Genotyping of *Vibrio Parahemolyticus* Isolated from Some Marine Fish

Marwa Abd El-Hak[*], Ezzat M.[**] & Gihan M. O. Mohamed[***]

[*] *Veterinarian, fish farming and technology institute Suez Canal University*
***Department of Bacteriology, immunology Mycology- Faculty of Veterinary Medicine Suez Canal University*
****Department of Bacteriology Animal Health Research Institute Port-Said branch*

Abstract

A total 180 marine fish samples, 60 of each mullet (*Mugil cephalus*), seabream (*Sparus aurata*) and seabass (*Dicentrarchus labrax*), were randomly collected from different fish farms in Port-Said Governorate. Some of collected fishes displayed hemorrhages on the external body surface, base of fins and operculum (gill cover) with congested gills, pale liver and corneal opacity were noticed. Bacteriological examination of samples was collected from fish surface, liver, kidney, spleen, muscle and gills revealed that the highest level of the isolates were recorded in liver followed by kidney, spleen, muscle and gill.

Biochemical identification results of the isolated ***Vibrio*** species were revealed that 38 samples (21.11%) from the total collected samples were positive for the prevalence of ***Vibrio*** species at a percentage of (28.33%), (18.33%) and (16.67%) for mullet, seabream and seabass respectively.

Molecular identification of ***Vibrio*** species isolated from some marine fishes using 16S rRNA and *toxR* revealed that 32 strains of the isolated ***Vibrio*** were belong to ***V. parahaemolyticus*** with a prevalence of 15, 8 and 9 from mullet, seabream and seabass respectively.

Meanwhile molecular characterization of the identified ***V. parahaemolyticus*** by using PCR showed that all 32 isolates were harbored *recA* gene and only 25 isolates were harbored *tlh* gene. The public health hazards of this microorganism, as well as improve quality status of marine farms fishes were discussed.

Keywords: ***Vibrio parahaemolytius***, virulence, mullet, seabream, seabass fish, PCR, Public health.

Introduction

Fish are enriched with high nutritional components and concentrated source of energy and due to their high palatability and good digestibility, fish considered a

major food component for humans and animals ***(Kawarazuka, 2010)***. Within the past fifteen years, annual global production of aquaculture has more than tripled, that given about 39% of total global seafood production ***(Sapkota et al., 2008).***

The genus ***Vibrio*** is a ubiquitous bacteria gram-negative, curved-rod shape, comma shape bacteria occur naturally in estuarine or marine environments, which inhabit estuarine ecosystem ***(Schärer et al., 2011).*** Causing infection in human ***(Baker-Austin et al., 2018).***

Vibrio species are inhabitants of healthy fish and aquatic systems that can become pathogenic when conditions are stressful ***(Smith et al., 2012).*** They constitute a part of the indigenous microflora of aquatic habitats of various salinity and the major causative agents for some of the most serious diseases in fish, shellfish and shrimp ***(Sung et al., 2001).***

Vibriosis is one of the major and important bacterial diseases caused by ***Vibrio*** species, affecting marine fish results in high mortalities with severe economic losses worldwide ***(Samuelsson et al., 2006).*** This disease burden and mortality was contributors particularly in developing countries that have disputable sanitary conditions ***(Song et al., 2013).***

The classical form of vibriosis among the infected fish is described as a generalized septicemia with hemorrhages on the base of fins, exophthalmia and corneal opacity. Moribund fish were frequently anorexic with pale gills that reflect a severe anemia. Edematous lesions centered on the hypodermis were often observed ***(Toranzo et al., 2005).***

The outbreaks of vibriosis were a common problem among cultured marine fish particularly at summer season, as a result of the deterioration of basic water parameters as temperature, pH, dissolved oxygen, and salinity ***(Albert and Ransangan, 2013).***

A good example is ***V. parahaemolyticus***, a member of ***Vibrio*** species from the Vibrionaceae family. ***V. parahaemolyticus*** is a Gram-negative halophilic bacterium that is widely disseminated in estuarine, marine and coastal surroundings ***(Zhang and Orth, 2013).***

V. parahaemolyticus is a common cause of foodborne illness that causes gastroenteritis, diarrhea among population all over the world and responsible for 20-30% of the food poisoning cases in Japan and seafood borne diseases in many Asian countries ***(Christopher et al., 2011).*** Effective control measures at food service establishments and proper handling are required to prevent cross-contamination and to ensure food safety ***(Hunang et al., 2012).***

Infection in human is usually occur from exposure to seawater or consumption of raw or undercooked fish ***(Altekruse et al., 2000),*** causing a symptoms characterized

by diarrhea, primary septicemia, wound infections, or other extra-intestinal infections ***(Daniels et al., 2000).*** Once consumers eat undercooked or contaminated fish, illness is inevitable ***(Rahimi et al., 2010).***

Accurate identification is the basic step for diagnosis and implementation of appropriate preventive, curative and prophylactic measures in any aquaculture system. Thus to avoid false-positive results, all the biochemical identifications should be confirmed by means of molecular methods ***(Croci et al., 2007)*** such as polymerase chain reaction (PCR) that avoid the short comings of conventional methods. Thus the aim of this work was carried out to assessment the phenotypic and genotypic identification of ***V. parahaemolyticus*** in marine fishes with detection of some virulence genes from the isolates that contribute to the pathogenicity and may act as alarming public health concern using PCR.

Material and Methods

1-Samples collection:

A total of 180 marine fish samples, 60 of each *Mugil cephalus* (mullet), *Sparus aurata (*seabream) and *Dicentrarchus labrax* (seabass) were collected randomly from different fish farms in Port-Said Governorates. The samples were put in sterile polythene bags and transferred to the laboratory in an ice-box and examined bacteriologically as soon as possible for detection of ***V. parahaemolyticus*** from lesions, external body surface, gills, liver, kidneys, muscle and spleen of each sample.

2-Bacteriological examination:

2-1 Isolation and identification of *V. parahaemolyticus*:

Twenty-five grams of each of the examined fish samples (mullet, seabream and seabass) were weighed aseptically and homogenized in a stomacher 400 Circulator at 120 rev/for 2 min in stomacher bags containing 225 ml of alkaline peptone for water (APW) pH 8.6. The homogenate was incubated at 35°C for 18-24 hr ***(Kaysner and DePaola 2004).*** At the end of incubation period, and without shaking flask, 3-5 mm loopful of culture from pellicle (surface growth) of each flask was then streaked onto TCBS agar plates and incubated at 35°C for 18-24 hr.

Colonies at least 2 mm in diameter and green in case of non-sucrose fermenters were selected randomly from each TCBS plate after 18-24 hr incubation. Colonies were examined quickly after removal from the incubator. Colony of ***V. parahemolyticus*** grows on TCBS agar was blue to green centered colors according to ***MacFaddin (1985)***. Green colonies of suspected colonies were sub-cultured on TCBS medium then each purified isolates were streaked on tryptic soy

agar supplemented with 3 % NaCl and incubated at 35ºC for 18-24 hr then stained by Gram stain ***(Anon, 1957)*** and confirmed by the oxidase ***(COLA, 2004),*** catalase reactions ***(MacFaddin, 2000),*** salt tolerance ***(West and Colwell, 1984),*** motility ***(Koneman et al., 1988),*** growth at 42ºC, sugar fermentation ***(Elliot et al., 1995),*** sensitivity to vibriostatic agent 10 µg and 150 µg O/129 ***(Elliot et al., 2001),*** Kligler Iron Agar ***(Kligler, 1917),*** Methyl red test ***(Lee et al., 1979),*** Voges – proskauer test ***(Lee et al., 1979),*** urease test ***(MacFaddin, 2000),*** Citrate utilization test ***(Anon, 1995),*** Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase ***(Koneman et al., 1988)*** and ONPG test ***(Elliot et al., 1995).***

3-Molecular confirmation of *V. parahaemolytius*:

3-1 DNA extraction:

DNA extraction from isolate was performed using the instructions of QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. 200 µl of the strain suspension was incubated with 10 µl of proteinase k and 200 µl of lysis buffer at 56°c for 10 min. After incubation, 200 µl of ethanol (100%) was added to the lysate. The strain suspension was washed and centrifuged following manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide primer: Primers used were supplied from Metabion (Germany) are listed in tables (1) and (2).

3-2 PCR amplification:

Primers were utilized in 25 µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), Code No. RR310Akit, 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water and 6 µl DnA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

3-3 Analysis of the PCR products:

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5 V/cm. For gel analysis, 15 µl of the PCR products were loaded in each gel slot. A gene ruler 100 bp and DNA Ladder (Fermentas, Thermo Scientific, Germany) and Gel pilot 100 bp plus DNA ladder (Qiagen, Germany, GmbH) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1): *Primers sequences, target genes, amplicon sizes and cycling conditions.*

Target gene	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
		Secondary denaturation	Annealing	Extension		
Vibrio 16S rRNA	94˚C 5 min.	94˚C 30 sec.	56˚C 45 sec.	72˚C 45sec.	72˚C 10 min.	**Tarr *et al*., (2007)**
V. parahaemolyticus toxR	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45sec.	72˚C 10 min.	**Kim *et al*., (1999)**
recA	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	**Casandra *et al*., (2013)**
tlh	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	

Table (2): *Oligonucleotide primers sequences.*

Target gene	Primers sequences 5'-3'	Amplified segment (bp)	Reference
Vibrio 16S rRNA	CGGTGAAATGCGTAGAGAT	**663bp**	**Tarr *et al*., (2007)**
	TTACTAGCGATTCCGAGTTC		
V. parahaemolyticus toxR	GTCTTCTGACGCAATCGTTG	**368 bp**	**Kim *et al*., (1999)**
	ATACGAGTGGTTGCTGTCATG		
recA	TGARAARCARTTYGGTAAAGG	**793 bp**	**Casandra *et al*., (2013)**
	TCRCCNTTRTAGCTRTACC		
tlh	AAAGCGGATTATGCAGAAGCACTG	**449 bp**	
	GCTACTTTCTAGCATTTTCTCTGC		

Results

Postmortem examination of fish for vibriosis:

The clinical investigation of collected fishes displayed hemorrhages on the external body surface, base of fins and operculum (gill cover) with congested gills, pale liver and corneal opacity were noticed as shown in Figure (1)

Figure (1): A, B, C and D showed hemorrhages on the external body surface, base of fins and operculum (gill cover) with congested gills and corneal opacity

Table (3): *Prevalence of Vibrio species in the examined mullet, seabream and seabass fishes' samples*

Types of fish	Examined samples	Positive samples for *Vibrio* spp.		Negative samples of *Vibrio* spp.	
	No.	No.	%	No.	%
Mullet	60	17	28.33	43	71.67
Seabream	60	11	18.33	49	81.67
Seabass	60	10	16.67	50	83.33
Total	180	38	21.11	142	78.89

Table (4): *Prevalence of Vibrio parahaemolyticus in the examined mullet, seabream and seabass fishes' samples*

Types of fishes	Positive samples for *Vibrio* species		Prevalence of *Vibrio* species		
	No.	%	*Vibrio* species	N0.	%
Mullet	17	28.33	*V. parahaemolyticus*	15	88.23
Seabream	11	18.33	*V. parahaemolyticus*	8	72.72
Seabass	10	16.66	*V. parahemolyticus*	9	90

Table (5): *Frequency distribution of V. parahaemolytius in the different organs of the examined mullet fish samples*

Vibrio spp.	No. of strains	Mullet fish											
		Surface		Internal organs						Muscle (Flesh)		Gills	
				Liver		Kidney		Spleen					
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
V. parahaemolyticus	15	2	13.33	6	40	2	13.33	2	13.33	2	13.33	1	6.66

Table (6): Frequency distribution of *V. parahaemolytius* in the different organs of the examined seabream fish samples

Vibrio spp.	No. of strains	Seabream fish											
		Surface		Internal organs						Muscle (Flesh)		Gills	
				Liver		Kidney		Spleen					
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
V. parahaemolyticus	8	0	0	4	50	2	25	2	25	0	0	0	0

Table (7): *Frequency distribution of V. parahaemolytius in the different organs of the examined seabass fish samples*

Vibrio spp.	No. of strains	Seabass fish											
		Surface		Internal organs						Muscle (Flesh)		Gills	
				Liver		Kidney		Spleen					
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
V. parahaemolyticus	9	1	11.11	3	33.33	1	11.11	2	22.22	2	22.22	0	0

Table (8): *Molecular identification of V. parahaemolyticus in the examined mullet fish samples*

Genes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
16S rRNA Genral for *Vibrio* spp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
toxR specific for *V. parahaemolyticus*	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+

Table (9): *Molecular identification of V. parahaemolyticus in the examined seabream fish samples*

Genes	18	19	20	21	22	23	24	25	26	27	28
16S rRNA Genral for *Vibrio* spp.	+	+	+	+	+	+	+	+	+	+	+
toxR specific* for *V. parahaemolyticus	+	+	+	+	-	+	-	+	-	+	+

Table (10): *Molecular identification of V. parahaemolyticus in the examined seabass fish samples*

Genes	29	30	31	32	33	34	35	36	37	38
16S rRNA Genral for *Vibrio* spp.	+	+	+	+	+	+	+	+	+	+
toxR specific* for *V. parahaemolyticus	+	+	+	+	+	+	+	+	-	+

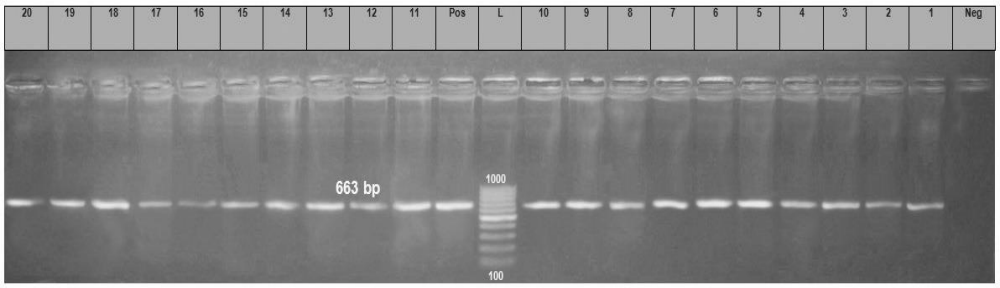

Figure (2)

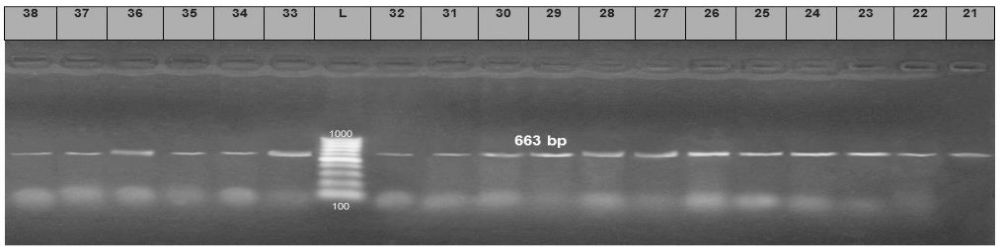

Figure (3)

Figure (2), (3): Agarose gel electrophoresis of PCR products after amplification of: 1- 16SrRNA gene for *Vibrio* species, MWM-molecular weight marker (100 – 1500 bp DNA ladder), + control (Positive, Negative) + different strains of *Vibrio* species. (16S rRNA gene products at 663 bp.).

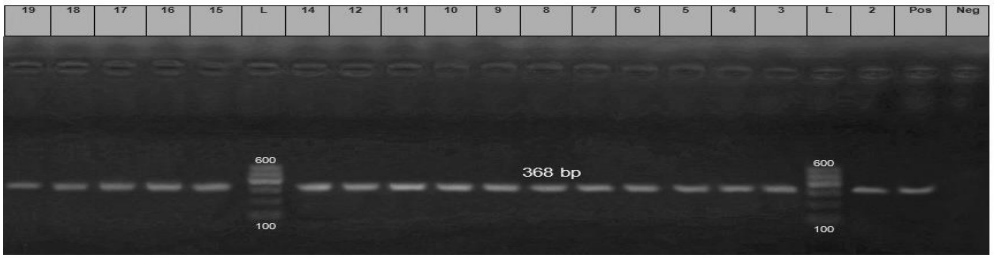

Figure (4)

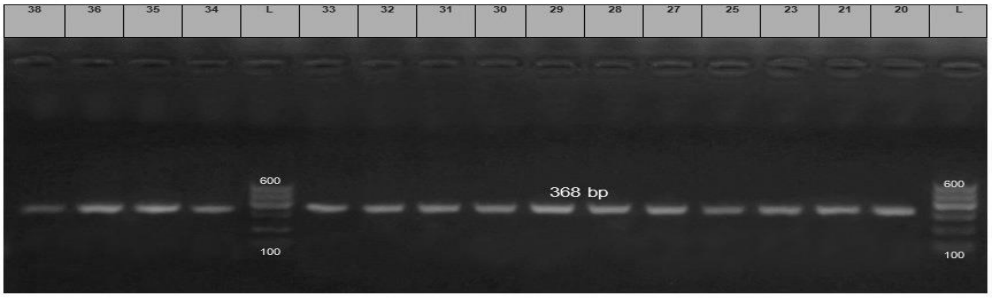

Figure (5)

Figure (4), (5): Agarose gel electrophoresis of PCR products after amplification of: 1- *toxR* gene for *V. parahaemolyticus*. MWM-molecular weight marker (100 – 600 bp DNA ladder), + control (Positive, Negative) + different strains of *V. parahaemolyticus*. (*toxR* gene products at 368 bp.).

Table (11): *Molecular characterization, virulence genes present of V. paraheamolytius strains in mullet fish samples*

***V. parahaemolyticus* genes**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
recA		+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
tlh		+	+	-	-	+	+	+	+	+	+	+		+	+	+	+

Table (12): *Molecular characterization, virulence genes present of V. paraheamolytius strains in seabream fish samples*

***V. parahaemolyticus* genes**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
recA	+	+	+	+		+		+		+	+
tlh	+	-	-	+		+		+		-	+

Table (13): *Molecular characterization, virulence genes present of V. paraheamolytius strains in seabass fish samples.*

***V. parahaemolyticus* genes**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
recA	+	+	+	+	+	+	+	+		+
tlh	+	-	+	+	-	+	+	+		+

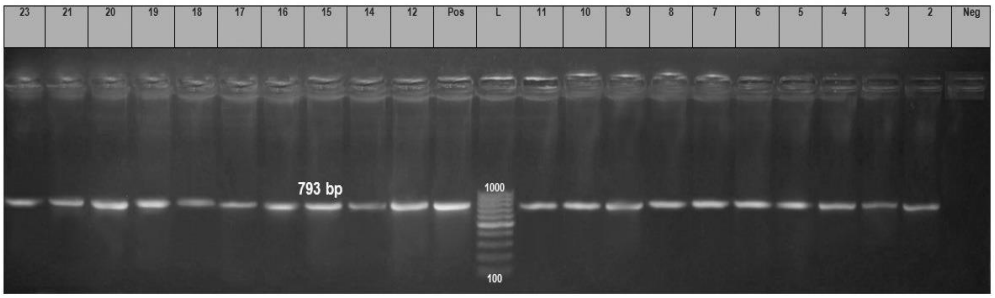

Figure (6)

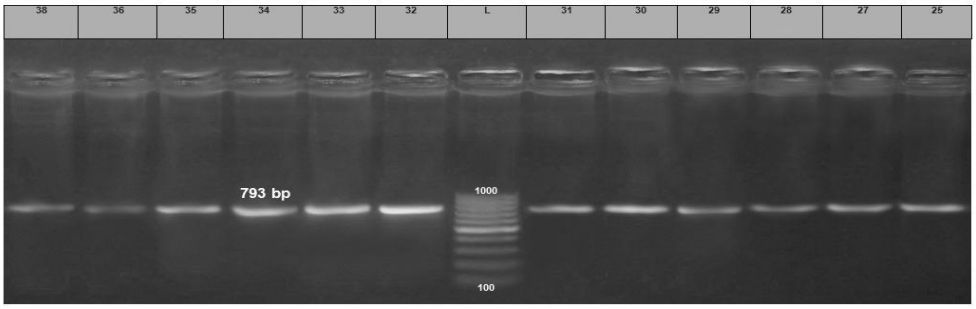

Figure (7)

Figures (8), (9): Agarose gel electrophoresis of PCR products after amplification of: 1- *recA* gene for *V. parahaemolyticus.* MWM-molecular weight marker (100 – 1000 bp DNA ladder), + control (Positive, Negative) + different strains of *V. parahaemolyticus*. (*recA* gene products at 793 bp.).

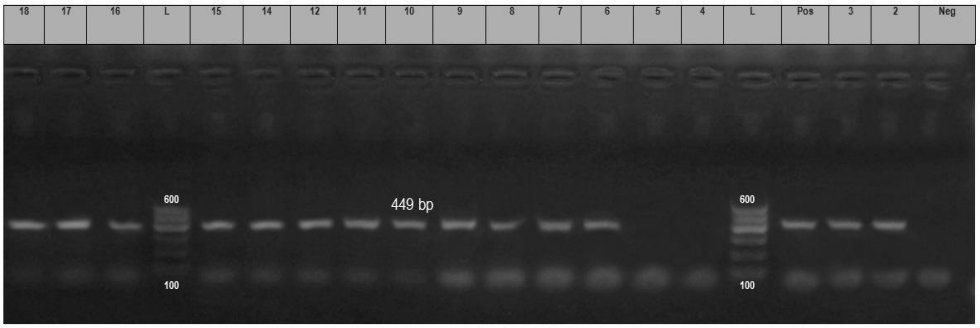

Figure (8)

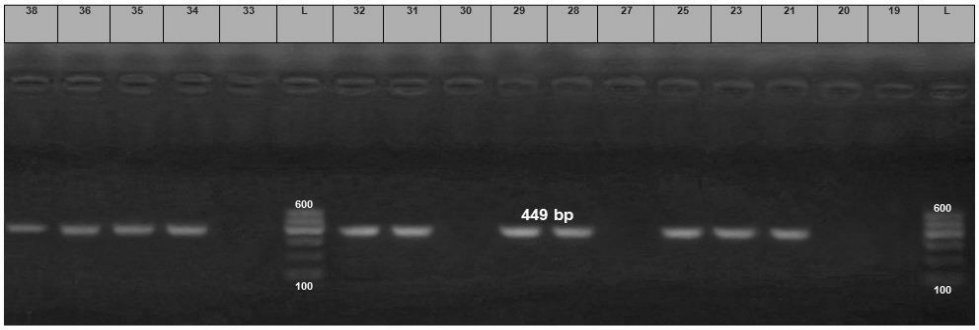

Figure (9)

Figures (8), (9): Agarose gel electrophoresis of PCR products after amplification of: 1- *tlh* gene for ***V. parahaemolyticus.*** MWM-molecular weight marker (100 – 600 bp DNA ladder), + control (Positive, Negative) + different strains of ***V. parahaemolyticus*****.** (*tlh* gene products at 449 bp.).

Discussion

Vibrosis is a globally threating bacterial disease affecting mariculture with high mortalities and sever economic losses. In Egypt, mariculture represents an important investment but diseases and high feeding cost are the main obstacles facing the sustainability and profitability of this sector ***(Mohamed et al., 2017)***. From the public health significance ***V.parahaemolyticus*** considered the most important species affecting human being fed on fish and crustacean meals ***(Schärer et al., 2011).***

Vibrio species are one of the most important groups of bacteria that cause food borne diseases as a result of the consumption of contaminated fish or partially cooked fish or shellfish. They cause seafood borne gastroenteritis and watery diarrhea in humans ***(Anjay et al., 2014).***

The obtained results in **Table (3)** showed that a total 180 samples of marine fish, 60 of each mullet (*Mugil cephalus*), seabream (*Sparus aurata*) and seabass (*Dicentrarchus labrax)* fish samples were examined bacteriologically for presence of ***Vibrio*** species. The prevalence of ***Vibrio*** species was 21.11% (38 isolates out of 180 samples) in the examined marine fish while for each of mullet, seabream and seabass fish samples were 17 (28.33%), 11 (18.33%) and 10 (16.67%) respectively. The current results were less than that of ***Saad et al. (2015)*** who isolated ***Vibrio*** species from marine fish in a percentage of 48% and that of ***Zorrilla et al. (2003)*** who isolated ***Vibrio*** species from seabream fish in a percentage of 69.90%.

Table (4) cleared that the isolated ***Vibrio*** spp. were identified biochemically as ***V. parahaemolyticus*** strains. The

highest incidence of ***V. parahaemolyticus*** isolates were 90%, 88.23% and 72.72% in seabass, mullet and seabream respectively. The current results were more than that of ***Saad et al. (2015)*** who isolated ***V. parahaemolyticus*** in percentages of 10%. While these results were less than that of ***Serracca et al. (2011)*** who isolated ***V. parahaemolyticus*** from mullet fish in proportion of 55%. Difference in prevalence percentages may be related to the difference between the geographical location of the samples, fish species, health condition and immune system of the fish and water and environmental quality which include pollution, pH, salinity and temperature of water.

The frequency distribution of the ***V. parahaemolyticus*** in the different examined samples presented in **Tables (5), (6) and (7)** showed that the highest incidences were recorded in the liver with a percentage of 50%, 40% and 33.33% for seabream, mullet and seabass respectively. These results were agreed with the results recorded by ***Sadok et al. (2013)*** who isolated the ***V. parahaemolyticus*** from internal organs of seabass.

The results of agarose gel electrophoresis using 16S rRNA gene in **Tables (8), (9), (10)** and **Figures (2) and (3)** revealed that the tested 38 isolates were ***Vibrio*** strain with molecular weight 663bp. These results are approximately similar to that recorded by ***Mohammed et al. (2107)*** who used 16S rRNA gene sequence as accurate identification and confirmation of all tested strains. While **Boudewijns *et al.* (2006)** used the 16S rRNA sequencing to identify bacterial in clinical microbiology laboratories. On other hands **Abd Ell-razeq and *Khaliel (2014)*** confirmed the positive isolates of ***V. parahaemolyticus*** by using 16S rRNA gene.

PCR assays were developed with specific primers for the detection of ***Vibrio*** species. These results in **Tables (8), (9)**, **(10)** and **Figures (4)** and **(5)** showed that 32 strains from the total 38 strains were positive for *toxR* which specific for ***V. parahaemolyticus***. This results agree with results recorded by ***Mohammed et al. (2107)*** who used species-specific primers of *toxR* genes for molecular identification of ***Vibrio*** species and categorizing 32 isolates belonged to ***V. parahaemolyticus*** Which confirmed through well conserved *toxR* gene at 368 bp. ***Zulkifli et al., (2009)*** and ***Cabrera-Garcia et al. (2004)*** who determined the characterization of the ***V. parahaemolyticus*** genotypically by using *toxR* gene at 368 bp and detected in 46 strains from seawater, fish, and oysters samples. ***Terzi and Martinez (2016)*** used PCR analysis for conformation of ***V. parahaemolyticus*** (*toxR*) and found that their incidence in the examined fish samples were 8.3%.

Also **Table (8), (9), (10)** showed that the molecular detection of ***Vibrio*** species by using 16S rRNA were 17, 11 and 10 strains for mullet, seabream and seabass fishes respectively. Meanwhile prevalence of ***V.parahaemolyticus*** strains through detection of *toxR* were 15, 8 and 9 strains for mullet, seabream and seabass fishes respectively.

The results of molecular detection and determination of the some virulence genes by PCR as in **Tables (11), (12), (13)** and **Figures (6), (7), (8) and (9)** showed that virulence genes *recA* and *tlh* were detected in 32 and 25 strains of isolated ***V. parahaemolyticus*** respectively. These results agree with the results recorded by ***Serracca et al. (2011)*** who identified the virulence genes (*toxR*, *trh* and *vvh*) of ***V. parahaemolyticus*** and ***V. vulnificus*** by using PCR and detect *trh*+/*tdh*- in 16% (47/295) of samples and only one strain resulted *trh*+/*tdh*+. Also ***Casandra et al. (2013)*** confirmed that the virulence factor genes encoding the thermostable direct hemolysin (*tdh*) and the thermostable direct hemolysin-related hemolysin (*trh*) are strongly correlated with virulence of the emergent human pathogen ***V. parahaemolyticus***. The gene encoding the thermolabile hemolysin (*tlh*) is also considered a signature molecular marker for the species.

Virulence genes *recA* and *tlh* of the isolated strains from mullet, seabream and seabass fishes samples in **Tables (11), (12), (13)** revealed that the isolates were confirmed to be virulent ***V. parahaemolyticus***. The isolated ***V. parahaemolyticus*** strains was harbored *recA* and *tlh* genes. The *recA* gene was detected in 15, 8 and 9 of ***V. parahaemolyticus*** strains isolated from mullet, seabream and seabass samples respectively. While *tlh* gene was detected in 13, 5 and 7 of ***V. parahaemolyticus*** strains isolated from mullet, seabream and seabass samples respectively.

The present work highlights the incidence of ***Vibrio*** species in mullet (*Mugil cephalus*), seabream (*Sparus aurata*) and seabass (*Dicentrarchus labrax)* fish intended for human consumption. The share of ***Vibrio*** species could poses a risk for human health after consumption of undercooked or cross-contaminated fish. Conventional microbiological methods needed to identify bacteria from fish are often limited due to time exhausted to complete the assays. In recent years, PCR have overcome problems associated with culture-based techniques, enabling the detection of bacteria directly in clinical samples without the need for previous culturing ***(Gonzalez et al., 2004).*** Regulating the extent of antimicrobial usage is aimed to guarantee the safety and efficacy of the antibiotics used to treat animal diseases and to protect consumer health ***(Scarano, 2014).***

Thus this study we could concluded that, PCR is considered as a rapid, reliable and sensitive tool which will help in diagnosis of ***V.paraheamolyticus*** and detection some of their virulence genes consequently prevent the infection of marine fish.

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الملخص العربي

التصنيف الجيني لميكروب الفيبريو باراهيموليتكس المعزولة من بعض أسماك المياه المالحه

مروه عبد الحق محمود * ، محمود عزت السيد ** ، جيهان محمد عمر محمد***

** طبيب بيطري– معهد الاستزراع السمكي وتكنولوجيا الأسماك – جامعة قناة السويس*

*** قسم البكتريولوجي و المناعة و الفطريات - كلية الطب البيطري- جامعة قناة السويس*

**** قسم البكتريولوجي - معهد بحوث الصحة الحيوانية - معمل فرعي بورسعيد*

تم جمع 180 عينة من أسماك المياه المالحة عشوائياً من مزارع الاسماك المختلفة من مدينة بورسعيد بواقع 60 عينة من كل من اسماك البوري (*Mugil cephalus*) ، الدنيس (*Sparus aurata*) والقاروص (*Dicentrarchus labrax*) . و بالكشف الظاهري كان هناك نزيف على سطح الجسم الخارجي ، نهايات الزعانف ، وغطاء الخياشيم , و الخياشيم محتقنه ، و الكبد شاحب و عتامة القرنية في عينات الأسماك موضع الدراسة.

و بالفحص البكتريولوجي للعينات التي تم جمعها من سطح السمك والكبد والكلى والطحال والعضلات والخياشيم أظهرت النتائج أن أعلى نسبه لعزل ميكروب الفيبريو كانت من الكبد تليها الكلية والطحال والعضلات والخياشيم. و بالتصنيف البيوكيميائي للمعزولات أوضحت النتائج أن 38 عينة (21.11٪) من مجموع العينات التي تم جمعها كانت إيجابية لميكروب الفيبريو بنسب (28.33٪) ، (18.33٪) و (16.67٪) للبوري ، الدنيس والقاروص على التوالي.

و باستخدام اختبار تفاعل انزيم البلمرة المتسلسل تم التصنيف الجيني لميكروب الفيبريو المعزولة من عينات أسماك المياه المالحة باستخدام 16S rRNA و *toxR* تبين أن العترات المعزولة كانت فيبريو باراهيموليتكس بواقع 32 عترة و متواجدة بعدد 15، 8 ، 9 عترة في اسماك البوري و الدنيس و القاروص على التوالي،

وبالتوصيف الجزيئي لـميكروب الفيبريو باراهيموليتكس باستخدام اختبار تفاعل انزيم البلمرة المتسلسل لتحديد جينات الضراوة *recA* ، *tlh* اسفرت النتائج ان جميع عترات الفيبريو باراهيموليتكس 32 كانت ايجابية لجين *recA* و 25 عترة فقط كانت ايجابية لجين tlh .

تم مناقشة النتائج و بيان أهمية الميكروب المعزول و خطورته علي الصحة العامة للمستهلك و علي مستوي انتاجية الأسماك و عمل التوصيات للتقليل من مخاطره.