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Vibrio Species in Fish and Shell Fish

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A B S T R A C T

A grand total of 100 random samples of fresh water fish with average weight 100-250g *(Oreochromis niloticus),* marine water fish *(Mugil cephalus),* and shell fish (shrimp and crab) with average weight 10-50g were collected from different markets in Gharbia governorate for detection of *Vibrio* spp. The obtained results revealed that the incidence of *Vibrio spp.* in fresh water fish (*Oreochromis niloticus*) were 8 (32%), with frequency of 2 (8%), 2 (8%), 2 (8%), 1 (4%) and 1 (4%) for *V. parahaemolyticus, V. mimicus, V. vulnificus, V. alginolyticus* and *V. fluvialis, respectively.* In marine fish *(Mugil cephalus),Vibrio* spp. were 10 (40%), the overall incidence in the samples was for *V. parahaemolyticus* 3(12%), *V. mimicus* 2(8%), *V. alginolyticus* 2 (8%), *V. vulnificus* 1 (4%) and *V. fluvialis 2* (8%). In shell fish (shrimp), *Vibrio* spp. were 13 (52%) while the overall incidence in the samples was *V. parahaemolyticus* 4 (16%), *mimicus* 3 (12%), *V. alginolyticus* 2 (8%), *V. vulnificus* 1 (4%), *V. fluvialis* 2 (8%) and *V. cholera* 1 (4%). Regarding crab, *Vibrio* spp. were 11 (44%) while the overall incidence in the samples was *V. parahaemolyticus* 3 (12%), *mimicus* 2 (8%), *V. alginolyticus* 2 (8%), *V. vulnificus* 1 (4%), *V. fluvialis* 2 (8%) and *V. cholera* 1 (4%).

Keywords: *Vibrio* spp., fresh water fish, marine fish, shellfish, *V. paraheamolyticus*

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1.INTRODUCTION

Fish is a nutrient-rich part of a healthful diet and its consumption is associated with potential health benefits, including neurological development during gestation and infancy (Hibbeln *et al.,* 2007) and reduce risk of heart disease (Mozaffarian and Rimm, 2006).

The degeneration of fish is accelerated by microorganism associated with aquatic environment as well as contamination during post -harvest handling. When fish dies, microorganisms on the surface as well as gut and gills begin to utilize the fish protein and food nutrient resulting in loss of nutritional

value as well as creating undesirable changes like off-flavors, texture and appearance (Jhonstone *et al.*,1994).

Seafood may be a vehicle for most of known bacterial pathogens as *Vibrio* spp. (Huss, 1997). Various outbreaks of bacterial disease associated with the consumption of seafood have been reported (Friesema *et al*., 2012). From these seafood-borne bacteria, *Vibrio* spp. which are Gram-negative rod-shaped, oxidase positive, non-spore forming bacteria and halophilic bacteria that generally widespread in the coastal and estuarine environments (Austin, 2010).

The members of the genus *Vibrio* are considered as one of the main causes of gastroenteritis in humans. The majority of infections are attributed to consumption of raw or insufficiently cooked seafood products. The number of *Vibrio* spp. classified as pathogenic strains is at least 11strains (Holmberg *et al.*, 1992), including *V. cholerae* as the main cause of diarrhea; *V. parahaemolyticus* as the cause of foodborne gastroenteritis (Ozer *et al.*, 2008) and *V. vulnificus* which is known to cause 95% of all deaths associated with seafood consumption (Rosche *et al.*, 2006). Other pathogenic species includes *V. alginolyticus; V. damsela; V. fluvialis; V. furnissii; V. hollisae; V. metschnikovii; V. cincinnatiensis* and *V. mimicus* (Pruzzo *et al.*, 2005).

The typical clinical symptoms of *V. parahaemolyticus* poisoning are acute dysentery and abdominal pain, accompanied with diarrhea, nausea, vomiting, fever, chills and water like stools (Shimohata and Takahashi, 2010). The faces of patients are mixed with mucus or blood and their blood pressure decreases dreamily leading to shock (Broberg *et al.*, 2011). *V. parahaemolyticus* is very sensitive to heat (killed at 47^0- 60^0 C) and to ionizing radiation, as well as to halogens (Adams and Moss, 2008). Most of *vibrios* secret enterotoxins in food, water or in the gastrointestinal tract (Nishibuchi and Depaola,2005).

Numerous studies have been conducted to determine the relationship between *Vibrio* spp. abundance and environmental factors such as temperature, salinity, nutrients and dissolved oxygen. As a result, these water quality characteristics can be used in a predictive manner to determine when these pathogens may be present (Gayatri, 2011).

The present work was planned out to determine the level of contamination of some fish (*Mugil cephalus & Oreochromis niloticus*) and shell fish (shrimp & crab) with *vibrio* species.

2. MATERIAL AND METHODS

A grand total of 100 random samples of fresh water (*Oreochromis niloticus*), marine water fish *(Mugil cephalus),* and shell fish (Shrimp and crab) were collected from different markets in Gharbia governorate. All samples were collected and transferred with a minimum of delay to the laboratory in ice box. All samples were subjected to the bacteriological examination.

2.1. *Preparation of samples:*

The scales and fins of the fish samples were removed, the skin was sterilized by alcohol and flamed by sterile spatula. The muscles above the lateral line were removed, while in shell fish (shrimp and crab) were washed with water then sterilized by alcohol and flamed and then the carapace was removed aseptically to expose the flesh. Ten grams were taken under aseptic conditions to sterile homogenizer containing 90ml of sterile alkaline peptone water (3%Nacl and pH 8).

2.2 Screening of Vibrio sp

It was done according to FDA (2004)

Isolation: Loopfuls from each previous cultured tube were separately streaked onto Thiosulfate citrate bile and sucrose agar (TCBS), then the medium was incubated at37^0 C for 24hrs. Typical colonies of *V. mimicus, V. parahaemolyticus* and *V. vulnificus* were appeared as smooth and green (sucrose negative), while colonies of *V. cholerae, V. furnissii, V. alginolyticus* and *V. fluvialis* were appeared as smooth and yellow (sucrose positive).

Presumptive identification: This was done according to the protocol recommended by ISO/ TS 21872-1 (2007) and ISO/ TS 21872-2 (2007).

2.3 *Confirmation of the results by multiplex PCR:*

It was done according to Tarr et al., (2007) and Rao and Surendran (2013).

The biochemically identified isolates and food samples were further verified genetically by PCR for detection of16S rRNA for all *Vibrio* spp, flaE for *V.parahaemlyticus*, hsp for *V.vulnificus*, sodB for *V.mimicus* and sodB for *V.*cholera.

3. **RESULTS**

Incidence of *Vibrio* spp. isolated from the examined samples of fish recorded in Table (1) were 32% ,40%, 52% and 44% for freshwater (*Oreochromis niloticus)*, marine water fish *(Mugil cephalus)*, and shell fish (shrimp and crab), respectively.

Table (2) revealed that incidence in the *Oreochromis niloticus* samples were 1(4%) for *V. fluvialis* and *V. alginolyticus* and were 2(8%) for *V. vulnificus, V. parahaemolyticus* and *V. mimicus* while *V. cholerae* failed to be detected biochemically.

Table (3) which revealed that incidence in the samples *Mugil cephalus* were2(8%) for each of *V. alginolyticus, V. fluvialis* and *V. mimicus*, and was 1 (4%) for *V. vulnificus*. For *V. parahaemolyticus* was 3 (12%).

Table (4) revealed that the of *Vibrio*.spp. incidence in shrimp samples was 4(16%) for *V. parahaemolyticus* and were 2(8%) for *V. fluvialis* and *V. alginolyticus* ; 3 (12%) for *V. mimicus* and 1(4%) for *V. cholerae* and *V. vulnificus*.

The incidence of *Vibrio spp.* in table (5) revealed that the incidence of *Vibrio spp*.in crab samples was 3(12%) for *V. parahaemolyticus*; 2(8%) for each of *V. fluvialis*; *V. alginolyticus* and *V. mimicus*.While, the incidence was 1 (4%) for each of *V. cholerae* and *V. vulnificus.*

Table (1): Incidence of *vibrio* spp. isolated from the examined samples of fish and shell fish (n=25of each)

Fish types	*Vibrio spp.*	
	No.	%
Oreochromis niloticus	8	32
Mugil cephalus	10	40
Shrimp	13	52
Crab	11	44
Total	42	42

Table (2): Incidence of *vibrio* spp. isolated from *Oreochromis niloticus.*

Isolates	Number	%
V. vulnificus	2	8
V. mimicus	2	8
V. fluvialis	1	4
V. cholerae	0	0
V. parahaemolyticus	2	8
V. alginolyticus	1	4

Table (3): Incidence of *vibrio* spp. isolated from *Mugil cephalus.*

Isolates	Number	%
V. vulnificus	1	4
V. mimicus	2	8
V. fluvialis	2	8
V. cholerae	0	0
V. parahaemolyticus	3	12
V. alginolyticus	2	8

Table (4): Incidence of *vibrio* spp. isolated from Shrimp.

Isolates	Number	%
V. vulnificus	1	4
V. mimicus	3	12
V. fluvialis	2	8
V. cholerae	1	4
V. parahaemolyticus	4	16
V. alginolyticus	2	8

Table (5): Incidence of *vibrio* spp. isolated from Crab

Isolates	Number	%
V. vulnificus	1	4
V. mimicus	2	8
V. fluvialis	2	8
V. cholerae	1	4
V. parahaemolyticus	3	12
V. alginolyticus	2	8

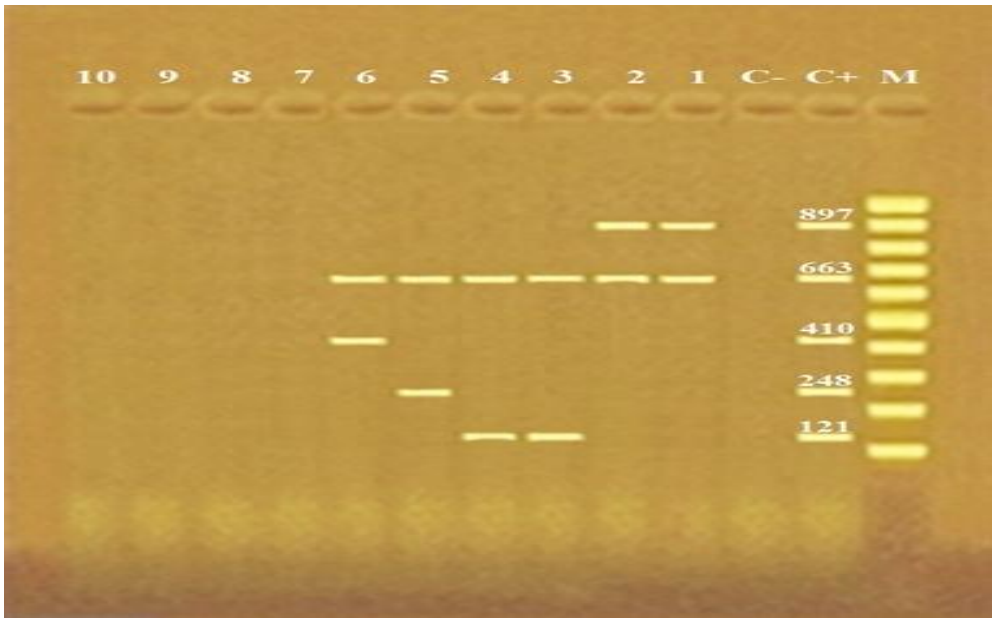

Fig (1): Agarose gel electrophoresis of multiplex PCR for characterization of *Vm.sodB* (121 bp) for *V.mimicus*, *Vc.sodB* (248 bp) for *V.cholera*, *Vv.hsp* (410 bp) for *V.vulnificus*, *16S rRNA* (663bp) for all Vibrio Spp. and *Vp.flaE* (897 bp) for *V.parahaemolyticus.*
Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive for *Vm.sodB, Vc.sodB, Vv.hsp, 16S rRNA* and *Vp.flaE* genes.
Lane 2: Control negative.
Lanes 1 & 2: Positive *V.parahaemolyticus* for *16S rRNA* and *Vp.flaE* genes.
Lanes 3 & 4: Positive *V.mimicus* for *16S rRNA* and *Vm.sodB* genes.
Lane 5: Positive *V.cholera* for *16S rRNA* and *Vc.sodB* genes.
Lane 6: Positive *V.vulnificus* for *16S rRNA* and *Vv.hsp* genes.
Lanes 7, 8, 9 &10: Negative samples for Vibrio species.

4. Discussion

Vibrio spp. inhabit marine environments and are associated with aquatic animals including fish, shellfish, shrimp, oyster, squid, prawn, and other freshwater animals (Sujeewa *et al.*, 2009).

Incidence of *Vibrio* spp. isolated from the examined samples of fish recorded in Table (1) were 32% ,40%, 52% and 44% for freshwater (*Oreochromis niloticus),* marine water fish *(Mugil cephalus),* and shell fish (Shrimp and crab), respectively. It is evident from the results recorded in table (1) that the high level of *Vibrio* spp. was in shrimp and crab when compared with samples from (*Oreochromis niloticus*) and *(Mugil cephalus),* this explained by Colakoglu *et al.* (2006) who found that shellfish make an excellent substrate for the microorganisms to live in the

aquatic habitats due to lose texture of their flesh. When the aquatic system was contaminated with pathogenic *vibrio*, these bacteria become part of shellfish microflora.

Table (2) revealed that incidence in the *Oreochromis niloticus* samples were 1(4%) for *V. fluvialis* and *V. alginolyticus* and were 2(8%) for *V. vulnificus, V. parahaemolyticus* and *V. mimicus* while *V. cholerae* failed to be detected biochemically. These results lower than those reported by Noorlis *et al.,* (2011) who found that *Vibrio* spp. could be detected at a prevalence of 98.67%, whereas *V. parahaemolyticus* was detected at a prevalence of 24% from examined fresh water fish. The presence of *Vibrio* spp. in samples of freshwater fish suggests that foodborne illness could arise if these fish are consumed in the uncooked or found that *Vibrio* undercooked state. They could also cross- contaminate ready-to-eat foods that are in the same environment.

Table (3) declared that incidence in the samples *Mugil cephalus* were 2 (8%) for each of *V. alginolyticus*, *V. fluvialis* and *V. mimicus*, and was 1 (4%) for *V. vulnificus*. For *V. parahaemolyticus* was 3 (12%) while, *V. cholerae* failed to be detected biochemically. On the other hand, Sanjeev (2002) recorded that the incidence of *V. parahaemolyticus* in fresh, marine and brackish water fish varied from 35 to 55%. Also, higher results were reported by Jaksic et al. (2002) who isolated *V. alginolyticus, V. fluvialis* and *V. mimicus* from 14%, 9% and 28% of the examined samples of marine fish, respectively. Lower results were recorded by Raissy *et al.,* (2013) who revealed that 29.3 % of the examined fish were *Vibrio* positive. This high incidence probably reflects the nature of *Vibrio* spp. which is known as a halophilic waterborne bacterium that commonly inhabits environmental water sources worldwide.

Table (4) revealed that the of *Vibrio.*spp. incidence in shrimp samples was 4 (16%) for *V. parahaemolyticus* and were 2 (8%) for *V. fluvialis* and *V. alginolyticus*; 3 (12%) for *V. mimicus* and 1(4%) for *V. cholerae* and *V. vulnificus*. These results nearly similar to those of Amin *et al.* (2011) who isolated *Vibrio* spp. with a percentage of 57.3% from shrimps. These results are higher than results reported by Bakr-Wafaa *et al.,* (2011) who detect *Vibrio spp.* in 32 % of the total examined shrimp. This high result may indicate bad management practices (inadequate nutrition, overcrowding and overfeeding) in fish farms which can cause stress to the fish being cultured and thus make them more susceptible to microbial infection. Aquaculture in Egypt remains a growing, vibrant and important production sector for high-protein animal food that is easily digestible and of high biological value. However, a major setback in aquaculture is the outbreak of diseases, especially those caused by *Vibrio* spp. which considered significant economic and public health problems.

The incidence of *Vibrio spp.* in table (5) revealed that the incidence of *Vibrio spp*.in crab samples was 3(12%) for *V. parahaemolyticus*; 2(8%) for each of *V. fluvialis*; *V. alginolyticus* and *V. mimicus*.While were 1 (4%) for each of *V. cholerae* and *V. vulnificus*.

These results are higher than those reported by Utsalo (2008) who detect *Vibrio spp*.in 27.0% of the examined crabs.

The occurrence of *Vibrio* spp. in raw shellfish was common, especially shellfish from regions with temperate climates around the world from both natural and farm environments and all seafood types (Ducan and Su, 2005).

Fish were conditioned by their environment if the growing and harvesting environment of fish was polluted chemically or microbiologically, the fish were also polluted. During transportation of these types of fish to landing center and wholesale market, these fish may also infect associate people during handling and when the consumers purchase those fishes, the associated microorganisms could be transferred to them (Begum *et al.*,2010).

The positive samples and its biochemically positive isolates also negative samples were subjected to 16S rRNA and multiplex PCR as shown in Fig (1). This fig. illustrated that positive samples or isolates give two bands, one at 663bp and the 2nd at its specific amplicon. *Vm. sodB* (121 bp) for *V.mimicus*, *Vc.sodB* (248 bp) for *V.cholera*, *Vv.hsp* (410 bp) for *V.vulnificus*, *16S rRNA* (663bp) for all *Vibrio* Spp. and *Vp.flaE* (897 bp) for *V.parahaemolyticus* while negatives not showed any band. Similar results showed by Tarr *et al.*, (2007) and Rao and Surendran (2013).

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