–427.

El-Sharaby, S.M.A., Abd-Elgaber, M., Tarabees, R., Khalil, R.H., Ali, M.N., El-Ballal, S., 2018. Bacteriological and histopathological studies on *Vibrio* species isolated from naturally infected freshwater fish in delta region, Egypt. Adv. Anim. Vet. Sci. 6, 17-26.

FDA's Bacteriological Analytical Manual, 2004. Bacteriological Analytical Manual Food and Drug Administration. BAM Chapter 9: *Vibrio*. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-9-*Vibrio*.

ISO/TS 21872-1, 2007. Specifies a horizontal method for the detection of the two main pathogenic *Vibrio* species causing intestinal illness in humans: *V. parahaemolyticus* and *V. cholerae*. https://www.iso.org/standard/38278.html

ISO/TS 21872-2, 2007. Specifies a horizontal method for detection of the enteropathogenic *Vibrio* species, causing illness in or via the intestinal tract, other than *Vibrio parahaemolyticus* and *Vibrio cholerae*. Include *Vibrio fluvialis*, *Vibrio mimicus*, and *Vibrio vulnificus*. https://www.iso.org/standard/38279.html

Jakšić, S., Uhitil, S., Petrak, T., Bažulić, D., Karolyi, L.G., 2002. Occurrence of *Vibrio* spp. in sea fish, shrimps and bivalve molluscs harvested from Adriatic sea. Food Control. 13, 491-493.

Jiang, Y., Chu, Y., Xie, G., Li, F., Wang, L., Huang, J., Zhai, Y., Yao, L., 2019. Antimicrobial resistance, virulence and genetic relationship of *Vibrio parahaemolyticus* in seafood from coasts of Bohai Sea and Yellow Sea, China. Int. J. Food Microbiol. 290, 116-124.

Jun, J.W., Kim, J.H., Choresca, J.r., C.H., Shin, S.P., Han, J.E., Han, S.Y., Chai, J.Y., Park, S.C., 2012. Isolation, molecular characterization, and antibiotic susceptibility of *Vibrio parahaemolyticus* in Korean seafood. Foodborne Pathog. Dis. 9, 224-231.

Kumar, P., Jain, M., Goel, A., Bhadauria, S., Sharma, S., Kamboj, D., Singh, L., Ramamurthy, T., Nair, G., 2009. A large cholera outbreak due to a new cholera toxin variant of the *Vibrio cholerae* O1 El Tor biotype in Orissa, Eastern India. J. Med. Microbiol. 58, 234-238.

Lee, L.H., Ab Mutalib, N.S., Law, J.W.F., Wong, S.H., Letchumanan, V., 2018. Discovery on antibiotic resistance patterns of *Vibrio parahaemolyticus* in Selangor reveals carbapenemase producing *Vibrio parahaemolyticus* in marine and freshwater fish. Front. Microbiol. 9, 2513-2526.

Letchumanan, V., Chan, K.G., Lee, L.H., 2014. *Vibrio parahaemolyticus*: a review on the pathogenesis, prevalence, and advance molecular identification techniques. Front. Microbiol. 5, 705-718.

Letchumanan, V., Yin, W.F., Lee, L.H., Chan, K.G., 2015. Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from retail shrimps in Malaysia. Front. Microbiol. 6, 33-44.

Lin, W., Fullner, K.J., Clayton, R., Sexton, J.A., Rogers, M.B., Calia, K.E., Calderwood, S.B., Fraser, C., Mekalanos, J.J., 1999. Identification of a *Vibrio cholerae Rtx* toxin gene cluster that is tightly linked to the cholera toxin prophage. Proc. Natl. Acad. Sci. USA. 96, 1071–1076.

Moore, S., Thomson, N., Mutreja, A., Piarroux, R., 2014. Widespread epidemic cholera caused by a restricted subset of *Vibrio cholerae* clones. Clin. Microbiol. Infect. 20, 373-379.

Morshdy, A.E.M., El-Ghandour, A.R., Hussein, M.A., El Bayomi, R.M., 2022a. Prevalence of Antibiotic-Resistant *Vibrio* Isolated from Some Marketed Fish in Egypt with a Decontamination Trial by Lemon juice. J. Adv. Vet. Res. 12, 353-357.

Morshdy, A.E.M., Hussein, M.A., Mohamed, M.A.A., Hamed, E., El-Murr, A.E., Darwish, W.S., 2022b. Tetracycline residues in tilapia and catfish tissue and the effect of different cooking methods on oxytetracycline and doxycycline residues. J. Cons. Protect. Food Saf. 17, 387-393.

Morshdy, A.E., Darwish, W.S., Hussein, M.A., Mohamed, M.A., Hussein, M.M., 2021. Lead and cadmium content in Nile tilapia (*Oreochromis niloticus*) from Egypt: a study for their molecular biomarkers. Sci. Afr. 12, 1-8.

Morshdy, A.E., Hafez, A.E., Darwish, W.S., Hussein, M.A., Tharwat, A.E., 2013. Heavy metal residues in canned fishes in Egypt. Jpn. J. Vet. Res. 61, S54-S57.

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

Nishibuchi, M., DePaola, A., 2005. *Vibrio* species. In: Fratamico, M., Bhunia, A.K., Smith, J.L. (Eds.), Foodborne Pathogens: Microbiology and Molecular Biology. Caister Academic Press, pp. 251–271.

Nordstrom, J. L., Vickery, M. C., Blackstone, G. M., Murray, S. L., DePaola, A., 2007. Development of a multiplex real-time PCR assay with an internal amplification control for the detection of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters. Appl. Environ. Microbiol. 73, 5840–5847.

Pfeffer, C.S., Hite, M.F., Oliver, J.D., 2003. Ecology of *Vibrio vulnificus* in estuarine waters of eastern North Carolina. Appl. Environ. Microbiol. 69, 3526-3531.

Provenzano, D., Schuhmacher, D.A., Barker, J.L., Klose, K.E., 2000. The virulence regulatory protein ToxR mediates enhanced bile resistance in *Vibrio cholerae* and other pathogenic *Vibrio* species. Infect. Immun. 68, 1491-1497.

Rivera, I. N., Chun, J., Huq, A., Sack, R.B., Colwell, R.R., 2001. Genotypes associated with virulence in environmental isolates of *Vibrio cholerae*. Appl. Environ. Microbiol. 67, 2421-2429.

Ruenchit, P., Reamtong, O., Siripanichgon, K., Chaicumpa, W., Diraphat, P., 2017. New facet of non-O1/non-O139 *Vibrio cholerae* hemolysin A: a competitive factor in the ecological niche. FEMS Microbiol. Ecol. 93, 1-12.

Saravanan, V., Sanath Kumar, H., Karunasagar, I., Karunasagar, I., 2007. Putative virulence genes of *Vibrio cholerae* from seafoods and the coastal environment of Southwest India. Int. J. Food Microbiol. 119, 329–333.

Suresh, Y., Subhashini, N., Kiranmayi, C.B., Srinivas, K., Ram, V.P., Chaitanya, G., Rao, T.S., 2018. Isolation, molecular characterization and antimicrobial resistance patterns of four different *Vibrio* species isolated from fresh water fishes. Int. J. Curr. Microbiol. Appl. Sci. 7, 3080-3088.

Tan, C.W., Rukayadi, Y., Hasan, H., Thung, T.Y., Lee, E., Rollon, W.D., Hara, H., Kayali, A.Y., Nishibuchi, M., Radu, S., 2020. Prevalence and antibiotic resistance patterns of *Vibrio parahaemolyticus* isolated from different types of seafood in Selangor, Malaysia. Saudi J. Biol. Sci. 27, 1602-1608.

Tanil, G.B., Radu, S., Nishibuchi, M., Rahim, R.A., Napis, S., Maurice, L., Gunsalam, J.W., 2005. Characterization of *Vibrio parahaemolyticus* isolated from coastal seawater in peninsular Malaysia. Southeast Asian J. Trop. Med. Public Health. 36, 940-946.

Thongjun, J., Mittraparp-Arthorn, P., Yingkajorn, M., Kongreung, J., Nishibuchi, M., Vuddhakul, V., 2013. The trend of *Vibrio parahaemolyticus* infections in Southern Thailand from 2006 to 2010. Trop. Med. Health 41, 151-156.

Trinanes, J., Martinez-Urtaza, J., 2021. Future scenarios of risk of *Vibrio* infections in a warming planet: a global mapping study. Lancet Planet. Health 5, e426-e435.

Tsai, S.E., Jong, K. J., Tey, Y.H., Yu, W.T., Chiou, C.S., Lee, Y.S., Wong, H.C., 2013. Molecular characterization of clinical and environmental *Vibrio parahaemolyticus* isolates in Taiwan. Int. J. Food Microbiol. 165, 18-26.

Xie, T., Wu, Q., Xu, X., Zhang, J., Guo, W., 2015. Prevalence and population analysis of *Vibrio parahaemolyticus* in aquatic products from South China markets. FEMS Microbiol. Lett. 362, 1-7.

Xie, T., Wu, Q., Zhang, J., Xu, X., Cheng, J., 2017. Comparison of *Vibrio parahaemolyticus* isolates from aquatic products and clinical by antibiotic susceptibility, virulence, and molecular characterisation. Food Control 71, 315–321.

Xu, M., Wu, J., Chen, L., 2019. Virulence, antimicrobial and heavy metal tolerance, and genetic diversity of *Vibrio cholerae* recovered from commonly consumed freshwater fish. Environ. Sci. Pollut. Res. 26, 27338–27352.

Yan, L., Pei, X., Zhang, X., Guan, W., Chui, H., Jia, H., Yang, D., 2019. Occurrence of four pathogenic *Vibrio*s in Chinese freshwater fish farms in 2016. Food Control. 95, 85-89.

Yano, Y., Hamano, K., Satomi, M., Tsutsui, I., Ban, M., Aue-Umneoy, D., 2014. Prevalence and antimicrobial susceptibility of *Vibrio* species related to food safety isolated from shrimp cultured at inland ponds in Thailand. Food control. 38, 30-36.

Yücel, N., Balci, Ş., 2010. Prevalence of Listeria, Aeromonas, and *Vibrio* species in fish used for human consumption in Turkey. J. Food Prot. 73, 380-384.

Zago, V., Zambon, M., Civettini, M., Zaltum, O., Manfrin, A., 2017 .Virulence-associated factors in *Vibrio cholerae* non-O1/non-O139 and *V. mimicus* strains isolated in ornamental fish species. J. Fish. Dis. 40, 1857–1868.