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Detection of Virulence Determinants and Antimicrobial Susceptibility of *Vibrio* species Isolated from Raw Fish Markets

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Abstract

*Vibrio*sis is a major cause of death in farmed aquaculture systems around the world caused by an infection with *Vibrio* spp. This study was performed to detect the prevalence of pathogenic *Vibrio* species from various fish species (n=200) collected from Port-Said fish markets as well as to assess the antimicrobial resistance profile and virulence determinants of the isolated *Vibrio*. Fish samples were inoculated onto *Vibrio*-selective medium (TCBS), and phenotypically identified using biochemical tests and the suspected isolates were then confirmed by PCR targeting collagenase gene followed by sequencing of the amplified gene. Disc diffusion method was used to investigate the antimicrobial susceptibility of the isolated strains as well as the virulence determinants were detected by PCR assay. Out of 200 fish samples, 80 *Vibrio* isolates including *V. parahaemolyticus* (22 /80) and *V. alginolyticus* (58 /80) were recovered. While *V. vulnificus* couldn't be identified. *Vibrio* isolates displayed high resistance to beta-lactams antimicrobials and a lower resistance were displayed against tetracycline, quinolones and carbapenems. Additionally, *tlh* gene was present in all *V. parahaemolyticus* isolates, while the *tdh* gene was present in 27.1% (6/22). In conclusion, the results of this study provide information on the hazards that certain fish and shellfish could bring by transferring virulent and genetic resistance of *V. parahaemolyticus* and *V. alginolyticus* to people through food., therefore, consumers should be more alert, prepare food properly, and avoid undercooked or cross-contaminated fish.

KEYWORDS
V. parahaemolyticus, *V. alginolyticus*, Seafood, Virulence Determinants, Antimicrobial susceptibility.

INTRODUCTION

Vibrio species are Gram-negative bacilli can survive in freshwater and estuarine environments with a variety of salinity and temperature levels (Pruzzo *et al*., 2005; Ramalingam and Ramarani, 2006; Alam *et al*., 2009). The maximum and minimum growth temperatures for pathogenic *Vibrio* are 43°C and 5°C, respectively. The optimal temperature for pathogenic *Vibrio* growth is around 37°C. Because they are acid-sensitive, *Vibrio* species well survive at pH values between 7.5 and 8.5, or just above neutrality (Adams and Moss, 2000). There are about 100 species of *Vibrio*, most of which are present in surface and marine waters. *Vibrio vulnificus*, *Vibrio tubiashi*, *Vibrio parahaemolyticus*, and *Vibrio fluvial* are the dangerous *Vibrio* species that are typically spread through water and seafood (Hoffmann *et al*., 2010; Hassan *et al*., 2012). *Vibrio* spp. have developed into a serious threat to human health. The infection is closely related to *Vibrio* outbreaks that are brought on by consuming raw seafood and drinking water that has been contaminated with sewage, as well as by exposing aquatic animals and environments to harm (Lee *et al*., 2002).

V. parahaemolyticus is the most prevalent foodborne gastroenteritis pathogen in several countries about 25% in coordination with other *Vibrio* spp. (Su and Liu, 2007). It recognized as potential pathogenic, in rare cases can be a life threating causing acute gastroenteritis or invasive septicemia through consumption of contaminated raw or undercooked seafood (Zarei *et al*., 2012).

V. alginolyticus cause human illness, significant morbidity and mortality (Scallan *et al*., 2011; Morris and Black, 1985) and can cause cases of otitis externa and traumatic wound infections after exposure to seawater (Gomez *et al*., 2003; Hornstrup and Gahrn-Hansen, 1993). It was recorded as the third most common *Vibrio* spp. cause human illness, but nowadays, it has been reported as the second most common one (CDC, 2014). However, most *Vibrio* spp. are transmitted via food, *V. alginolyticus* can be predominantly transmitted via water or food (Newton *et al*., 2012; Dechet *et al*., 2008).

Numerous virulence factors are present in *V. parahaemolyticus*, including thermostable direct hemolysin (*Tdh*) and thermostable direct hemolysin-related hemolysin (*Trh*), which are encoded by the *tdh* and *trh* genes, respectively (Bej *et al*., 1999; Alipour *et al*., 2014). Similar to the *tdh* gene, which exhibits hemolytic, enterotoxic, and cytotoxic action in the host cell, the *trh* gene is also pathogenic (Broberg *et al*., 2011; Nelapati *et al*., 2012; Zheng *et al*., 2014). The *trh* gene is now the most widely used putative virulence factor among pathogenic *V. parahaemolyticus* strains (Honda *et al*., 1988). Additionally, there are other hemolysin genes,encodes the thermolabile hemolysin (*tlh*) (Zhang and Austin, 2005 Although this gene is thought to be an indicator for *V. parahaemolyticus* (DePaola *et al*., 2003).

Antimicrobial resistance increases as a result of the extensive usage of different antibiotics in aquaculture for improving growth rate and preventing bacterial infections, which has become a serious threat to veterinary medicine and worldwide

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public health (Ahmed *et al*., 2018). A possible concern to human health is the direct transmission of antibiotic-resistant bacteria from animal to human through the food chain or mobile genetic elements (Shakerian *et al*., 2017). The goal of this study was to ascertain the frequency of *Vibrio* spp. in the fish market in Port-Said Governorate, evaluate their antibiotic susceptibility, and assess the existence of virulence factors in the isolate *Vibrio* strains.

MATERIALS AND METHODS

Sample collection

A total of 200 fish species samples of marine and freshwater fish that are often consumed in Egypt were examined. From January to May 2020, from eight wet markets in Port-Said, Egypt, different fish samples were collected. Sea bass (*Dicentracus labrax*) (n = 50), crab (*Brachyura*) (n = 50), and Nile tilapia (*O. niloticus*) (n = 100) were the three fish species that were chosen. For bacteriological examination, each sample was preserved individually in a clean, labelled plastic bag and delivered right away in an icebox to the lab of the Bacteriology, Mycology, and Immunology Department, Faculty of Veterinary Medicine at Mansoura University, Egypt.

Isolation of Vibrio species

Fish were examined for any possible lesions on the inside and outside before being sampled for a bacteriological study. All fish samples appeared to be healthy and free from any evident lesions. 10 g of fish or crustacean flesh were blended in 90 ml of alkaline peptone water with 2% sodium chloride (NaCl) and incubated for 18 h at 37°C for a total of 18 hours. A loopful of the previously incubated broth was streaked onto TCBS agar (HiMedia, Mumbai, India) and kept in incubated 37°C for 18 hours. Purification of the suspected colonies was done on the Trypticase Soy Agar (TSA) plates with 2% NaCl (HiMedia) and incubating for 24 hours at 37°C. Biochemical tests (Alsina and Blanch, 1994) and morphological traits (Letchumanan *et al*. 2015) are used to identify different *Vibrio* species. For further storage, purified *Vibrio* spp. colonies were kept in 30% glycerol at 20°C for further identification.

PCR identification of suspected Vibrio spp.

Following a previously documented PCR procedure (Kim *et al*., 1999), two collagenase-targeted primer pairs (VP and VA) from Invitrogen, Carlsbad, California, were employed. The following PCR cycle conditions were used: initial denaturation at 95°C for 15 min; 35 cycle (94°C for 30 s; 57°C for 30 s; 72°C for 60 s); and final extension at 72°C for 5 min. A uniplex PCR assay that targets the *vvh* gene was used to identify *V. vulnificus* as it was described in an earlier work (Neogi *et al*., 2010). Table 1 includes a list of the primer sets and amplicon sizes. The following cycling conditions were used to accomplish PCR amplification in a 96-well 2720 thermocycler from Applied Biosystems in Norwalk, Connecticut: 5 minutes of initial denaturation at 94 degrees then 30 cycle of 30 s at 94˚C, 30 s at 55˚C, 30 s at 72˚C and a final extension for 5 min at 72˚C.

Antimicrobial susceptibility testing

According to Clinical and Laboratory Standards Institute states, Kirby-Bauer disc diffusion method was used to assess the antimicrobial susceptibilities of *Vibrio parahaemolyticus* and *V. alginolyticus* isolates against various antimicrobial drugs (CLSI, 2017). A total of nine antimicrobial discs (Oxoid, Basingstoke, UK) presenting different antimicrobial class including ciprofloxacin (CIP; 5 µg), sulfamethoxazole-trimethoprim (SXT; 25 µg), erythromycin (E; 15 µg), tetracycline (TET; 30 µg), imipenem (IMP; 10 µg), ampicillin (AMP; 10 µg), and penicillin (P; 10 µg) were used. Multidrug resistance, or MDR, is the term used to describe resistance to at least three different classes of antibiotics. Results were interpreted using the Clinical Laboratory Standards Institute chart (CLSI 2017).

Molecular characterization of virulence genes

Three virulence-related genes were investigated using PCRs. The *tdh* and/or *trh* genes were investigated using a duplex PCR strategy under the following conditions: 72°C for 7 min, 94°C for 1 min, 35 cycles (94°C for 1 min, 55°C for 1 min, 72°C for 1 min). While *tlh* gene was tested by a uniplex PCR. The PCR processes were completed in a 96-well Applied Biosystems 2720 thermal cycler. The following cycle PCR conditions were used: 94°C for 3 min, 30 cycle (94°C for 1 min, 58°C for 1 min, 72°C for 1 min), and 72°C for 5 min (Bej *et al*., 1999). The oligonucleotide sequences of the used primers are listed in Table 1.

RESULTS

Phenotypic and genotypic characterization of Vibrio species

In this study, 200 fish samples including Nile tilapia (*O. niloticus*) (n = 100), crab (*Brachyura*) (n = 50), and sea bass (*Dicentracus labrax*) (n = 50) were collected from Port-Said raw markets for screening the presence of *Vibrio* species targeting *V. para-*

Table 1. The Oligonucleotides and PCR cyclic conditions for PCR used in this study.

Target gene	Primer direction and sequence	Amplicon size (bp)	Reference
VA	5′-CGA GTA CAG TCA CTT GAA AGC C-3′ 5′-CAC AAC AGA ACT CGC GTT ACC-3′	737	Di pinto *et al*. (2004)
VP	5′-GAA AGT TGA ACA TCA TCA GCA CGA-3′ 5′-GGT CAG AAT CAA ACG CCG-3′	271	Di pinto *et al*. (2004)
vvh	5′-ACTCAACTATCGTGCACG-3′ 5′-ACACTGTTCGACTGTGAG-3′	366	Neogi *et al*. (2010)
tdh	5′-GTAAAGGTCTCTGACTTTTGGAC-3′ 5′-TGGAATAGAACCTTCATCTTCACC-3′	251	Tada *et al*. (1992)
trh	5′-TTGGCTTCGATATTTTCAGTATCT-3′ 5′CATAACAAACATATGCCCATTTCCC-3′	373	Tada *et al*. (1992)
tlh	5′-AAAGCGGATTATGCAGAAGCACTG-3′ 5′-GCTACTTTCTAGCATTTTCTCTGC-3′	450	Bej *et al*. (1999)

haemolyticus, *V. alginolyticus* and *V. vulnificus*. Fish samples were first subjected to tradition isolation of *Vibrio* spp. by cultivating the samples on TCBS agar. Yellow colonies were characteristic for *V. alginolyticus* while, blue-green colonies indicated the presence of *V. parahaemolyticus* and *V. vulnificus*. Out of 200 samples, 80 *Vibrio* isolates were recovered with a total infection rate of 40% (80/200). The infection rate of fish samples collected from freshwater fish (Nile tilabia n=100) was 31% (31/100), while the infection rate in marine fish (carb and sea bass) samples (n=100) was 49% (49/200) including 9.5% for carb and 15% for sea bass (Table 2).

The recovered isolates were then confirmed by PCR targeting collagenase gene which used as a genetic marker for identification *Vibrio* spp. *VP* and *VA* was tested by using multiplex PCR, *VP* was successfully identified at 271bp in 22 isolates and *VA* was amplified at 737 bp which confirm the V. aliginolyticus in 58 isolates (Figure 1, Table 2). Regarding *V. vulnificus*, a uniplex PCR targeting *vvh*- collagenase gene using *vvh* primer was failed to be amplified. Sequencing of *VP*, *VA* collagenase gene DNA products have been performed and the isolated showed more than 97% similarity with database sequences. Regarding the distribution of *Vibrio* spp. from the fish samples, the isolation rate of *V. parahaemolyticus* was 11% (22 /80) which produces green colonies on the used media and *V. alginolyticus* 29% (58 /80) showed yellow color on the isolation media were recovered (Table 2).

The distribution of *V. parahaemolyticus* isolates in Nile tilapia, Carb and Sea bass was 18.18% (4/22), 36.36% (8/22), 45.45% (10/22) respectively, while, the frequency of *V. alginolyticus* was 46.5% (27/58), 18.9% (11/58) and 34.5% (20/58) in Nile tilapia, Carb and Sea bass respectively.

Antimicrobial susceptibility testing results

Phenotypic antimicrobial susceptibility was investigated using nine antimicrobial agents. *V. parahaemolyticus* isolates displayed 100% resistant against penicillin and ampicillin respectively. A relative lower resistance was displayed against tetracycline 27.3% (6/22), imipenem 18.2 % (4/22) and nalidixic acid 9.1 % (2/22) (Table 4). Similarly, *V. alginolyticus* isolates were found to be highly resistance to ampicillin (100%, 58/58) and penicillin (94.8%, 55/58). An intermediate resistance was determined against erythromycin (55.2%, 32/58), nalidixic acid (30/58, 51.7%), trimethoprim-sulfamethazole (24/58, 41.4%) and a lower resistance was displayed against tetracycline (18/58, 31.03%), ciprofloxacin (6/58, 10.3%) and gentamycin 2/58 (3.44%) and imipenem (0/58, 0.00%) (Table 4).

Molecular identification of Virulence associated genes

The identified *V. parahaemolyticus* strains were tested for the presence of toxin genes, the results of the PCR assay revealed the *tlh* identified in all *V. parahaemolyticus* isolates. While *trh* was identified in 6 *V. parahaemolyticus* isolates, while none of *V. parahaemolyticus* isolates carried *tdh* gene (Figure 4, 5).

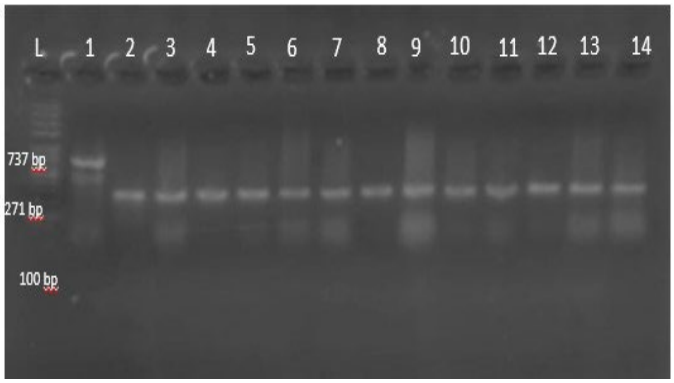

Fig. 1. Agarose gel electrophoresis showing multiplex PCR amplification of collagenase gene in *V. haemolyticus* at 271bp (lane 1) and in *V. alginolyticus* at 737bp (lane 2-14), L: DNA ladder.

Table 2. The prevalence of *Vibrio* species in the examined fish types.

Fish type	Species	Number of examined samples (n=200)	No. of positive samples
Fresh water fish	Nile tilapia (*O. niloticus*)	100	31
Marine water fish	Crab (*Brachyura*)	50	19
	Sea bass (*Dicentracus labrax*)	50	30

Table 3. Distribution of *Vibrio* species in the examined fish species.

Fish type	*V. parahaemolyticus* (n=22)	*V. alginolyticus* (n=58)
Nile tilapia (*O. niloticus*)	4 (18.18%)	27 (46.5%)
Crab (*Brachyura*)	8 (36.36%)	11 (18.9%)
Sea bass (*Dicentracus labrax*)	10 (45.45%)	20 (34.5%)

Table 4. Antimicrobial susceptibility testing results of *V. parahaemolyticus* and *V. alginolyticus* isolates.

Antimicrobial agent	Antimicrobial class	Concentration	*V. parahaemolyticus*		*V. alginolyticus*	
			Resistant	Sensitive	Resistant	Sensitive
Penicillin	b-lactam	10	22 (100%)	0 (0.00%)	55 (94.8%)	3 (5.2%)
Ampicillin	b-lactam	10	22 (100%)	0 (0.00%)	58 (100%)	0 (0.00%)
Trimethoprim-sulfa methazole	Folate pathway Inhibitors	25	11 (50%)	11 (50%)	24 (41.4%)	34 (58.6%)
Ciprofloxacin	Fluoroquinolone	5	14 (63.4%)	8 (35.4%)	6 (10.3%)	52 (89.7%)
Erythromycin	Macrolide	15	9 (40.9%)	13 (59.1%)	32 (55.2%)	26 (44.8%)
Tetracycline	Tetracycline	30	6 (27.3%)	16 (72.7%)	18 (31.03%)	40 (68.9 %)
Gentamycin	Aminoglycoside	10	13 (59.1%)	9 (40.9%)	2 (3.44%)	56 (96.6%)
Nalidixic acid	Quinolone	30	2 (9.1 %)	20 (90.9%)	30 (51.7 %)	28 (48.3%)
Imipenem	Carbapenem	10	0 (00.0 %)	22 (100%)	0 (0.00 %)	58(100%)

Fig. 2. Phylogenetic tree showing the genetic relatedness among *V. parahaemolyticus* based on nucleotide sequence analysis of the collagenase gene. Strain in this study is labeled with yellow color.

Fig. 3. Phylogenetic tree showing the genetic relatedness among *V. alginolyticus* based on nucleotide sequence analysis of the collagenase gene. Strain in this study is labeled with purple color. OR396991

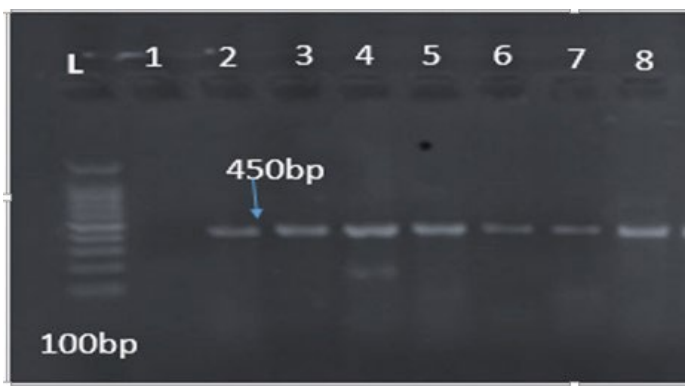

Fig. 4. Agarose gel electrophoresis showing amplification of *tlh* gene in *V. haemolyticus* at 450 bp.

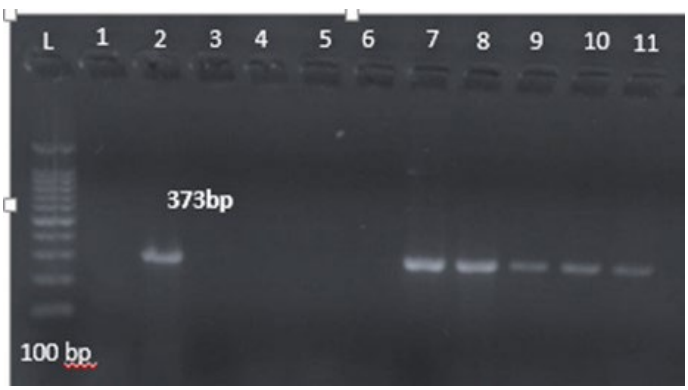

Fig. 5. Agarose gel electrophoresis showing amplification of *trh* gene in *V. haemolyticus* at 373 bp.

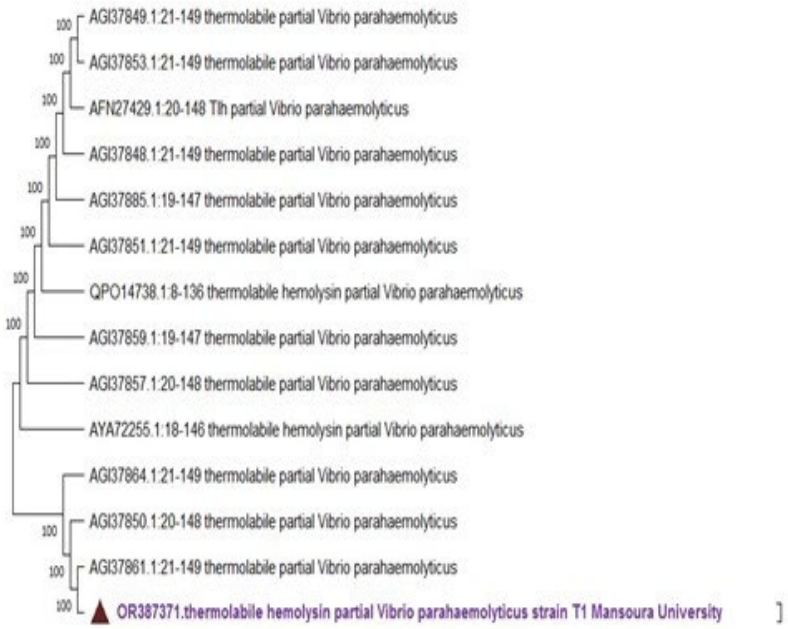

Fig. 6. Phylogenetic tree showing the genetic relatedness among *V. parahaemolyticus* based on nucleotide sequence analysis of the *tlh* gene. Strain in this study is labeled with yellow color. OR387371.

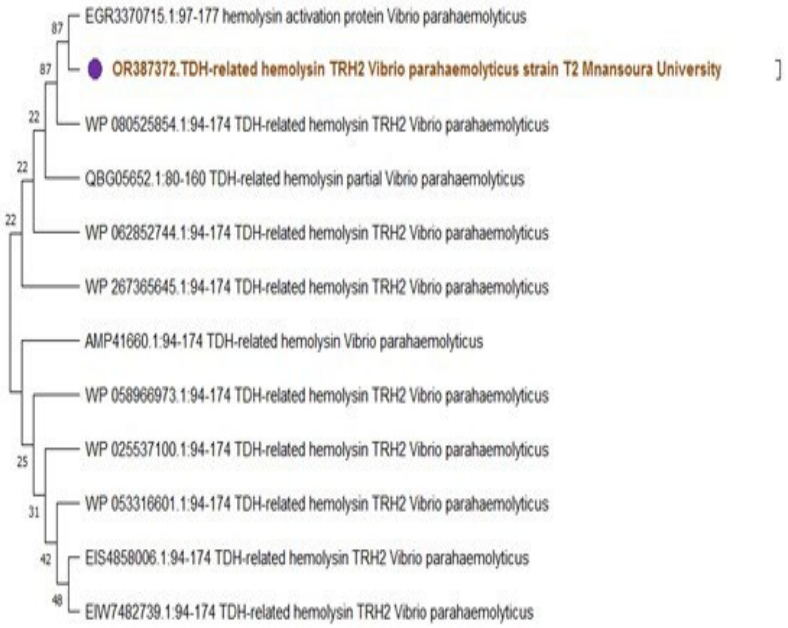

Fig. 7. Phylogenetic tree showing the genetic relatedness among *V. parahaemolyticus* based on nucleotide sequence analysis of the *trh* gene. Strain in this study is labeled with yellow color. OR387372

DISCUSSION

*Vibrio*sis is commonly responsible for the substantial mortality rate of fish in marine and aquaculture systems across the world with high economic losses (Dar *et al*., 2020; Sanches-Fernandes *et al*., 2022; Xu *et al*., 2022). The main reason for the infection with this pathogen could be related to consumption of raw or under cooked seafood. Therefore, *Vibrio* spp. infection poses significant threats to the public health (Ziarati *et al*., 2022). The current study performed to assess the prevalence, virulence determinants and antimicrobial resistance profile of *Vibrio* spp. from fishes widely sold in Egyptian seafood markets, such as Nile Tilapia (*O. niloticus*), Crab (*Brachyura*), and seabass (*Dicentracus labrax*) to study their involvement in transferring *Vibrio* spp. to people. The rapid spread of resistance among harmful bacteria, especially *Vibrio* spp., is a serious issue for public health and the development of antibiotics (Zhu *et al*., 2017; Abdelaziz *et al*., 2017; Helmi *et al*., 2020; Pepi and Focardi, 2021).

The current study verified that 40% (80/200) of naturally infected fish contaminated with *Vibrio* species based on bacteriological assessment using TCBS which is a selective medium giving green colonies with *V. alginolyticus* as it is a sucrose fermenter while yellow colonies with V. parahaemoloyticus as it is a non-sucrose fermenter (Letchumanan *et al*., 2014). Which is similar to El-Sharaby *et al*. (2018) who reported the infection rate of 39% (79/200) in the delta region. While Abd-Elghany and Sallam (2013) and Sadat *et al*. (2021) reported that infection rate of 33.3% and 30.67% respectively from Mansoura City. The lower infection rate was found in China (12.14%) of 420 samples tested positive for *V. parahaemolyticus*, including 19 (9.05%) and 32 (15.24%) samples from restaurants and supermarkets in China, respectively (Xie *et al*., 2019) and 34.7% in Korea (Mok *et al*., 2021). The highest infection rate of *V. parahaemolyticus* in Malaysia was 79.5% (310/390) in short mackerel gills. The current study demonstrated that the

isolation rate of *V. parahaemolyticus* was 11% (22 /80) and *V. alginolyticus* 29% (58 /80) so the predominant species was *V. alginolyticus* followed by *V. parahaemolyticus* which similar to Gobarah *et al*. (2022) that revealed that the most common species was *V. alginolyticus* (16.00%), followed by V. cholerae (7.33%) and *V. parahaemolyticus* (5.33%). The predominance of *V. alginolyticus* was also reported by many other studies worldwide (Abdel-Aziz *et al*., 2013; Al-Taee *et al*., 2017; Deng *et al*., 2020; Sadat *et al*., 2021). *V. vulnificus* was not isolated from this study which is similar to Sadat *et al*. (2021) and Haque *et al*. (2023).

The isolation rate in marine water fish (Crab and Sea Bass) was 24.5% higher than in freshwater fish (*O. niloticus*) 15.5% consistent with Das *et al*., (2009) who recorded high infection rate from marine fish (21.74%) and estuarine fish (16.04%) than freshwater fish (13.83%). Similarly, Lee *et al*. (2018) reported that marine fish samples had a greater prevalence (58%) than freshwater species (42%), on the contrary to Sadat *et al*. (2021)demonstrated that freshwater fish had a greater isolation rate of *V. parahaemolyticus* (32%; 16/ 42) than marine species (12.67%).

The frequency distribution of *V. parahaemolyticus* isolates in Nile tilapia, Carb and Sea bass was 4 (18.18%), 8 (36.36%), 10 (45.45%) respectively, while the frequency of *V. alginolyticus* was 27 (46.5%), 11 (18.9%) and 20 (34.5%) in Nile tilapia, Carb and Sea bass respectively. *V. alginolyticus* was noted to infect various marine creatures such as silver sea bream, stone crab, and prawn (Xie *et al*., 2005; Jia *et al*., 2010; Martins *et al*., 2010; Xu *et al*., 2013).

V. alginolyticus was found to be more common than 50% in studies on the frequency of *Vibrio*s in seafood from Morocco (Bouchriti *et al*., 2001). The prevalence of *V. alginolyticus* has been found in earlier research to be 72% in fisheries goods sold in Casablanca (Cohen *et al*., 2007) and 71% in the marine ecosystem of the Bay region Tamouda, with peak concentrations during the warmer months. This resulted in the temperature is the main element controlling the concentration of *V. alginolyticus* (Sabir *et al*., 2011).

Antimicrobial resistance has emerged as a major public health concern, and *V. parahaemolyticus* is becoming more and more resistant to antibiotics in marine habitats (Elmahdi *et al*., 2016; Zhao *et al*., 2018). The public health may be seriously threatened by multidrug resistance in *V. parahaemolyticus* strains (Al-Othrubi *et al*., 2014; Shaw *et al*., 2014; Ryu *et al*., 2019). In this study, penicillin and ampicillin were the least effective against *V. parahaemolyticus* (resistance rate = 100 and 92%, respectively) and *V. alginolyticus* (94.8%, and 100%, respectively). Significant incidence of ampicillin resistance in *V. parahaemolyticus* strains was reported worldwide (Kang *et al*., 2016; Obaidat *et al*., 2017; Mok *et al*., 2021; Sadat *et al*. 2021; Meng *et al*., 2023). The increased ampicillin resistance may be related to the production of β-lactamase and decreased peptidoglycan (PG) synthesis activity as a result of decreased penicillin-binding protein (PBP) transcription (Han *et al*., 2007; Melo *et al*., 2011; Ottaviani *et al*., 2013).

The current study, *V. parahaemolyticus* showed the moderate resistant against gentamycin, trimethoprim-sulfamethazole, ciprofloxacin and erythromycin were 59.1% (13/22), 50% (11/22), 63.4% (14/22) and 40.9% (9/22), respectively. A relative lower resistance was displayed against tetracycline 27.3% (6/22), imipenem 18.2 % (4/22) and nalidixic acid 9.1 %. Contrarily to Venggadasamy *et al*. (2021), the *V. parahaemolyticus* isolates were extremely susceptible to nalidixic acid, 95.3%; gentamicin, 93% and tetracycline, 74%. While Igbinosa (2016) demonstrated that *Vibrio* isolates were 50.8% resistant to nalidixic acid and 91% resistant to erythromycin. In addition to other beta-lactams including carbenicillin, aztreonam, cefotaxime, ceftazidime, cefuroxime, and imipenem, a large percentage of the isolates was also sensitive to additional quinolones such ciprofloxacin and norfloxacin.

In this study, *V. alginolyticus* isolates were found to be highly resistance to ampicillin (100%, 58/58) and penicillin (94.8%, 55/58) which is Similar to Korun& Karaca (2013) and Sadat *et al*. (2021). While an intermediate resistance was determined against erythromycin (55.2%, 32/58), nalidixic acid (30/58, 51.7%), trimethoprim-sulfamethazole (24/58, 41.4%) and a lower resistance was displayed against tetracycline (18/58, 31.03%), ciprofloxacin (6/58, 10.3%) and gentamycin 2/58 (3.44%), imipenem (0/58, 0.00%) consistent with Sadat *et al*. (2021). *V. alginolyticus* revealed that the bacterium was extremely resistant to naldixic acid, erythromycin, ciprofloxacin, and chloramphenicol, on the other hand, showed moderate sensitivity. Additionally, it was shown that the bacteria was extremely susceptible to oxytetracyclin, gentamycin (Abdel-Aziz *et al*., 2013; Khalil *et al*., 2014; Younes *et al*., 2016; Hernández-Robles *et al*., 2016; Khafagy *et al*., 2018).

The thermolabile hemolysin (*tlh*) gene is a biomarker that has been used to detect *V. parahaemolyticus* in a variety of food systems (Su and Liu, 2007). A PCR experiment showed that *tlh* was detected in all *V. parahaemolyticus* isolates. *Tdh* and *Trh* are the major virulence factors in *V. parahaemolyticus* and they are primarily responsible for *V. parahaemolyticus* infections (Matsuda *et al*., 2010; Shimohata and Takahashi, 2010; Yanagihara *et al*., 2010). The presence of *tdh* and/or *trh*-positive isolates in freshwater and marine fish is indicative of human health (Lee *et al*. 2018). In this study, *trh* was found in 6 isolates (3%, 6/200) in *V. parahaemolyticus*. Similarly, Letchumanan *et al*. (2015) reported that only 6.5% (13/200) of the isolates of *V. parahaemolyticus* obtained from shellfish samples were *trh*-positive and none of the samples were *tdh*-positive. On the contrary, Tran *et al*. (2020) reported absence of *tdh* and *trh*. Therefore, it is discovered that the majority of *V. parahaemolyticus* strains recovered from seafood samples lack the *tdh* and *trh* genes. Also obtained from clinical tissues and described in several investigations are *V. parahaemolyticus* strains lacking the virulence genes *tdh* and *trh* (FDA, 2005; Bhoopong *et al*., 2007; Jones *et al*., 2012; Li *et al*., 2014; Gutierrez West *et al*., 2013; Pazhani *et al*., 2014). Also, many investigations have revealed a low prevalence rate (less than 5%) of pathogenic *V. parahaemolyticus* isolates containing *tdh* and/or *trh* genes in the environment and food sources (Parveen *et al*., 2008; Zulkifli *et al*., 2009; Tsai *et al*., 2013). The *tdh* gene was also detected at a lower level than the *trh* gene, were also obtained by Bilung *et al*. (2005); Ottaviani *et al*. (2013) and Yen *et al*. (2021). On the other hand, a higher prevalence of the *tdh* gene than the *trh* in *V. parahaemolyticus* marker was observed in Spain and in Thailand (Roque *et al*., 2009; Mala *et al*., 2016).

CONCLUSION

The wide spread of virulence genes across the studied strains suggested a possible concern for humans, requiring consumers to raise their awareness, assure correct seafood preparation, and steer clear of undercooked or contaminated fish. Misuse of antibiotics should be avoided as antimicrobial resistance will be developed and affect the aquaculture. Additionally, when temperatures rise and the climate changes, bacteria adapt and an enormous increase in antibiotic resistance occurs. Keeping the guidelines of sustainability is vital for a beneficial expansion of the resources given by the aquaculture industry protecting human and animal health and in harmony with the environment, taking into consideration the recommendations of the 2030 Agenda based on Global Development Goals. By adopting the strictest hygienic regulations, maintaining clean fish farms, introducing new antibiotics against infections, and eventually developing vaccines that minimize of infections in fish.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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