

MOLECULAR CHARACTERIZATION OF *VIBRIO PARAHAEMOLYTICUS* ISOLATED FROM SOME MOLLUSKS, SHELLFISH AND THEIR HARVESTING WATER FROM SUEZ CANAL AREA

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ABSTRACT

The study included 410 samples of shellfish (164 clams, 86 mussels, and 160 shrimps) collected from Suez Canal area as the following: 156 from Port Said, 164 from Ismailia and 90 from Suez governorates. The harvesting water samples (48) were collected from the three sites. All samples were collected during the warmest season (June, July, and August) through two years. After extraction of muscle tissue and homogenization, enriched samples were identified by plating onto TCBS agar medium. Presumptive *V. parahaemolyticus* colonies were selected, purified, and further identified by API20 and PCR techniques targeting *toxR* gene. The pathogenicity of the isolates was examined by detection of Thermostable Direct Haemolysin (TDH) and related Haemolysin (TRH) genes. Results revealed that the overall prevalence of *V. parahaemolyticus* in shellfish was 38/410 (9.27%), where as in water was 12/48 (25%). Higher contamination rate was detected in shrimp (15%), and the highest prevalence was recorded in Ismailia governorate (12.2%). The detection rate of TDH and TRH genes was 21.05% and 5.26% consequently indicating its health hazards to the consumers. This study concluded that the examined shellfish may have the potential human health risk associated with the presence of pathogenic *V. parahaemolyticus*. The preventative measures should be considered.

Key words: *Vibrio parahaemolyticus*, Suez Canal, Shellfish, Water, Zoonoses.

INTRODUCTION

Vibrio parahaemolyticus is a Gram-negative halophilic and mesophilic bacterium, commonly found in estuarine environment (McCarter 1999; Su and Liu, 2007). *V. parahaemolyticus* is considered a natural pathogen of the aquatic environment and is also inhabited by fish, shellfish, shrimp, crayfish, oysters, and lobster and other aquatic organisms (Cook *et al.*, 2002; Lee *et al.*, 2008; Yamamoto *et al.*, 2008). It has been reported that the bivalves are filter feeders which accumulate environmental bacteria in their gills and digestive glands becoming potential vectors for many pathogens including *Vibrio* species (Potasman *et al.*, 2002).

Twelve *Vibrio* species have been recognized as potential foodborne disease agents in humans, of which, *V. parahaemolyticus* is the most common (Adams and Moss, 2008). Several *Vibrio* bacteria

species can cause serious disease in humans or animals. Although strains of *V. parahaemolyticus* are environmental, many strains are pathogenic to humans. *Vibrio vulnificus* and *V. parahaemolyticus* are the most common types of foodborne and wound infections. Virulent strains can cause wound infections, septicemia, or more commonly acute gastroenteritis which is acquired through the consumption of raw or undercooked seafood, especially shellfish.

Outbreaks of V. parahaemolyticus has now been detected worldwide (Nair, 2007). In Egypt, few studies on *V. parahaemolyticus* in shellfish have been conducted and almost all of them in the north of Egypt or markets that sell shellfish and oysters hunted from the Northern Egypt with an incidence ranged from 2.6 to 16.6% reached 31% (Eissa *et al.*, 2011, Merwad *et al.*, 2011, Abdel-Elghany and Sallam, 2013).

Not all *V. parahaemolyticus* strains have the same pathogenic potential. Pathogenicity is strongly correlated with two well-characterized hemolysins, the thermostable direct hemolysin (TDH) and the TDH-related hemolysin (TRH). To determine the real

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risk posed to human health by the presence of *V. parahaemolyticus* in seafood, the identification of this microorganism should be followed by the detection of the genes responsible to produce TDH and TRH toxins. The TDH and TRH genes are known to occur in 99% of clinical strains whereas; their presence in environmental strains is relatively rare (2–3%) (Nishibuchi and Kaper, 1995). The virulence of *V. parahaemolyticus*, among other virulence attributes, depending on the presence of a thermostable direct hemolysin (TDH gene) and/or the thermostable direct hemolysin related (TRH, gene) (Ceccarelli *et al.*, 2013; Raghunath, 2015).

Recognized infections from *Vibrio* species are on the rise, and although there is some uncertainty, most researchers predict that climate change will increase cases (Burge *et al.*, 2014). Outbreaks have been reported in many countries such as the USA, France, and New Zealand because of the increase in seafood consumption and the global warming, which may be resulting in a higher prevalence of *Vibrio* species and increase the risk of *Vibrio*-borne infections (Cruz *et al.*, 2015; Esteves *et al.*, 2015).

The present study aimed to investigate the prevalence of *V. parahaemolyticus* in mollusks, shellfish and its harvesting water at Suez Canal area, and to assess its pathogenicity.

MATERIALS AND METHODS

Sample collection and preparation

This study included the analysis of a total of 410 samples of wild shellfish consisted of 164 clams, 86 mussels and 160 white shrimps (*Penaeuslatisulcatus*). Samples were collected from the three Governorates of the Suez Canal area (Port Said, Ismailia and Suez) Governorates. Shellfish samples were collected from the Suez Canal Gulf and Suez Canal and the Mediterranean Sea that were routinely sold out at the fish market. In addition, 48 harvesting water samples were collected from the same collection areas. All samples were collected during the warmest seasons (June, July, and August) each sampling year through two years. Samples were transported with minimal delay under chilling conditions to the laboratory. Samples were analyzed and processed quickly upon arrival to the laboratory. The term sample was represented by pooled shellfish to provide 25g of the soft tissue to be prepared for *Vibrio* isolation, one sample represented by 25 grams of the shellfish after un-shelling under sterile condition which was collected from an average from 5-10 shell fish according to the species and the size.

Isolation and biochemical identification of *V. parahaemolyticus* was carried out according to Kaysner and DePaola, (2004). Samples were

disinfected externally before being shelled on a clean sterile surface using sterile forceps and scissors.

Fifty grams of shelled samples and 50 ml water from water samples was placed in a stomacher bag containing 450 ml sterile PBS (Oxoid) and mixed in a stomacher for 60 s. This constitutes the 1:10 dilution. Prepare 1:100, 1:1000, 1:10,000 dilutions.

The homogenated samples were enriched by incubation at 35 ±2°C for 24 h. Alkaline peptone water (APW) tubes were incubated overnight at 35 ±2°C. A loopful from the top 1 cm of APW tubes containing the three highest dilutions of sample showing growth were streaked on to plates of Thiosulfate Citrate Bile Sucrose agar (TCBS) (Oxoid) and incubated at 35 ±2°C for 24 h. Presumptive *Vibrio sp.*, that appear round, opaque, green or bluish colonies, 2 to 3 mm in diameter on TCBS agar were selected and purified on new TCBS agar plates. Each single colony was screened for Gram staining, motility, cytochrome oxidase, urease activity, NaCl requirement (0%, 1%, 3%, 6%, 8%, or 10%), citrate utilization test, triple sugar iron agartest, arginine dehydrolase test, lysine and ornithine decarboxylase tests, O/129 sensitivity test, Vogese Proskauer test, indole test, Onitrophenyl-b-d- galactopyranoside (ONPG) hydrolysis and acid production from sucrose, lactose, arabinose, cellobiose, mannitol and mannose. The suspected colonies were further confirmed by both API20E test (BioMerieux, France). The reference of *V. parahaemolyticus* strains (ATCC 17802) was used as positive control for the PCR reaction.

Molecular identification of *V. parahaemolyticus* and detection of TDH and TRH genes.

DNA extraction

DNA extraction from enriched samples was carried out by the boiling method as described by Tunung *et al.* (2012). For the isolation of chromosomal DNA, 5 ml from each bacterial culture in nutrient broth (Oxoid CM0001) were incubated over night at 37°C, followed by centrifugation at 3000 rpm for 15 min., the supernatant was discarded and the collected cells were suspended in 1.5 ml TE (Trise-EDTA) buffer and transferred into 2 ml centrifuge tubes and centrifuged for 1 min at 8000 rpm to collect the washed cell pellet. The supernatant was then discarded and the bacterial pellet was resuspended with 500 µl of TE and incubated at 37°C for 2 h after the addition of lysozyme (10 mg/ml) and RNase (50mg/ml). The molecular experiments were performed in the Laboratory of Zoonoses, Department of Animal Hygiene and Zoonoses, Faculty of veterinary medicine, Suez Canal University.

PCR amplification reactions

All the identified colonies as *V. parahaemolyticus* were subjected to PCR targeting species-specific gene *toxR* gene to be confirmed as *V. parahaemolyticus*.

The biochemically identified *V. parahaemolyticus* isolates were further verified by molecular characterization using PCR tool for the presence of species-specific gene (*toxR* gene).

In this study, Chromosomal DNA from all the conventionally identified *V. parahaemolyticus* (n ¼ 89) was used as a template for PCR amplification using two sets of specific oligonucleotide primers for *toxR* gene. Amplified bands of the expected molecular size of 251 bp, agarose gel electrophoresis for PCR analysis of TDH gene (A) and TRH gene (B) in *V. parahaemolyticus* isolated from different shellfish samples. Chromosomal DNA from the *toxR* gene-positive isolates was used as a template for PCR amplification using a specific oligonucleotide primers for TDH and TRH genes.

The molecular experiments were performed in the Laboratory of Zoonoses, Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Suez Canal University, using primers sequences Forward: 5-GTCTTCTGACGCAATC GTTG-3 and Reverse: 5-ATACGAGTGGTTGCTGT CATG-3, which amplify an amplicon of 367 bp as described by Kim *et al.* (1999). In addition, TDH and TRH pathogenicity genes were also examined in the isolates using primers sequences as following TDH Forward: 5-CCACTACCACTCTCATATGC-3, 5-GGTACTAAATGGCTGACATC-3, amplifying an amplicon size of 251bp and TRH forward: 5-GGCTCAAAATGGTTAAGCG-3, REVERSE 5-CATTTCCGCTCTCATATGC-3 amplifying an amplicon size of 250 bp as described by Tada *et al.* (1992) and cited in the following table.

The primers were ordered from Operon Company, (Operon, Japan) as nucleotide sequence. All primers were diluted according to the company instructions using sterile distilled water.

Target gene	Forward primers sequences	Reverse primers sequences	Amplicon size	References
ToxR	5-gtcttctgacgcaatcgttg-3	5-atacgagtggtgctgtcatg-3,	367	Kim <i>et al.</i> (1999)
TDH	5-ccactaccactctcatatgc-3	5-ggtactaaatggctgacatc-3,	251	Tada <i>et al.</i> (1992).
TRH	5-ggctcaaaatggttaagcg-3,	5-catttccgctctcatatgc-3	250	Tada <i>et al.</i> (1992).

Each PCR reaction mixture consisted of a final volume of 25 µl divided to 5 µl of the extracted DNA, 12.5 µl of 2X PCR Master Mix (Bioteke corporation) [20µl of Phosphate buffered saline (PBS) pH 7.3., Ten volumes of Tris-EDTA buffer (TE) pH 8.0, 1.5 mM MgCl₂, 10 mM Deoxy Nucleotide Triphosphate solution (dNTPs) and Ampli Taq DNA polymerase (1unit/µl)], 0.5 µl of each primer (5 pmol concentration) and 6.5 µl sterile distilled water. The PCR assays were performed using a Thermal Cycler (Eppendorf).

The amplification procedure consisted of an initial denaturation step at 94 °C for 2 min, followed by 30 cycles with denaturation at 94°C for 30 secs, annealing for 45 s at 57°C and extension at 72 °C for 30 s. A final extension step was carried out at 72 °C for 5 min. Aliquots from amplification reactions were analyzed by 1% agarose gel electrophoresis and viewed and photographed under UV light using gel documentation system (Biospectrum 310 imaging system). The reference of *V. parahaemolyticus* strains (ATCC 17802) was used as positive control for the PCR reaction.

Statistical analysis

Chi-square was used for calculation of significance between the prevalence at P <0.01 using Graph Pad Quick Calcs program.

RESULTS

Total prevalence of *Vibrio* species from shellfish examined

As tabulated in Table 1, results revealed that the total prevalence of *Vibrios* species isolated from the shellfish examined was 70/410 (17.07%). The total isolation rates of *Vibrio* species were 14.63% from clams, 11.63% from mussel, and 22.5% from shrimp. The isolation rate of *Vibrio* species was highest in Port Said (20.51%), followed by Ismailia 19.12% and Suez 6.67% Governorate.

Prevalence of *V. parahaemolyticus* among Governorates of Suez Canal area

As tabulated in Table 2, results revealed that the total prevalence of *V. parahaemolyticus* isolated from the shellfish examined was 38/410 (9.27%).

The total isolation rates of *V. parahaemolyticus*, were (6.01%) from clams, (4.65%) from mussels, and (15%) from shrimp. The isolation rate of *V.*

parahaemolyticus was highest in Ismailia Governorate (12.2%) followed by Port-said Governorate (8.94%) and Suez (4.44%). There were no significant differences between the two years of sampling using Chi-square.

Percentage of the prevalence of *V. parahaemolyticus* in relation to *Vibrio* spp.

As illustrated in Table3, out of 70 isolates of *Vibrio* species, 38 (54.29%) was confirmed as *V. parahaemolyticus* by microbiological and molecular techniques. The percentage of isolation of *V. parahaemolyticus* to *Vibrio* species was 24 out of 36 (66.67%), where as it was nearly similar in clams 10/24 (41.67%), and 4/10 (40%) in mussels. Regarding the location, the highest percentage of *V. parahaemolyticus* was in Suez 4/6 (66.67%), followed by Ismailia 20/32 (62.5%), and Port Said 14/34 (41.18%).

Detection of the virulence genes TDH and/or TRH genes among the isolates

As tabulated in Table4, the total detection rate of the TDH gene was 8/38 (21.05%), whereas the detection rate of TRH gene was 2/38 (5.26%). The positive TRH gene isolate was also TDH gene positive.

The Isolation rate of *V. parahaemolyticus* and distribution of TDH and TRH genes in the water samples

Among 48 water samples, 20 (40%) was *Vibrio* species positive. Of them, 12 (25%) was identified as *V. parahaemolyticus*. TDH gene was detected in 2/12(16.67%) of water *V. parahaemolyticus* samples where as TRH gene was not detected.

Fig. (1) illustrates the Lane L that indicated the 100 bp DNA ladder, lanes number 1,2, and 3 indicated positive samples yielding an amplified band size of 251bp, Lane C+ and C- indicated control positive and control negative samples, respectively. And Fig. (2) illustrates the Lane L that indicated the 100 bp DNA ladder, lanes number 1,2, and 3 indicated positive samples yielding an amplified band size of 367 bp, Lane C+ and C- indicated control positive and control negative samples, respectively.

Table 1: Total prevalence of *Vibrio* species from shellfish examined.

Shellfish	Geographical location			Total
	Port Said	Ismailia	Suez	
Clams	14/56 (25%)	10/80 (12.5%)	0/28 (0%)	24/164 (14.63%)
Mussel	6/24 (25%)	4/28 (14.29%)	0/34 (0%)	10/86 (11.63%)
Shrimp	12/76 (7.89%)	18/56 (32.14%)	6/28(21.43%)	36/160 (22.50%)
Total	32/156 (20.51%)	32/164 (19.12%)	6/90 (6.67%)	70/410 (17.07%)

Table 2: Total isolation rate of *V. parahaemolyticus* from shellfish.

Shellfish	Geographical locations			Total
	Port Said	Ismailia	Suez	
Clams	4/56 (7.14%)	6/80 (7.5%)	0/28(0)	10/164(6.01%)
Mussel	2/24 (8.33%)	2/28 (7.14%)	0/34 (0)	4/86 (4.65%)
Shrimp	8/76 (10.53%)	12/56 (21.43%)	4/28 (14.29%)	24/160 (15%)
Total	14/156 (8.94)	20/164 (12.20%)	4/90 (4.44%)	38/210 (9.27%)

Table 3: Percentage of *V. parahaemolyticus* in relation to *Vibrio* spp.

Shellfish	Geographical locations			Total
	Port Said	Ismailia	Suez	
Clams	4/14 (28.57%)	6/10 (60%)	0/0 (0)	10/164 (6.10%)
Mussel	2/6(33.33%)	2/4 (50%)	0/0 (0)	4/86 (4.65%)
Shrimp	8/12(66.67%)	12/18(66.67%)	4/6(66.67%)	24/160 (15%)

Total	14/32 (41.18%)	20/32 (62.5%)	4/6(66.67%)	38/410 (9.27%)
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Table 4: Detection of the virulence genes TDH and/or TRH genes among the *V. parahaemolyticus* isolates.

Shellfish	Pathogenicity genes		
	TDH	TRH	TDH/TRH
Clams	2/10(20%)	0/10 (0%)	0 (0%)
Mussel	0/4 (0%)	0/4 (0%)	0 (0%)
Shrimp	6/24(25%)	2/24 (8.33%)	2/24 (8.33%)
Total	8/38 (21.05%)	2/38 (5.26%)	2/24 (8.33%)

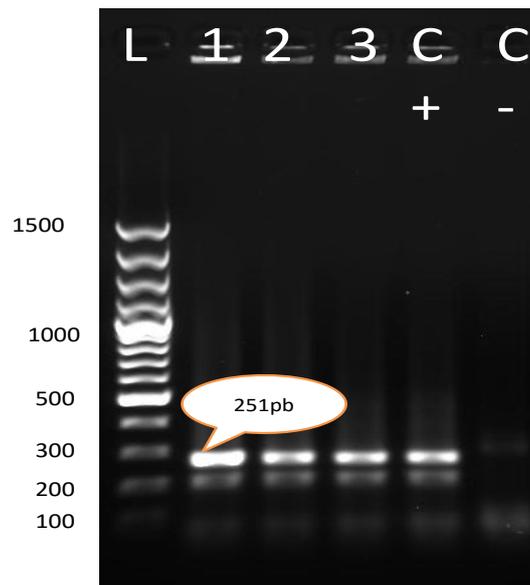


Fig. (1): Agarose gel electrophoresis for PCR analysis of *toxR* gene in *V. parahaemolyticus* isolated from different shellfish samples. Lane L indicated the 100 bp DNA ladder, lanes number 1,2, and 3 indicated positive samples yielding an amplified band size of 251bp, Lane C+ and C- indicated control positive and control negative samples.

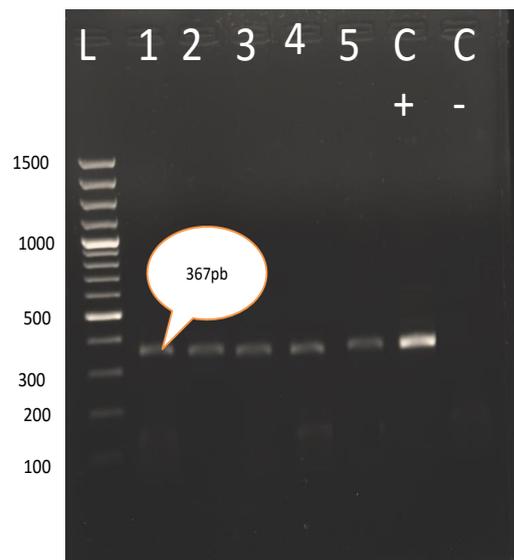


Fig. (2): Agarose gel electrophoresis for PCR analysis of TDH gene (A) and TRH gene (B) in *V. parahaemolyticus* isolated from different shellfish samples. Lane L indicated the 100 bp DNA ladder, lanes

number 1,2, and 3 indicated positive samples yielding an amplified band size of 367bp, Lane C+ and C- indicated control positive and control negative samples.

DISCUSSION

V. parahaemolyticus is a zoonotic pathogen and is one of the most significant foodborne pathogens causing gastroenteritis, wound infections, and septicemia (Pinto *et al.*, 2008; Letchumanan *et al.*, 2014). Although *V. parahaemolyticus* is disseminated throughout the world (Yeung and Boor, 2004; Su and Liu, 2007), rare pathogenic variants of *V. parahaemolyticus* can cause human gastric infections most of ten from the consumption of raw or improperly handled seafood and wound infections from recreational aquatic activities (McCarter, 1999; Scallan *et al.*, 2011).

In the north Egypt, shellfish is widely eaten in the Suez Canal area as, seafood dishes are preferred for both residents and visitors. The overall high seafood diet, as well as the widespread practice of eating seafood raw, could be associated with a potential risk of food poisoning in that area. Moreover, the Egyptian dietary habits neither including the eating of raw fish nor seafood, but shellfishes could be eaten improperly cooked.

In the present study, the most common shellfish sold in the markets in the Suez Canal area fish markets were freshly purchased and examined for the hazards of zoonotic *Vibrio* spp. contaminations. The overall prevalence of *Vibrio* species was 22.5%. These results were lower than that reported by Eissa *et al.* (2011) in Egypt (31.1%). Another study revealed that the total prevalence of *Vibrio* spp. in the Northeast of Egypt was 57.3% in shrimp, 54% in oysters (Merwad *et al.*, 2011). Incidence was 43.4% in shrimp in China (Xu *et al.*, 2016). The high prevalence of *Vibrio* species isolation from shellfish that was detected in the study area could be attributed to the collection of samples during the hot season. It has been reported that, *V. parahaemolyticus* may be detected year-round in locations where water temperatures do not drop below 15°C with the number of organisms detected in water, sediment and oysters increasing as water temperatures rise and the risk of exposure to an infectious dose of pathogens increases (Su and Lu, 2007; Johnson *et al.*, 2010; Eissa *et al.*, 2011). On the other hand, oysters may have a concentration of *V. parahaemolyticus* up to 100-fold higher than surrounding waters due to filter feeding particularly in the summer season which increases the chances of infection (Morris 2003). However, temperature and total abundance do not fully explain contamination rates with *V. parahaemolyticus* as some infections occur when water temperatures and abundance of total *V. parahaemolyticus* are low (Zimmerman *et al.*, 2007).

Results of the current study revealed a high prevalence of *V. parahaemolyticus* in shellfish in Suez Canal areas. This result was lower matched with another study conducted in the same study area which were 22.5% in shrimps and 16.7% in shellfish samples (Abd-Elghany and Sallam, 2013). Higher incidences of *V. parahaemolyticus* in shellfish were also reported in many studies. The incidence of *V. parahaemolyticus* in shellfish was 36.2% in China (Xu *et al.*, 2016). The high detection rate of *V. parahaemolyticus* in shrimp detected in the present study 9.27% was higher compared to 2.6% of another study (Merwad *et al.*, 2011). Controversy, Eissa *et al.* (2011) detected high incidences of *V. parahemolyticus* in marine shrimp in Suez-bay (31.1%).

In this study, *V. parahaemolyticus* was isolated from water samples collected from the study area. Similarly, Hassanin, (2007) identified 40% *V. parahaemolyticus* in water samples of fishing farm in Abu-Kir, Egypt. The overall prevalence of *Vibrio* spp. in water samples from Suez Canal was 25% (5 out of 20) (Abdel-Ghany and Sallam, 2013). Another study showed contamination rates of marine water in Egypt with *V. parahaemolyticus* (25%) (Merwad *et al.*, 2011). In contrary, lower incidences of *V. parahaemolyticus* in water were noticed in other studies. Mohamed *et al.* (2000) reported an isolation rate of 6.25% from River Nile tributaries at Damietta Province, Egypt. On the contrary, these results disagreed with Merwad *et al.* (2011), who did not detect *V. parahaemolyticus* in the same study area. It has been reported that *V. parahaemolyticus* levels in water are strongly correlated with turbidity during summer (Zimmerman *et al.*, 2007).

It is worth mentioning that these results are not accepted concerning the Egyptian standard (ES,2005), as it should be free from *V. parahaemolyticus*. Climate global change can play an important role in the dissemination of pathogenic microorganisms in the marine environment (Letchumanan *et al.*, 2015). The difference in the incidence of *V. parahaemolyticus* among samples from both sampling sites could possibly be contributed by the original source from which the shellfish were collected, post-harvest practices and hygiene standards applied during handling, transportation, and storage of seafood products. Moreover, the lack of hygiene, improper handling, cross-contamination and difference in storage temperature could be the possible cause of variation in *V. parahaemolyticus* incidences in samples from the supermarket (Yang *et al.*, 2008).

Concerning the zoonotic aspect, the hazardous pathogenic *Vibrio* causes life-threatening foodborne

infections and poses a considerable public health threat as agents of sporadic and epidemic human infections to be represented an important microbial group in the field of food safety (Rippey, 1994). *V. parahaemolyticus* was identified in 4% in diarrheic patients in Egypt (Merwad *et al.*, 2011; Abdel-Ghany and Sallam, 2013). A lower percentage of 2.4% in Damietta province, Egypt was cited (Mohamed *et al.*, 2000).

To assess the actual risk to human health posed by the presence of *V. parahaemolyticus* in seafood, the incidence of pathogenic strains need to be identified by detection of the toxigenic genes responsible for causing diseases in humans. Strains of *V. parahaemolyticus* carrying *tdh* and/or *trh* are considered pathogenic (Turner *et al.*, 2013). Previous investigations revealed that 1–2% of the environmental strains harbored the *TDH* and *TRH* genes under natural conditions (Alam *et al.*, 2002; Hervio-Heath *et al.*, 2002; Abd Elghany and Sallam, 2013).

TDH gene has been recognized as a primary virulence factor in pathogenic *V. parahaemolyticus* (Okuda *et al.*, 1997; Pinto *et al.*, 2008). TDH genes currently used as pathogenicity marker since most clinical isolates of *V. parahaemolyticus* possess this gene (Bej *et al.*, 1999; Nordstrom *et al.*, 2007). Generally, 0.2 to 3% of environmental *V. parahaemolyticus* isolates are potentially pathogenic based on the presence of TDH gene (Nordstrom *et al.*, 2007).

In the present study, TDH and TRH genes were detected (Fig.1), these results were higher than that detected by Abd-Elghany and Sallam, (2013) who found that 3/120 (2.5%) seafood samples were positive for one or both *tdh* and *trh* virulent. In a previous study conducted in Italy, one and three strains were positive for *tdh* and *trh* genes, respectively out of 35 *V. parahaemolyticus* isolates from mussels (Ottaviani *et al.*, 2005). *TDH+* *V. parahaemolyticus* was detected in 3.4% of oysters from India (Raghunath *et al.*, 2015) and 3.4% of oysters from New Zealand (Kirs *et al.*, 2011). Another study in Malaysia identified higher *trh*-positive 6.5% (13/200) while none was *tdh*-positive. Conversely, higher incidences of virulent *V. parahaemolyticus* isolates from seafood were identified in the USA by Bej *et al.* (1999) who could detect *tdh* and *trh* genes in 32.56% (14/43) and 23.3% (10/43), respectively in examined seafood isolates. Likewise, Terzi *et al.* (2009) in Turkey confirmed that 24 (75%) out of the 32 *V. parahaemolyticus* isolated from mussel were *tdh* and *trh* genes positive. Much higher incidences of 85% (17/20) of shellfish samples tested in Chile were positive for *tdh* (Fuenzalida *et al.*, 2007). Additionally, both *tdh* and *trh* genes were detected in 44% (12/27) and 52% (14/27), respectively in oyster samples tested in

Alaska (Nordstrom *et al.*, 2007). The percent 44-56% of Eastern oysters from Mexico (Zimmerman *et al.*, 2007). In total, 145 *V. parahaemolyticus* isolates were confirmed and tested for the presence of *trh* and *tdh*. None of the isolates possessed these genes (Xu *et al.*, 2016). *V. parahaemolyticus*, of the 38/71 (53.5 %) isolates were positive for the *trh* virulence gene and 71 (100 %) were negative for the TDH gene (Kang *et al.*, 2016). The differences in the frequency of the TDH and/or TRH pathogenicity genes may depend on the location, sample source, and detection methods (Alam *et al.*, 2002; Cook *et al.*, 2002; Hervio-Heath *et al.*, 2002). Therefore, continued monitoring of both the prevalence of *V. parahaemolyticus*, with surveys expanded to the national level, is important to ensure shellfish safety.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the results of this study constitute an indicator of *V. parahaemolyticus* contamination of a variety of seafood. Although virulent strains of *V. parahaemolyticus* can cause distinct diseases, including wound infections, septicemia, or more commonly acute gastroenteritis, pathogenicity is strongly correlated with two well-characterized hemolysins, the thermostable direct hemolysin (TDH) and the TDH-related hemolysin (TRH). Genes responsible for producing these toxins were detected in this study. The presence of these pathogenic organisms in seafood and water could pose a serious threat and hazard to susceptible people through consumption of raw or undercooked seafood, especially shellfish. Thus, it is recommended that: Monitoring of harvesting areas for the prevalence of *V. parahaemolyticus*, with continued surveys, expanded to the national level, considering various locations, sample source, and detection methods is important to ensure shellfish safety. In addition, post-harvest practices and hygiene standards applied during handling, transportation, and storage of seafood products including measures for preventing cross-contamination. Seafood should be adequately cooked before consumption to identify and implement proper public health measures for the control and prevention of diseases caused by pathogenic *V. parahaemolyticus*.

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التصنيف الجزيئي للفيبريوباراهايموليتيكس المعزول من بعض القشريات والمحاريات ومياه الصيد من منطقة قناة السويس

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يعتبر الفيبريوباراهايموليتيكس احد اسباب التسمم الغذائي من الاغذية البحرية. الهدف الرئيسي من هذه الدراسة تحديد مدى تواجد العترات المعزولة من الفيبريوباراهايموليتيكس في القشريات والمحاريات في منطقة قناة السويس و كذلك تقييم الخصائص الجزيئية. شملت الدراسة فحص ٤١٠ من المحاريات (١٦٤ من الرخويات - ٨٦ من بلح البحر - ١٦٠ من الجمبري) وجمعت العينات من محافظات قناة السويس. كما يلي ١٥٦ من بورسعيد, ١٦٤ من الإسماعيلية و ٩٠ من السويس. كما جمعت عينات من المياه (٤٨) من الثلاث مناطق السابقة خلال سنتين في شهور الصيف يونيو ويوليو وأغسطس. تم عزل الفيبريوباراهايموليتيكس وتصنيفه باستخدام الإختبارات البيوكيميائية وإختبار تفاعل البلمرة المتسلسل بهدف الكشف عن tox R جين. تم الكشف عن جينات الضراوة للمعزولات TDH & TRH. وأثبتت الدراسة أن أعلى نسبة تلوث بالميكروب في المحاريات كانت ٢٧% وفي المياه ٢٥% وقد كانت أعلى نسبة عزل جيني في عينات الجمبرى ١٥% وفي المحافظات الإسماعيلية ٢%. ١٢% وعزل جينات الضراوة TDH & TRH ١٥,٧٨% و ٨,٣٣% على التوالي والتي تسبب خطورة على المستهلك. خلصت الدراسة إلى ان تواجد ميكروب الفيبريوباراهايموليتيكس في المحاريات يشكل خطورة على صحة المستهلك وقد نوقشت الاحتياطات والشروط اللازمة الواجب اتباعها لمنع تلوث القشريات والمحاريات بمثل هذا الميكروب وكذلك لتجنب الإصابة بهذا النوع من التسمم الغذائي.