Bacteriological and Histopathological Studies on Adult Shrimps (*Penaeus Japonicas*) Infected With Vibrio Species in Suez Canal Area

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Abstract

210 shell diseased shrimps (*Penaeus japonicas*) were collected and taken alive from port-said governorate. Shell diseased shrimps were taken monthly from January to October 2016 and subjected to clinical, postmortem, bacteriological and histopathological examinations. In addition of 15 apparently healthy shrimps, free from any shell lesions were collected and taken alive from port-said Governorate were used in the experimental infection (pathogenicity test). Results revealed that the isolated bacteria from shell diseased shrimps were identified as *Vibrio alginolyticus* and *Vibrio parahemolyticus.* The number of isolates for *Vibrio alginolyticus* was 568 isolates by incidence of 77.59%, *Vibrio parahemolyticus* was 131 isolates by incidence of 17.89%, It was found that *Vibrio alginolyticus, Vibrio parahaemolyticus,* were highly isolated from the muscles by ratio of 95.71%, the cuticle by ratio of 90% then from gills by ratio of 80% followed by the hemolymph by ratio of 51.90%, while it was less isolated from the hepatopancrease by ratio of 30.95%.The pathogenicity test of the isolated microorganisms showed nearly the same clinical picture and postmortem findings which observed in naturally infected shrimps and isolated vibrio species appear to be highly virulent gave 100% mortality in 60 hrs. of the experimental infected shrimps. *Vibrio alginolyticus* and *Vibrio parahemolyticus* were sensitive to Norofloxacin, Ciprofloxacin and Trimethoprim-Sulfamethoxazole and resistant to Amikacin and Rifamycin. The histopathological studies among naturally infected shrimps (*penaeus japonicas*) revealed changes in muscles, gills and hepatopancrease due to infection as intermuscular edema, inflammatory cells between muscle bundles and degeneration and necrosis of muscles, the gills

showed squamous metaplasia, and the hepatopancreas showed congestion in the hepatic vessels, advanced vacuolar degeneration with nuclear pyknosis of most hepatocytes were evident.
Key words: Shrimps, *Vibrio alginolyticus, Vibrio parahaemolyticus*, pathogenicity and Sensitivity.

Introduction

Bacterial disease outbreaks particularly vibriosis and black shell disease imposed a significant constrain on the sustainable production of shrimp***, Manilal et al. (2010)***. A crisis in the shrimp industry over the last few years is due to largely to an increase in virulence of pathogens, especially bacterial diseases, ***Lightner (1993).***

The cuticle of crustaceans consists of a thin outer layer, the epicuticle, consists of proteolipoidal material, covering three inner chitinous layer, the exocuticle, which is pigmented and calcified, the calcified endocuticle, and the non-calcified endocuticle, ***Dennell (1947)***. Chemically chitin is a polysaccharide, the outer cuticular layer of the cuticle, the epicuticle is biochemically inert, and shell erosion can occur when this layer is breached by chemical attack, injury, abrasive action of sediments, or possibly enzymatic digestion, exposing the underlying chitinous layers to a adequate numbers of chitin-destroying microorganisms, ***Schlotfeldt (1972), Stewart (1980).***

Because of the lipoidal nature of the epicuticle, microorganisms producing extracellular lipase may initiate lesions even in the absence of abrasions. In one series of experiments, all microorganisms able to infect healthy non-abraded crustaceans were lipolytic, ***Cipriani et al., (1980).*** And may become opportunistic pathogens of stressed or damaged crustaceans, and may increase in numbers dramatically in aquaculture facilities or in polluted water. Participating microorganisms have been shown to produce extracellular lipase, chitinase and proteases***, Lightner (1988a)***.

The black coloured lesions are the end-result of the melanization reaction, defense response triggered by cuticular damage. Pitting the top most layer of the exoskeleton in the form of irregular pits ranged from few millimeters up to different centimeters. In some cases, the pits coalesced or united together to form a foramen. The shells became soften and fragile so it was easily destructed, ***Lee and Söderhäll (2002)***. These mentioned above explained the occurrence of common clinical signs and postmortem lesions which observed in shell diseased shrimps in this study.

This study was planned to investigate the phenotypic characterization of vibrio species isolated from shrimps in Suez Canal area.

Material and methods

A total number of 210 shell diseased shrimps (*Penaeus japonicas*) were collected and taken alive from port-said governorate. Shell diseased shrimps were taken monthly from January to October 2016 and subjected to clinical, postmortem, bacteriological and histopathological examinations. In addition of 15 apparently healthy shrimps, free from any shell lesions were collected and taken alive from port-said Governorate were used in the experimental infection (pathogenicity test).

1. Clinical examination of naturally infected adult shrimps:

These were performed according to method described by ***Austin and Austin (1989)*** to observe the following. Abnormal coloration and lesions on carapace, Abnormal swimming movements, opacity of abdominal muscles tissue, erosions of appendages, eye abnormalities, gill abnormalities, hemolymph color. The external lesions then were recorded and photographed.

2. Postmortem examination of naturally infected adult shrimps:

According to ***Austin and Austin (1989)*** After cleaning the surface of cuticle by cotton socked in 70% ethyl alcohol using a pair of sterile scissors with fine points and a pair of fine tipped forceps, the carapace was separated from connective tissue and the hepatopancrease exposed in the situation the color and consistency were observed, examination of gills, foregut, midgut, hindgut, cardiac sinus, muscle, walking legs (periopods), swimming legs (pleopodes) and tail (uropods) were applied.

3. Bacteriological examination:

3.1. Collection of samples: taken from exocuticle, gills, hepatopancreas, haemolymph and muscles from adult shell diseased shrimps showing clinical symptoms of the disease. This is under full aseptic condition.

3.2. Isolation: adult shrimp exoskeleton was firstly cleaned with cotton socked in 70% ethyl alcohol. The following samples were separately collected;exocuticle,gills, hepatopancreas, haemolymph and muscles. The Hemolymph extracted from cardiac sinus by inserting insulin needle between cephalothorax and 1st abdominal segment after cleaning the site by ethyl Alcohol 70%, ***Brady and Ernesto (1992).*** Each sample were inoculated into Tryptic Soya broth, nutrient broth and peptone water (pH 8.2) all with 2% NaCl and incubated at 18-23°C for 24- 48 hrs. Then the inocula were streaked over Thiosulphate Citrate Bile Salt Sucrose agar (TCBS), nutrient agar with 2% NaCl and Tryptic Soya agar with 2% NaCl at 25-28°C for 48 hrs. The purified colonies were picked up and inoculated into nutrient agar slant and Tryptic Soya agar slant with 2% NaCl for further Identification.

3.3. Identification of bacterial isolates: the biophysical and biochemical characters were carried

according to ***Bergey manual of systematic bacteriology (2004).***

4. Experimental infection:

A total number of 15 apparently healthy adult shrimp (*Penaeus japonicus*), free from any shell lesions were collected and taken alive from the Suez Canal area (port-said Governorate).

Aquaria: shrimps were kept in previously prepared 3 fiber glass aquaria. These aquaria were used for holding the experimental infection throughout the period of investigation, and all tanks were filled with seawater (temperature 25°C and salinity 28%). The temperature was adjusted thermostatically by using heaters and all tanks were aerated by electric aerator pumps. shrimps were divided into 3 equal groups, each group (5 shrimps) in separate glass. shrimps put in the tanks for 5 days for adaptation and will be fed regularly on cocklets and chopped whitefish.

Preparation of bacterial suspension: the identified bacteria which isolated from shell diseased shrimps in this study were used in experimental infection (*Vibrio alginolyticus* and *Vibrio parahaemolyticus*). Strains were prepared by inoculation into nutrient agar slant and incubated at 25°C for 24 hrs, bacterial suspension 106cell/ml was estimated with (McFerland barium sulphate standard tube) (Difco). A dose of 0.05 ml of bacterial suspension using sterile distilled water containing 10^6cell/ml was injected with 1 ml tuberclin syringe into muscle of 3rd abdominal segment, ***Jiravanichpasial and Miyazaki (1994).***

Group (1) were injected with *Vibrio alginolyticus* strain suspension.

Group (2) were injected with *Vibrio parahaemolyticus* strain suspension.

Group (3) were injected with sterile saline.

5. Antibiotic sensitivity tests:

Sensitivity tests were performed using the disc diffusion method on Mueller-Hinton agar (oxoid) according to **the National Committee of Clinical Laboratory Standards (NCCLS) (2003).**

5.1. Preparation of the inoculums: isolated strains on slope agar from shrimps were streaked into a Muller-Hinton broth and incubated at 25-28^{o}C overnight, the isolated strains were Vibrio alginolyticus, Vibrio parahemolyticus, Isolates were subculture on Muller – Hinton agar and incubated at 25-28^{o}C overnight, 4 or 5 well isolated colonies selected with an inoculating needle or loop, and transferred to a tube of sterile saline and vortexes thoroughly. The bacterial suspension compared to 0.5 McFarland standards.

5.2. Inoculation procedures: within 15 minutes after adjusting the turbidity of the inoculums suspension, a sterile cotton swabs were dipped into the suspension. Pressing firmly against the inside wall of the tube just above the fluid level, the swab was rotated to

removes excess liquid, then the swab was streaked over the entire surface of the Muller- Hinton agar medium three times and the plate was rotated approximately 60 degrees after each application to ensure an even distribution of the inoculums. Finally, the edge of the agar surface was swabbed all around.

5.3. Antibiotic discs: sensitivity discs were stored in the refrigerator (4^oC). Upon removal of the discs from the refrigerator, the package containing the cartridges should was left unopened at room temperature for approximately one hour to allow the temperature to equilibrate. The discs were dispensed on the surface of the medium. All of them were under complete aseptic precautions, plates were incubated aerobically at 25-28^oC for 24h.

5.4. Recording and interpreting results: the results were recorded as resistant or susceptible by measurement of the inhibition zone diameter according to the interpretive standard of **National Committee for Clinical Laboratory Standards** ***(NCCLS 2003)*** that would be susceptible or intermediate or resistant.

6. Histopathological studies:

Samples for histopathological examination were taken from shell diseased shrimps, organs and tissues which taken were muscles, cuticle, gills and hepatopancrease. The samples were fixed in phosphate buffer formalin 10% according to ***Nash et al., (1987), Nash et al., (1988)*** *and* ***Anderson et al., (1990)*** and in Davidson,s fixative Acetic Acid Formalin Alcohol (AFA), ***Humason (1967)*** and ***Bell and Lightner (1988),***the samples fixed in (AFA) for 24 hrs then in 50% ethyl alcohol solution, then all samples fixed in phosphate buffer formalin 10% and Davidson,s fixative were embedded in paraffin wax and sectioned about 5 – 10 u and stained by Haematoxylin and Eosin (H&E) and examined histopathologically.

Result and discussion

The clinical examination of the moribund shrimps showed swimming lethargically, stop feeding, general weakness, loss of balance and whirling movement with varied degree of shell lesions, (photo 1) appeared as dark brown to black patches scattered all over the body surface including the cuticle of the carapace, abdominal segments, uropod, pereopod (walking legs) and pleopod (swimming legs), (photos 2). With necrotic focci most frequently located on the cuticle of the abdominal segments, Necrosis and destruction of the periopods, pleopods and the antennal flagellum, (photo 3). The eyes of some moribund shrimps were affected and became protruded and edematous (Exophthalmia), (photo 4). The drawn haemolymph changed their colour to reddish (bloody haemolymph). These

results agreed with ***El-bouhy et al. (2006)*** who reported that the clinical examination of the naturally infected adult shrimp with vibriosis showed black spots to brown spots on the carapace and the abdominal segments. Erosions and black spots on the uropode , pleopds and pereiopods . Dirty appearance and total blackness of all body surfaces. Necrosis and destruction of the pereiopods, pleopods and uropods, slow motion, loss of the appetite, swim around the pond wall, and the shell was attached by algae. the destruction of the exoskeleton and appendages may be attributed to the chitinolytic and proteolytic enzymes produced by vibrio sp. As described by ***Sharshar and Azab (2008)*** who described shrimps suffered from Vibrosis showed dark brown focal lesions and necrosis of appendage tips. Moribund prawns assembled at the edges of ponds and swim slowly near the surface.

Similar finding was previously obtained by ***Zhang et al. (2014)*** recorded that infected shrimp showed lethargy, swimming near the water surface and close to pond edges, breaking of antenna, and reduction in food consumption.These results concomitant with the previously reported by ***Kumaran and Citarasu (2016)*** recorded that the diseased shrimps with vibriosis showed symptoms such as lethargy, loss of balance, whirling movement and general weakness.

The common postmortem findings in the moribund shrimps revealed that the gill lamellae adhered each other and changed their colour to black, (photo 5) and there were swelling and congestion of hepatopancreas and heart. These results agreed with ***El-bouhy et al., (2006)*** mentioned that the naturally infected adult shrimp revealed that black spots of the gills. Hepatopancreas appears congested, swollen, soft and surrounded by congested fluid. Congestion of the heart and the intestine free from any food particles.

The isolates were identified according to the morphological and biochemical tests to *Vibrio alginolyticus* and *Vibrio parahaemolyticus,* as shown in (Tables 1& 2). The obtained results were nearly similar to that recorded by several studies including ***Baticados et al., (1986)*** isolated chitinolytic bacteria from tiger prawn *(penaeus monodon*) from brackish water pond suffered from soft shell, the isolates were Vibrio and Aeromonas species, ***Thakur et al. (2003)*** identified four species of Vibrios from the hepatopancrease of moribund shrimp with vibriosis namely *Vibrio parahaemolyticus, Vibrio alginolyticus* and others vibrio sp. based on their cultural, morphological, biochemical characteristics, ***Ferrini et al. (2008)*** identified ninety two Vibrio strains which isolated over a period of nine years from different sources (national and imported fishery

products, shellfish, seawater from aquaculture settings) and belonging to two species relevant for human health and fish pathology, *V.alginolyticus* and *V.parahaemolyticus* and many Vibrio species have which described as important fish and shellfish pathogens, ***Devi et al. (2009)*** identified *Vibrio parahaemolyticus* isolates from shrimp farms along the southwest coast of India, ***Heenatigala and Fernando (2016)*** identified 24 isolates belonged to *Vibrio* species which are responsible for vibriosis in shrimps. Those were *Vibrio alginolyticus, V. parahaemolyticus* and others *Vibrio.* Most frequently isolated species was *V. parahaemolyticus* during a bacteriological study which was undertaken in semi intensive shrimp (*Penaeus monodon*) culture ponds, ***Kumaran and Citarasu (2016)*** could isolate *V. parahaemolyticus* from the infected shrimp farms at Marakkanam, Kancheepuram district of Tamilnadu and Artemia franciscana culture tank at CMST campus, ***Mastan and Begum (2016)*** isolated five species of Vibrio bacteria from diseased shrimp, *Litopenaeus vannamei* with vibriosis, collected from commercial shrimp cultured ponds.The isolated bacterial species were identified as *Vibrio parahaemolyticus, Vibrio alginolyticus* and other vibrio species.

In this study, a total of 1050 Samples were collected from 210 naturally infected shrimp organs, 5 samples from each shrimp, as following, muscles, cuticle, gills, haemolymph and hepatopancreas. Results of isolation showed that the number of positive samples were 732 with rate of isolation 69.71% . The number of isolates for *Vibrio alginolyticus* was 568 isolates by incidence of 77.59% and *Vibrio parahaemolyticus* was 131 isolates by incidence of 17.89%.The result obtained showed that the highest prevalence of isolated bacteria was Vibrio species especially *V. alginolyticus* which explained by ***Baffone et al., (2001)*** *and* ***Saifedden et al., (2016)*** who mentioned that *Vibrio alginolyticus, V. parahaemolyticus* were a halophilic gram negative bacteria, widely spread geographically in marine and estuarine waters. And coincided with that obtained previously by ***Eduardo et al., (1998)*** who isolated 172 bacterial isolates from the hepatopancreas of *Penaeus monodon* and found that most 90.12% were *Vibrio* species, moreover ***Sudheesh et al., (2002)*** recorded that *V. alginolyticus* and *V. parahaemolyticus* are two important pathogenic species. They considered opportunistic pathogens and isolated from shrimps suffering from vibriosis, meanwhile ***El-bouhy et al. (2006)*** isolated about 173 bacterial isolates from the 135 samples (larvae, adults and water samples) of diseased shrimp with

vibriosis; all of them were belonging to the Vibrio species and represented by 31.8% *Vibrio alginolyticus,* 23.7% *Vibrio parahemolyticus,* 27.7% *Vibrio harveyi,* 8.1% *Vibrio anguillarum* and 8.6% *Vibrio campbelli. Vibrio alginolyticus* was isolated in 50%, 40%, 30% and 30% from the examined *P. japonicus, P. kerathurus, P. semisulcatus* and larvae respectively. *Vibrio parahemolyticus* was isolated in 43.3%, 26.7%, 30% and 25% from the examined *P. japonicus, P. kerathurus, P. semisulcatus* and larvae, respectively**,** finally by ***Abraham et al. (2013)*** Isolated Vibrio spp., Aeromonas spp. and Pseudomonas spp. from the hepatopancreas, hemolymph, intestine, gills and eroded portion of the exoskeleton of the cultured shrimp *Penaeus monodon*. Vibrio species were the dominant bacterial flora in the affected organs, followed by Aeromonas spp.

The distribution and prevalence of infection in different organs and tissues of shrimp showed 201 positive samples from muscles by ratio of 95.71%, 189 positive samples from cuticle by ratio of 90%, 168 positive samples from gills by ratio of 80%, 109 positive samples from haemolymph by ratio of 51.90% and 65 positive samples from hepatopancrease by ratio of 30.95%. it was found that the highest sampled level of infection was the muscles followed by the cuticle then from the gills and the haemolymph while it was less isolated from the hepatopancrease respectively. and the distribution of each bacterial isolates in different organs and tissues of shrimps as shown in (Table 3) revealed that Vibrio sp. was the predominant bacterial types in the muscles of infected shrimps, and these results were disagreed with ***El-bouhy et al. (2006)*** which recorded that *Vibrio alginolyticus* and *Vibrio parahaemolyticus* were highly isolated from hepatopancrease and less isolated from the muscles of infected adult *penaeus japonicas.*

Results of experimental infection of shrimp with isolated *V. alginolyticus* and *V. parahaemolticus* showed the Pattern of mortality in *penaeus japonicus* shrimp experimentally injected with isolated bacteria in relation to the time of death after inoculation was recorded within 4 days as shown in (Table 4) and it was clear that Vibrio species were highly pathogenic bacteria. *V. alginolyticus* causing 100% mortality within 60 hrs starts within 12 hrs post-injection, at 12 hrs gave 20% mortality, at 24 hrs gave 40% mortality, at 36 hrs gave 60% mortality and at 48 hrs gave 80% mortality. *V. parahemolyticus* causing 100% mortality within 60 hrs starts within 24 hrs post-injection, at 24 hrs gave 20% mortality, at 36 hrs gave 40% mortality and at 48 hrs gave 80% mortality. None of injected shrimp died in the control group. These

results agree with ***Lewis (1973a)*** who recorded that *vibrio alginolyticus* was pathogenic to normal adult shrimp within 24 hrs, also agree with ***Vera et al. (1992)*** who recorded that the intramuscular injection of adult shrimp with *Vibrio alginolyticus* and *Vibrio parahaemolyticus* resulted in 100% mortality, depend on the dose and time of exposure, also these results similar with ***Mastan and Begum (2016)*** who recorded that *Vibrio parahaemolyticus* is highly pathogenic and it produced disease symptoms within 24 hr. after injection, these results disagree with ***Thakur et al. (2003)*** who reported that *V. alginolyticus* causing 100% mortality within 96hrs post-injection while *V. parahemolyticus* causing 100% mortality within 24 hrs.

the clinical picture of the experimental infected shrimps was nearly similar to that present in naturally infected shrimps but varied only in the severity of the developed lesion, where the clinical abnormalities began to appear within 12 hrs post-injection, which includes weak antennal sensation, loss of escape reflex, weak limb sensation, swim lethargically, developed black spots on the the carapace and abdominal region, periopods, pleopods and uropods, these results simillar to that of ***Abou El-Atta (1998)*** who reported that, the clinical signs of experimentally infected prawns by the isolated Vibrio and Aeromonas strains were loss of escape reflex, reducing of swimming activity, swimming lethargically, developed of brownish to black patches or spots on the periopods, pleopods, uropod , also agree with ***Hassanin (2007)*** who mentioned that the clinical finding of experimentally infected adult shrimp (*Penaeus japonicus*) showed reducing activity in some cases but swim lethargically or lay motionless, developed of brownish to black spots on the exoskeleton in the abdominal region and in cephalothorax area. Congestion of all body and black spots occur on the walking and swimming legs.

Post mortem findings of experimentally infected shrimps revealed black coloration of the gills. The heart and hepatopancreas were congested and swollen, these results agree with ***Abou El-Atta (1998)*** who reported that, the postmortem finding of experimentally infected prawns were congestion of the internal organs especially hepatopancreas, accumulation of reddish fluid in the pericardium, also agree with ***Hassanin (2007)*** who recorded that the post mortem finding of experimentally infected adult shrimp with Vibrio spp showed congestion of hepatopancreas and fine black spots on the gills.

In this work, antibiotic sensitivity test for the isolated bacteria was applied as *Vibrio alginolyticus* as shown in **(**Table 5) and (Photo 6&7)was sensitive to Norofloxacin, Ciprofloxacin and Trimethoprim-

Sulfamethoxazole and resistant to Amikacin, Erythromycin, Rifamycin, Gentamycin and Cephradine while *Vibrio parahemolyticus* as in **(**Table 6) and (Photo 8&9) was sensitive to Norofloxacin, Ciprofloxacin, Trimethoprim-Sulfamethoxazole, Gentamycin and Cephradine and intermediate to Erythromycin and resistant to Amikacin, Amoxicillin and Rifamycin. These investigations were nearly similar to, ***Lio-po and lavilla-pitogo (1990)*** reported that vibrio species isolated from tiger prawn were sensitive to sulphamethoxazole trimethoprim and resistant to erythromycin, Amikacin and streptomycin then by ***Ruangpan and kitao (1990)*** were found vibrio species isolated from diseased shrimp were highly sensitive to ampicillin and ciprofloxacin while were resistant to streptomycin.

Xu Bing et al. (1993) recorded that *Vibrio alginolyticus* isolated from cultured shrimp was sensitive to sulphamethoxazol, then by ***Lee et al. (1996)*** recorded that the Swy strain which isolated from the hepatopancreas of kuruma prawns and identified as *Vibrio alginolyticus* during an outbreak of vibriosis was susceptible to ciprofloxacin.

In this study, it is proved that the isolates were most sensitive to Norofloxacin and Ciprofloxacin compared with the other antibiotics, these result harmonies with the previously mentioned by several studies including ***Vaseeharan et al. (2005)*** who mentioned that, thirteen species of Vibrio (N = 90) and two species of Aeromonas (N = 7) isolates were tested by agar disk diffusion. The results showed that Norofloxacin and Ciprofloxacin were found to be the most effective in controlling the isolates from hatcheries and ponds compared with the other antibiotics, ***Akinbowale et al. (2006)*** studied the resistance of bacteria isolated from crustaceans and found that all strains of Vibrio sp. and Aeromonas sp. were sensitive to Ciprofloxacin, Ttrimethoprime- sulfamethoxazole and resistant to amoxicillin, ***Jayasree et al. (2006)*** who studied the resistance of Vibrio spp. (*V. alginolyticus* and *V. parahaemolyticus*) associated with diseased shrimp from culture ponds to antibiotics and they recorded that all bacterial isolates were sensitive to Norfloxacin and Ciprofloxacin and resistant to Rifampacin andAmoxicillin, then ***Jayasree et al. (2008)*** studied the antibacterial sensitivity of Six species of Vibrio and found that all isolates were sensitive to Ciprofloxacin and Norfloxacin and resistant to Amoxicillin and Rifampacin.

Histopathological pictures among naturally infected shrimps (*penaeus japonicas*) revealed the following; there were changes in muscles as intermuscular edema, Photo (10&11), inflammatory cells between muscle bundles, Photo (12,13&14) and degeneration and

necrosis of muscles, Photo (15), The gills showed squamous metaplasia, photo (16) and the hepatopancreas showed congestion in the hepatic vessels, advanced vacuolar degeneration with nuclear pyknosis of most hepatocytes were evident. The necrotic cells were either ruptured or lacked their nuclei. The necrotic areas infiltrated with some hemocytes and mononuclear cells, photo (17), these results were similar to those mentioned by ***Khuntia et al., (2008)*** who observed that histopathological changes due to Vibrio infection were showed the muscle bundles were severely degenerated. Gill tissues showed moderate necrotic changes in lamellae. Branchial arches were thickened at places due to hyperplasia and sever haemolytic in alteration. At times, the Branchial arches were oedemateous and infiltrated with haemocytes. Cellular changes in hepatopancreatic tissues were more pronounced and characterized by dilation of tubules, vacuolation of hepatocytes and marked necrosis in acinar cells. There was necrosis of acinar cells with complete desquamation of haemocytes. There was severe infiltration of cells in intertubular spaces, then by ***Abraham et al. (2013)*** recorded that the histopathological examination of muscle of affected *penaeus monodon* which had symptoms of vibriosis, gill disease, shell disease, red discolouration showed bacterial infection resulting in edema, haemocytic infiltration and degeneration of cells and necrosis. Severe necrosis in hepatopancreas was noticed in the diseased shrimps, with hepatopancreatic epithelial cell damage and accumulation sloughed cells in the lumen. Hstopathological examination revealed extensive hepatopancreatic lesions, characterised by inflammatory sinuses with bacterial plaques and cell debris

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Photo (1): *Dead adult penaeus japonicas shrimps showed different shell lesions (dark brown to black patches scattered all over the body surface).*
Photo (2): *Diseased adult penaeus japonicas shrimp showed area of black discolouration in the carapace and bluish black discolouration of the periopods and pleopods with destruction of the pleopods.*
Photo (3): *Diseased adult penaeus japonicas shrimp showed eroded periopods.*
Photo (4): *Diseased adult penaeus japonicas shrimp showed protruded and edematous eyes (Exophthalmia) and discoloration in the eye iris with melanization of the carapace.*

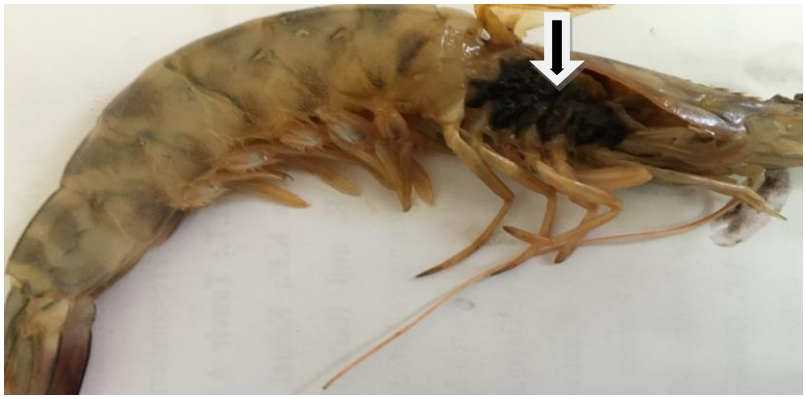

Photo (5): *Shell diseased adult penaeus japonicus shrimp showed black colored gills with adhesion of gills lamellae.*

Table (1): *Morphological, biochemical and culture characters of suspected Vibrio alginolticus isolated from naturally infected shrimps (Penaeus japonicus):*

Items	***Vibrio alginolticus***
Gram stain	Negative
Motility test	+
shape	Curved rods
Cytochrome Oxidase	+
Catalase	+
Growth on TCBS	+ Yellow colonies
Growth at 43^{o} c	+
Growth on media contain sodium chloride weight per volume:	
Growth at 0.0% NaCl	-
Growth at 3.0 %NaCl	+
Growth at 6 .0% NaCl	+
Growth at 8.0 %NaCl:	+
Growth at 10.0% NaCl	+
Lysin decarboxylase	+
Arginin dihydrolase	+
Ornithin decarboxylase	+
Esculin hydrolysis	-
Voges-proskauer test	+
ONPG hydrolysis	-
Nitrate reduction	+
Gas from glucose	-
Acid from L-Arabinose	-
Acid from inositol	-
Acid from sucrose	+
Acid from salicin	-

Table (2): *Morphological, biochemical and culture characters of suspected Vibrio parahemolyticus isolated from naturally infected shrimps (Penaeus japonicus):*

Items	***Vibrio parahemolyticus***
Gram stain	Negative
Motility test	+
shape	Curved rods
Cytochrome Oxidase	+
Catalase	+
Growth on TCBS	Green colonies
Growth at 43^{o} c	+
Growth on media contain sodium chloride weight per volume:	
Growth at 0.0% NaCl	+
Growth at 3.0 %NaCl	-
Growth at 6 .0% NaCl	+
Growth at 8.0 %NaCl:	+
Growth at 10.0% NaCl	-
Lysin decarboxylase	+
Arginin dihydrolase	+
Ornithin decarboxylase	+
Esculin hydrolysis	-
Voges-proskauer test	-
ONPG hydrolysis	-
Nitrate reduction	+
Gas from glucose	-
Acid from L-Arabinose	Different reaction
Acid from inositol	-
Acid from sucrose	-
Acid from salicin	-

Table (3): *The distribution of each bacterial isolates in different organs and tissues of shrimp:*

Bacterial isolates	Muscles		Cuticle		Gills		Haemolymph		Hepatopancrease		Total isolates
	No	%	No	%	No	%	No	%	No	%	
Vibrio alginolyticus	141	**24.82**	148	**26.05**	151	**26.58**	91	**16.02**	37	**6.51**	**568**
Vibrio parahemolyticus	49	**37.40**	38	**29**	10	**7.63**	15	**11.45**	19	**14.50**	**131**
Total	190	**27.45**	186	**25.81**	161	**22.95**	106	**14.89**	56	**8.87**	**699**

Table (4): *Pattern of mortality in penaeus japonicus shrimp experimentally injected with isolated bacteria:*

Groups	No.	Tested bacteria	Percentage of dead shrimp during 96 hours post I/M injection							
			12	24	36	48	60	72	84	96
1	5	*V. alginolyticus*	20	40	60	80	100	-	-	-
2	5	*V. parahaemolyticus*	-	20	40	80	100	-	-	-
3	5	Sterile saline	-	-	-	-	-	-	-	-

Table (5): *Antibiogram for Vibrio alginolyticus in mm***:**

Antibiotic disc	Bio-disc cod	Concentration	Zone diameter interpretation Standards (mm)			Resulted diameter inhibition zone	Description of inhibition zone
			R	I	S		
Amikacin	AK	30mcg	14≤	15-16	17≥	11	R
Norofloxacin	NOR	10mcg	12≤	13-16	17≥	23	S
Amoxicillin	AX	25 mcg	11≤	12-13	14≥	-ve	-ve
Bacitracin	B	10 U	8≤	9-12	13≥	-ve	-ve
Ciprofloxacin	CIP	5 mcg	15≤	16-20	21≥	22	S
Trimethoprim-Sulfamethoxazole	SXT	1.25 mcg 23.75 mcg	10≤	11-15	16≥	20	S
Erythromycin	E	15mcg	13≤	14-22	23≥	13	R
Rifamycin sv	RF	30mcg	16≤	17-19	20≥	9	R
Fucidic acid	FA	10mcg	17≤	18-21	22≥	-ve	-ve
Gentamycin	CN	10mcg	12≤	13-14	15≥	10	R
Cephradine	CE	30mcg	11≤	12-13	14≥	7	R

Table (6): Antibiogram for *Vibrio parahemolyticus* in mm:

Antibiotic disc	Bio-disc cod	Concentration	Zone diameter interpretation Standards (mm)			Resulted diameter inhibition zone	Description of inhibition zone
			R	I	S		
Amikacin	AK	30mcg	14≤	15-16	17≥	13	R
Norofloxacin	NOR	10mcg	12≤	13-16	17≥	35	S
Amoxicillin	AX	25 mcg	11≤	12-13	14≥	0.6	R
Bacitracin	B	10 U	8≤	9-12	13≥	-ve	-ve
Ciprofloxacin	CIP	5 mcg	15≤	16-20	21≥	40	S
Trimethoprim-Sulfamethoxazole	SXT	1.25 mcg 23.75 mcg	10≤	11-15	16≥	26	S
Erythromycin	E	15mcg	13≤	14-22	23≥	15	I
Rifamycin sv	RF	30mcg	16≤	17-19	20≥	15	R
Fucidic acid	FA	10mcg	17≤	18-21	22≥	-ve	-ve
Gentamycin	CN	10mcg	12≤	13-14	15≥	15	S
Cephradine	CE	30mcg	11≤	12-13	14≥	17	S

R=Resistant S=Sensitive I=intermediate -Ve= Negative

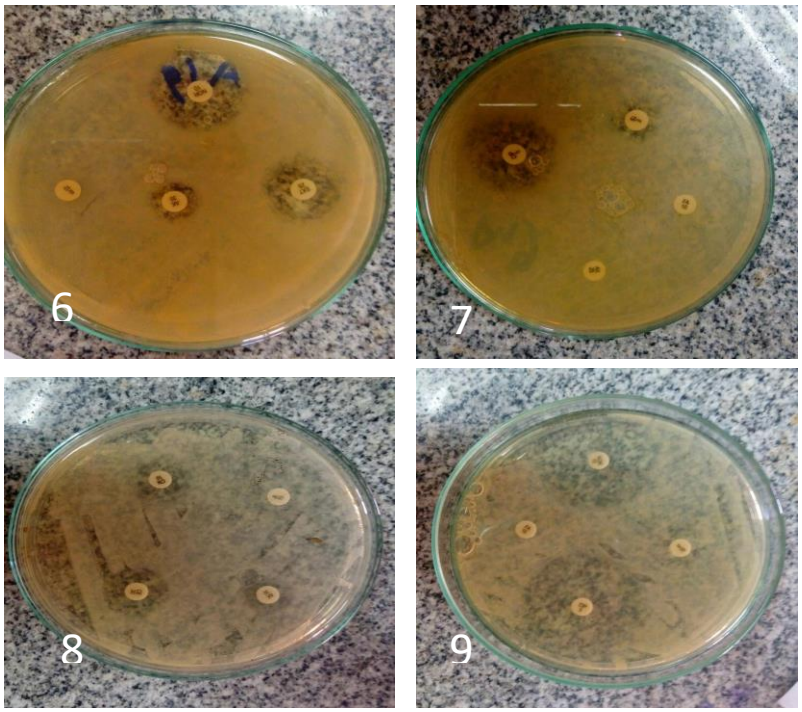

Photo (6): *Results of Antibiogram sensitivity test for V.alginolyticus*
Photo (7): *Results of Antibiotic sensitivity test for V.alginolyticus*
Photo (8): *Results of Antibiotic sensitivity test for Vibrio parahemolyticus*
Photo (9): *Results of Antibiotic sensitivity test for Vibrio parahemolyticus.*

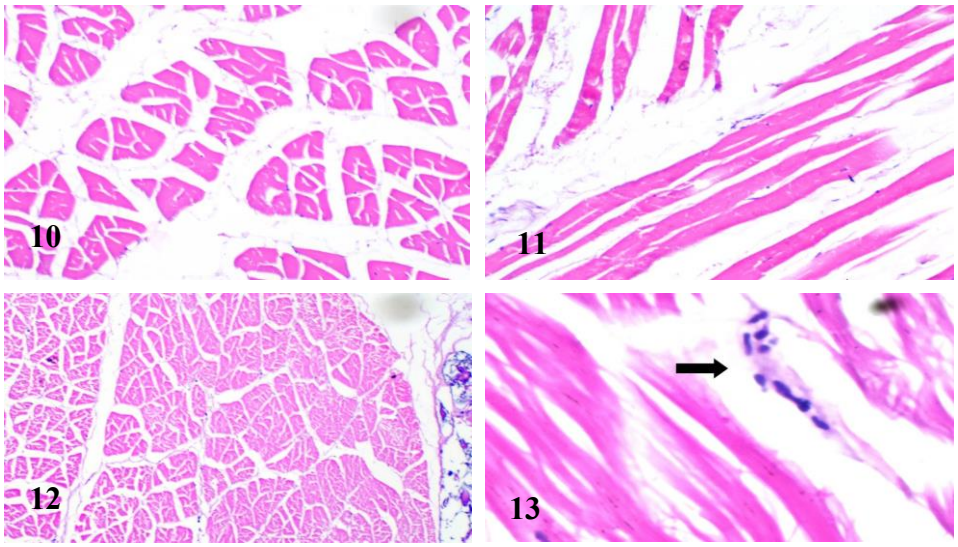

Photo (10): *Transverse section in smooth muscle of shell diseased shrimp (penaeus japonicas) with edema (H&E stained section x 400)*
Photo (11): *longitudinal section in smooth muscle of shell diseased shrimp (penaeus japonicas) with marked edema (H&E stained section x 400)*
Photo (12): *Muscle of shell diseased shrimp (penaeus japonicas) showed inflammatory cells between muscle bundles (H&E stained section x 400)*
Photo (13): *Muscle of shell diseased shrimp (penaeus japonicas) showed edema, congestion and inflammatory cells between muscle bundles (H&E stained section x 400)*

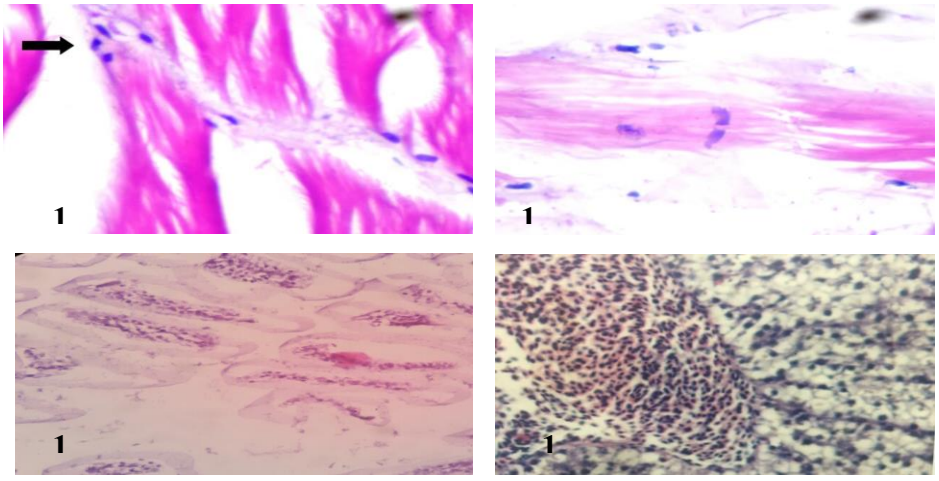

Photo (14): *Muscle of shell diseased shrimp (penaeus japonicas) showed inflammatory cells (H&E stained section x 400)*

Photo (15): *Muscle of shell diseased shrimp (penaeus japonicas) showed marked edema, degeneration and necrosis (H&E stained section x 400)*
Photo (16): *Gills of shell diseased shrimp (penaeus japonicas) showed squamous metaplasia (H&E stained section x 400)*
Photo (17): *Hepatopancreas of shell diseased shrimp (penaeus japonicas) showed advanced vacuolar degeneration with nuclear pyknosis (H&E stained section x 400)*

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الملخص العربي

تم جمع 210 عينة من الجمبرى المريض وأخذ وهو حي من محافظة بورسعيد في الفترة من شهر يناير الي أكتوبر 2016 وتم فحصها اكلينيكيا وتم عمل الصفة التشريحية والفحوص البكتريولوجية والهيستو باثولوجية وايضا قد تم جمع 25 عينة من الجمبرى السليم ظاهريا والخالي من العلامات المرضية على جميع أنحاء جسمه وأخذ وهو حي من محافظة بورسعيد لعمل عدوى صناعية بالبكتريا المعزوله من الجمبرى المصاب طبيعيا وقد تم التوصل في هذه الدراسه الى النتائج التاليه: تم تصنيف انواع البكتريا التي تم عزلها من الجمبرى من العضلات, الغطاء الكيتيني, الخياشيم ,الهيموليمف والهيباتوبنكرياس كالاتي: فيبريو الجينوليتكس و فيبريو باراهيموليتيكس وأخرى وكانت عدد العزلات البكتيرية بالنسبة للعدد الكلى (732) من العزلات كالأتى: عدد العزلات البكتيرية من الفيبريو الجينوليتكس 568 بنسبة 77.59% ومن الفيبريو باراهيموليتيكس 131 بنسبة 17.89% . وأن أكثرمعدل لانتشار العزلات البكتيرية وجد فى العضلات بنسبة 95.71% وفى الغطاء الكيتيني بنسبة 90% ثم فى الخياشيم بنسبة 80% ثم فى الهيموليمف بنسبة عدوى 51.90% بينما أقل معدل لانتشار العزلات البكتيرية وجد فى الهيباتوبنكرياس بنسبة عدوى 30.95%. تم عمل عدوى صناعية بالبكتريا المعزوله من الجمبري المصاب طبيعيا الى جمبري سليم ظاهريا وتبين ان الفيبريو الجينوليتكس والفيبريو باراهيموليتيكس كانوا الأكثر ضراوة حيث ادي الي نفوق 100% من الجمبري خلال60 ساعة وظهرت نفس الأعراض المرضية والصفة التشريحية علي المصاب صناعيا و تم عمل أختبار الحساسية للبكتريا المعزولة من الجمبري المصاب طبيعيا ووجد أن عترات الفيبريو الجينوليتكس و الفيبريو باراهيموليتيكس حساسة للنورفلوكساسين و السبروفلوكساسين و تريموثوبريم + سلفاميثاكسازول ومضادة للاميكاسين والريفاميسين وبفحص أنسجة الجمبري المصاب طبيعيا وجد تغيرات في العضلات والخياشيم والهيباتوبنكرياس نتيجة العدوي البكتيرية حيث وجد تورم وخلايا التهابية بين العضلات ووجد تورم وإحتقان بالهيباتوبنكرياس بالإضافة الي تغير في خلايا الخياشيم نتيجة الالتهاب .