ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(1): 51-58
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www.fisheriesjournal.com
Received: 13-11-2015
Accepted: 13-12-2015

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Environmental conditions/bacterial infections relationship and their impact on immune parameters of cultured *Fenneropenaeus indicus* with special refer to *in-vitro* antibiotic susceptibility

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Abstract
To investigate the relation between bad environmental conditions especially heavy metals contamination in water used for rearing Indian white shrimp (*Fenneropenaeus indicus*) and the enhancement of certain bacterial attack causing heavy mortalities throughout the period from March 2014 to January 2015, about 200 pieces of shrimp tissue samples were collected during grow out stages with the average body weight range from (4.7±1.2 to 22.2±5.2). The clinical, bacteriological, and immunological examinations and the histopathological changes in the intestine of the infected shrimp samples were recorded. Physico-chemical analysis of water samples, in addition to heavy metals concentration in water as well as shrimp tissue associated with such diseases conditions was also documented. Results revealed that Vibrio species especially *V. parahaemolyticus*, *V. anguillarum*, *V. alginolyticus*, *V. ordalii*, *V. harveyi* and *V. viscosus* were the most prevalent species with (82.17%) among all bacterial isolates, followed by equal percent of Salmonella species, Flavobacterium species, Shigella species (3.96%), and *Staphylococcus aureus* recorded the lowest bacterial isolate with (1.98%). Histopathological examination on the intestinal epithelium of the infected shrimp revealed marked degeneration, vacuolation and mild atrophy of epithelium lining the intestine. Measurement of immune parameters revealed that phenoloxidase activity, respiratory burst activity, superoxide dismutase (SOD) activity and phagocytic activity were lowered in diseased shrimp when compared to the apparently healthy shrimp. The sensitivity test applied to detect the effective antibiotic treatment to the different bacterial isolates revealed that the ciprofloxacin is the most effective antibiotic.

Keywords: environmental pollution, marine shrimp, immune response

1. Introduction
Farming of the Indian white shrimp (*Fenneropenaeus indicus*) in Egypt has been found to be of great potential in coastal areas of the Mediterranean Sea. Advances in the technology for rearing *F. indicus* including maturation, spawning, hatching, and larval rearing and grow out of *F. indicus* became a major concern in the private sectors. Vibrio species are part of the natural microflora of wild and cultured shrimps [1] that turned to an opportunistic pathogen when natural defenses mechanisms are immunosuppressed [2]. Pathogenic strains including *V. harveyi*, *V. vulnificus* and *V. parahaemolyticus* have caused massive epidemics in Thailand [3] and Philippines [4]. Luminescent strains of *V. harveyi* appear to release exotoxins [5] and may cause 80-100% mortality in *Penaeus monodon* hatcheries [6]. *V. anguillarum*, *V. campbelli, V. nereis*, *V. cholerae* (no. 01) and *V. splendidus* have also been reported in association with disease outbreaks in shrimps [7-9].

The relationship between luminescence and toxicity of *V. carchariae* in shrimp was also reported by [10]. The occurrence of five types of diseases: tail necrosis, shell disease, red disease, loose shell syndrome (LSS) and white gut disease (WGD) is by Vibrio spp. in *Penaeus monodon* from culture ponds of coastal Andhra Pradesh [11]. Among these, LSS, WGD, and red disease caused mass mortalities in shrimp culture ponds. Six species of *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V. vulnificus* and *V. splendidus* are associated with the diseased shrimp. Systemic vibriosis typically results in the formation of septic haemocytic nodules in the lymphoid organ, heart and connective tissues of the gills, hepatopancreas, antennal gland, nerve cord, telson and muscle [12].

Vibriosis in *P. monodon* is associated with the formation of "spheroids" in the lymphoid organ [13].The effect of copper concentration on the expression of both luminescence and toxin of *V. harveyi* was investigated [14]. They found copper concentration of less than 40 ppm had no effect on the growth of shrimp. While *V. harveyi* cultured with 40 ppm copper concentration showed decreased luminescence. Therefore, the combination of prebiotics, probiotics, immune stimulants and non-antibiotic substances has superior specificity against vibriosis and Luminescent Bactria (LB) coupled with Best Aquaculture Practices (BAP), which makes it an effective management tool for the control of luminescence bacterial toxicity in aquaculture. Initially, the role of bacteria was suggested to be secondary; bacterial colonization was prominent at the latter stage of the disease. The role of bacteria *V. parahaemolyticus* came up on several occasions. *V. parahaemolyticus* was consistently isolated from EMS/AHPNS-infected shrimp. Based on the work done in China [15], Chinese researchers reported a virulent strain of *V. parahaemolyticus* isolated from *P. vannamei* suffering from this early mortality disease in 2010 in Guangxi Province.

Immune parameters in shrimp including total haemocyte count, phenol oxidase activity, respiratory burst activity and lysozyme assay and phagocytosis which is a common cellular defense reaction, and is generally recognized as a central and important way to eliminate micro-organisms or foreign particles. Once a pathogen enters the hemolymph, the host's NADPH-oxidase is activated, which in turn reduces oxygen molecules and subsequently produces several reactive oxygen intermediates (ROIs), such as superoxide anion (O2−), hydroxyl radical (OH), singlet oxygen (1O2), and hydrogen peroxide (H_2O_2). This process is known as respiratory burst, and plays an important role in microbicidal activity [16]. The decrease in all immune responses under stress conditions and pathological injuries in cultured shrimp for the validation of health monitoring at population level [17]. A farm on Negros, in the Philippines, which had been devastated by luminous Vibrio disease while using heavy doses of antibiotics in feed, achieved survival of 80-100% of shrimp in all ponds treated with probiotics [18]. In addition, the extensive use of antimicrobial agents in finfish farming and the consequent selective pressure lead to the acquisition of antibiotic resistance in aquaculture environment bacteria [19].

The rational use of antimicrobial agents and surveillance on antibiotic administration may reduce the acquisition of resistance by microorganisms of aquatic ecosystems. It was noted that bad environmental conditions, especially heavy metals can enhance certain bacteria attack and nutritional deficiencies that lead to black gill disease through weaken the immune system of shrimp [20]. The aim of the present study is to investigate the relationship between bad environmental parameters present in water used in rearing shrimp especially heavy metals and mortalities caused by such condition and its impact on the immune parameters associated with such disease conditions.

2. Material and Methods

2.1. Sample collection, clinical examination and Postmortem findings

A total of 200 specimens of shrimp tissue samples were collected from different shrimp farms in Damietta region during the period from March 2014 to January 2015 of the grow out stages with body weight range from (4.7± 1.2 to 22.2±5.2 g).

Clinical examination of naturally infected shrimp was performed to investigate any clinical abnormalities and necropsy was performed on variable number of freshly dead and moribund shrimp for detection of PM lesions according to the method described by [21].

For bacteriological examination, hepatopancreas was the predilection site for different microbiological assessments of shrimp samples, hepatopancreas samples were processed and analyzed following procedures applied by [22].

2.2. Determination of water physico-chemical parameters:-

Water samples were taken parallel to shrimp samples and subjected to complete water analysis according to [23] including temperature, dissolved oxygen, water salinity using Salinometer, pH values using PH meters and Kits for measure the level of unionized ammonia in the water (USA, Virginia Company, lot. No. 201134) according to manufacture instructions).

2.3. Bacteriological examination

2.3.1. Enumeration of viable bacteria and total coliform count

The number of viable bacteria in water sample was counted by using the standard pour plate method [24] while total coliform bacteria count was determined according to [25]. Shrimp samples have been thorough rinsed, macerating and smashing with distilled water for measuring bacterial growth immediately by spread plate method using different culture media as follow:-

About 15 ml of plate count Agar (PCA), Membrane Fecal Coliforms (mFC) Agar, MacConkey Agar, Eosin Methylene Blue (EMB) Agar, Xylose Lysine Deoxycholate (XLD) Agar, Salmonella- Shigella Agar (SSA) and Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar has been melted and poured at 45° C into the 6 sterile Petri plates and different initiatives were taken for equal distribution of the media. Subsequently solidifying and the inoculated plates were inverted and incubated at 37°C for 48 hours for confirmation of growth. Sixteen samples have been analyzed in terms of aerobic plate count (APC), Salmonella-Shigella (SS) counts and enterobacteriaceae counts. Biochemical tests were used for identification of pure isolates of resulting growth were done according to [26] such as Indole production test, Voges-Proskauer (VP) test, Methyl Red (MR) test, Citrate utilization test, salt tolerance test and Carbohydrate fermentation test.

2.3.2. Bacterial culture

From the collected samples, isolation was carried out directly by swabbing from hepatopancreas under complete aseptic condition and striking on thiosulfate citrate bile-salt sucrose (TCBS) agar media [26].

The criteria used for identification of the isolates are based on colonial characteristics (colony morphology and arrangement) and gram staining of the microorganisms according to the methods described by [27]. All bacterial isolates were used as antigens in the agglutination tests [28] slants with the appropriate salt concentration. One antigen preparation was examined simultaneously with the different antisera using a multi well glass slide [29].

2.4. Histopathological studies of intestine

Specimens for histopathological techniques were freshly taken from infected intestine. Samples were soaked in Davidson's fixative to be prepared for histological examination by standard

procedures for paraffin sections stained with hematoxylin and eosin (H&E) and examined microscopically [30].

2.5. Measurement of immunological parameters

Hemolymph was sampled individually at the beginning of the mortality from all collected shrimp including apparently healthy and diseased shrimp. Hemolymph (100 µl) was withdrawn from the ventral sinus of each shrimp into a 1 ml sterile syringe (25 gauge) containing 0.9 ml anticoagulant (30 mM trisodium citrate, 0.34 M sodium chloride, 10 mM EDTA, 0.12 M glucose, pH 7.55, osmolality 780 mosM kg /1).

2.5.1. Phenoloxidase activity measurement

Phenoloxidase activity was measured spectrophotometrically by recording the formation of dopachrome produced from l-dihydroxyphenylalanine (l-DOPA)**.** The optical density of phenoloxidase activity was measured by recording the formation of dopachrome produced from L-dihydroxyphenylalanine (L-DOPA) [31]. Briefly, the diluted hemolymph was centrifuged at 300 × g at 4°C for 10 min. The supernatant fluid was discarded and the pellet was rinsed, resuspended gently in 1 ml cacodylate-citrate buffer (sodium cacodylate 0.01 M, sodium chloride 0.45 M, trisodium citrate 0.10 M, pH 7.0) and then centrifuged again. The pellet was then resuspended with 200 µl cacodylate buffer (sodium cacodylate 0.01 M, sodium chloride 0.45 M, calcium chloride 0.01 M, magnesium chloride 0.26 M, pH 7.0).

2.5.2. Respiratory burst activity of hemocytes quantification

The respiratory burst activity of hemocytes was quantified using the reduction of nitroblue tetrazolium (NBT) to formazan as a measure of superoxide anion [32].

2.5.3. Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was measured by its ability to inhibit superoxide radical dependent reactions using the Ransod Kit (Randox, Crumlin, UK). The details of the measurements [31].

2.5.4. Phagocytic activity

Phagocytic activity was carried out after collection of 100 µl of hemolymph from the ventral sinus, and mixed with 100 µl and 900 µl of sterile anticoagulant for the measurement of phagocytic activity and clearance efficiency, respectively. Phagocytic activity was measured following the method described by [32] in which Percentage phagocytosis = {(phagocytic hemocytes) / (total hemocytes)} x100.

2.6. Detection of heavy metals levels in water and shrimp hepatopancreas

The method for analysis of the heavy metals in the water was carried out according to [33] and in the hepatopancreas was carried out according to [34] spectrophotometrically using Atomic Absorption (Thermo Electron Corporation, S. Series AA Spectrometer with Gravities furnace, UK). The concentrations of heavy metals were expressed as mg/l for water and µg/g dry wt. for tissues.

Hepatopancreas tissues were dissected separately and minced using a domestic blender, then approximately 1.0 gm was placed in a 150 ml beaker and 10 ml concentrated nitric acid was added. After a short soaking period, 5 ml of 60% perchloric acid was added and the mixture was gradually heated on a hot plate until the conclusion of growth (approximately 2hrs). The mixture was then heated until the appearance of dense white fumes that indicate the nitric acid had evaporated and perchloric acid had reached its boiling point. The mixture then was cooled; 10 ml of 25% hydrochloric acid was added then, the solution was transferred to a 100 ml volumetric flask that was then brought to volume with de ionized water. Blank solution was prepared for the background correction. Atomic absorption spectrophotometer instrument was used to determine Fe, Zn, Cu, Pb, Hg, Ni and Cd concentrations which were expressed as µg / g dry weight.

2.7. Sensitivity test of the bacteria *(In vitro antibiotic susceptibility)*

Antibiotic discs used for Antimicrobial susceptibility test were: Ciprofloxacin (5 Mg), Gentamycin (10 Mg), Flumequine (30 MG) Enrofloxacin (5Mg), Doxycycline (30 Mg), Sulfamethoxazole/Trimethoprim (23.7+1.25 Mg), Oxytetracycline (30 Mg), Amoxicillin (10 Mg) and Erythromycin (15 Mg). This antimicrobial disc obtained from (Oxoid, England). The graduated ruler to 0.5 mm was used for reading the diameter of the zones of inhibition twice at right angles.

2.8. Data analysis

Parameters of bacteriological, immunological, water quality and heavy metals during grow out were compared using Student t tests to determine if there were significant differences. As indicator of mixed infection, analysis of variance (ANOVA) [35] (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Clinical signs of examined shrimp

Examination of adult shrimp pond revealed mass mortalities in cultured shrimp up to nearly 100% of affected population, reddening of the body with red to brown gills. Other examined adult shrimps showing tissue and appendage necrosis (Figure 1).

Fig 1: Shrimp pond revealed mass mortalities in cultured shrimp up to nearly 100% of affected population (A). Adult shrimp showing reddening of the legs (swimmers). Diseased shrimps showing tissue and appendage necrosis at tail area (uropods) (C).

3.2. Microbiological assay of the diseased shrimp

The highly total count (CFU/ml) was reported in second sampling of shrimp (5.9×10^3) compared to the other sampling count (Table1). In addition, different types of bacteria were isolated from the shrimp samples were explained in Table (2); where the Vibrio species were the most predominant among all bacterial groups as they were (82.17 %) whereas other

bacterial groups were; (7.9 %) for Salmonella species, (3.96 %) for both Flavobacterium spp. and Shigella spp. (1.96%) for *Staphylococcus aureus*. While the results observed in Fig. (2) showed that, the incidence of bacterial isolates among examined of naturally infected *F. indicus* differed significantly ($P < 0.01$). The incidence of bacterial serotypes includes *V. parahaemolyticus* (77.11%), *V. anguillarum* (8.44%), *V. alginolyticus* (4.82%), *V. ordalii* (4.82%), *V. harveyi* (3.61%) and *V. viscosus* (1.20%).

Table 1: Total bacteria counts, total coliform counts and Salmonella-Shigella (SS) counts isolated from the collected *Fenneropenaeus indicus* shrimp samples

Sample	Total bacterial count (CFU/ml)	Total coliform count (CFU/ml)	Salmonella-Shigella count (CFU/ml)
First sampling	2.04×10^2	5.4×10^2	0.15×10^2
Second sampling	5.9×10^3	5.3×10^2	0.45×10^3
Third sampling	4.5x10	4.5 x10	0.2 x 10

Table 2: The different types of bacteria isolated from the collected *Fenneropenaeus indicus* shrimp samples

Bacterial Isolates	Sample No	%
Salmonella spp	8	7.9
Shigella spp.	4	3.96
Staphylococcus aureus	2	1.98
Vibrio spp.	83	82.17
Flavobacterium spp.	4	3.96
Total	101	100

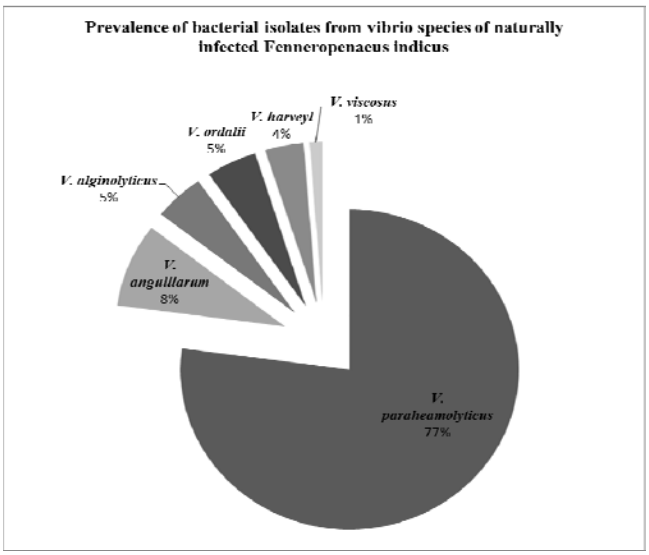

Fig 2: Prevalence of *Vibrio* species isolated from naturally infected *Fenneropenaeus indicus*

3.3. Histopathological results of naturally infected *F. indicus*

Results of histopathological examination showing marked degeneration and vacuolation of lining epithelium of intestine (figure 3-A), mild vacuolation of epithelium lining intestine (figure 3-B), mild atrophy of epithelium lining intestine (figure 3-C).

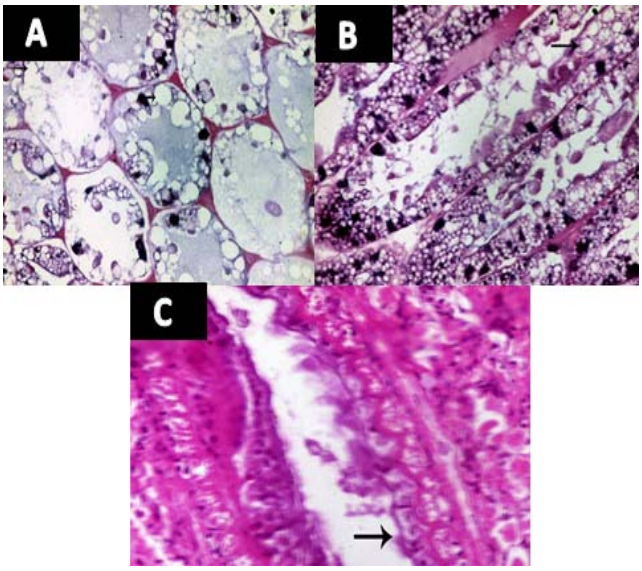

Fig 3: Marked degeneration and vacuolation (black arrow) of lining epithelium of intestine (A). Shrimp intestine showing mild vacuolation of epithelium lining intestine (black arrow) (B). Mild atrophy of epithelium lining intestine (black arrow) (C).

3.4. Immunological parameters

The results observed in Fig. (4) Indicates that phenoloxidase activity, respiratory burst activity, superoxide dismutase (SOD) activity and phagocytic activity were generally decreased in diseased shrimp compared to apparently healthy shrimp. The levels of Phenoloxidase activity, Respiratory burst activity of hemocytes; Superoxide dismutase (SOD) activity and Phagocytic activity % were 0.077, 0.034 and 4.52, 1.36 and 20.33, 62.87 and 87.21, 22.37 in both apparently healthy and diseased shrimp respectively.

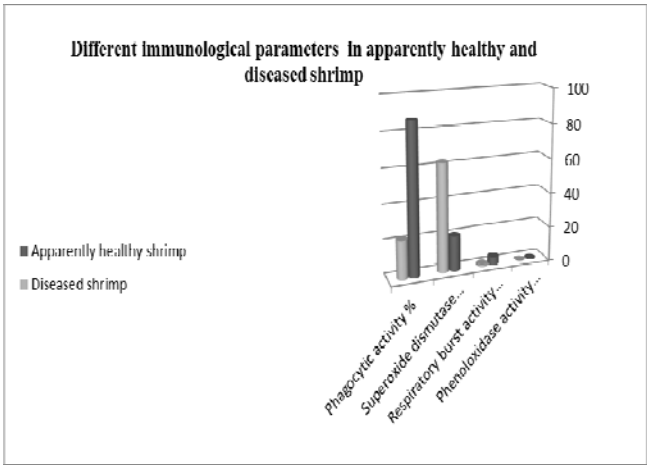

Fig 4: Different immune parameters in apparently healthy and diseased shrimp (Phenoloxidase activity, Respiratory burst activity, Superoxide dismutase (SOD) activity and Phagocytic activity (%) after appearance of mortality.

3.5. Sensitivity test of different Vibrio species isolated during this study

The results observed in Table (3) revealed that the ciprofloxacin is the most effective antibiotic on different isolated bacteria. On the other hand the other tested antibiotics appeared as resistant to all tested bacterial isolates.

Table 3: The sensitivity of different bacterial isolates identified from the diseased *Fenneropenaeus indicus* to different antimicrobial discs

Bacterial serotype	Antimicrobial discs								
	CIP	CN	UB	ENR	DO	SXT	OT	ANL	E
Vibrio parahaemolyticus	S	IM	IM	IM	R	R	R	R	R
Vibrio anguillarum	S	R	R	R	R	R	R	R	R
Vibrio alginolyticus	S	S	IM	IM	R	R	R	R	R
Vibrio ordalii	S	IM	IM	IM	R	R	R	R	R
Vibrio harveyi	S	S	IM	IM	R	R	R	R	R
Vibrio viscosus	S	R	R	S	IM	R	R	IM	R

R: resistant IM: Intermediate S: Sensitive

3.6. Results of water quality

The results of water quality were illustrated in Table (4). It revealed that the levels of Unionized ammonia (NH3) (mg/L.), Nitrite (NO2) (mg/L.) and Hydrogen Sulphate (mg/L.) in all tested water samples were higher than the permissible limits.

Table 4: Physicochemical parameters of water used for rearing of marine shrimp *Fenneropenaeus indicus*

Parameters	Detected limits	* Permissible limits
Dissolved oxygen (mg/L)	5.5	5-6
Unionized ammonia (NH3) (mg/L)	0.04	0.01
Nitrite (NO2) (mg/L)	0.03L	0.01
PH	8.4	8.0 - 8.5
Salinity (PPT)	34	40
Organic matter (mg/L)	2.79	2-3
Hydrogen Sulphate (mg/L)	176.3	70-120
Temperature °C	23-24	25

** WHO (1984).* Means within the same row of different litters are significantly different at ($P < 0.01$).

3.7. Heavy metal concentrations (µg/g dry weight) in hepatopancreas of *F. indicus*

According to Table (5) and Fig. (5); In comparison with the permissible levels (PL) of heavy metals; Zinc (Zn) and Copper (Cu) levels were lower in hepatopancreas than the PL While Iron (Fe) and Cadmium (Cd) levels were higher in Gill samples over the PL and their levels in hepatopancreas are the lowest one. On the other hand; Mercury (Hg), Lead (Pb) and Nickel (Ni) levels were higher in hepatopancreas of Fenneropenaeus indicus over the permissible levels.

Table 5. Heavy metal concentrations (mg/L) in water samples used for rearing of marine shrimp *Fenneropenaeus indicus*

Heavy metals	Detected limits	Permissible limits*
Copper (Cu) mg/ Liter	0.34±0.001^{c}	0.2
Zinc(Zn) mg/ Liter	0.73±0.001 ^{b}	2.0
Cadmium (Cd) mg/ Liter	ND	0.004
Lead (Pb) mg/ Liter	0.004±0.001^{e}	0.050
Mercury (Hg) mg/ Liter	ND	0.001
Nickel (Ni) mg/ Liter	0.002±0.001^{d}	0.001
Iron (mg/L.)	1.23±0.001^{a}	1.00

*WHO (1984). Means within the same row of different litters are significantly different at ($P < 0.01$).

Table 6: Heavy metal concentrations (µg/g dry weight) (Means ±SE) in hepatopancreas of diseased *Fenneropenaeus indicus*

Metals	Heavy metal concentration (µg/g dry weight) Mean± ±SE	Permissible limits *
Zinc (Zn)	58.15±2.04 ^{b}	60.0
Copper (Cu)	0.63±0.0038 ^{b}	3.0
Iron (Fe)	59.82±1.08 ^{a}	50.0
Nickel (Ni)	1.59±0.29 ^{a}	0.50
Mercury (Hg)	1.70±0.89 ^{a}	0.50
Cadmium (Cd)	0.59±0.75 ^{a}	0.20
Lead (Pb)	4.33±0.059 ^{a}	0.20

**WHO (1984).* Means within the same row of different litters are significantly different at ($P < 0.01$).

4. Discussion

Environmental stress caused by heavy metal appear to be necessary factor for determining decrease in immunocompetence and is signaled by the appearance or the high prevalence of diseases in shrimp [36]**.** The determination of coliforms of fecal origin and E. coli provides safe information regarding the hygiene-sanitary conditions of both the oysters and the cultivation water, since *E. coli* accounts for 90% of fecal coliforms and is an indicator microorganism of fecal contamination [37]**.** Total counts, coliform counts and Salmonella-Shigella (SS) counts isolated from shrimp samples revealed a highly total count (CFU/ml, the coliform count (CFU/ml) Salmonella-Shigella count (CFU/ml) in all examined samples that means fecal contamination in the water used for rearing of the shrimp**.** Vibrio species were the most prevalent among all bacterial groups. These results are agreement with the results showed that the Vibriosis was the most prevalent bacteria in marine shrimp while the seasonal prevalence was the highest in summer and the lowest percent was in winter [38].

The results of prevalence of Vibrio species isolates of naturally infected *F. indicus* in the present study revealed that the incidence of bacterial serotypes includes *V. parahaemolyticus* (77.11%), *V. anguillarum* (8.44%), *V. alginolyticus* (4.82%), *V. ordalii* (4.82%), *V. harveyi* (3.61%) and *V. viscosus* (1.20%). The prevalence of shrimp bacterial pathogens was found to be as following; *V. harveyi* (56%), *V. parahaemolyticus* (12%), *V. vulnificus* (17%), *E. faecalis* (7%) and *Ps. fluorescence* (8%) [39].

Also, Vibrio species from the marine shrimp *P. japonicus* were identified as *V. alginolyticus* and *V. mimicus* and *V. parahaemolyticus*. Vibrios are among the most important

bacterial pathogens of cultured shrimp causing number of diseases, and mortalities up to 100% have been reported due to Vibriosis [40, 41]. *Vibrio harveyi* (luminous Vibrio) is the main cause of shrimp death infecting larva in the hatchery also in the cultivation pond [42].

Regarding to the effect of water contamination on immune system of shrimp, phenoloxidase activity was decreased in diseased shrimp than apparently healthy shrimp. These results confirmed by [43-45] who documented the significant decrease in phenoloxidase activity in the infected shrimp. On the same manner the respiratory burst activity of hemocytes were also decreased in diseased shrimp than apparently healthy shrimp. similar to what is published by [46-48] who recorded that the both phenoloxidase activity and respiratory burst decreased significantly in the infected shrimp up to 36 h, and then remained steady for the remainder of the treatment. Similar to our results of phagocytic activity % in the present study that is decreased in diseased shrimp than apparently healthy shrimp, [49] found that a great number of immune parameters, such as lymphocyte proliferation, cytokine synthesis, natural killer cell activity and phagocytosis were decreased in diseased shrimp. In addition, a significant increase in NBT values as well as serum bactericidal activity were detected in apparently health shrimps when compared with the diseased shrimps [50] agreeing with the data of the present study. On overview of the sensitivity of different Vibrio species isolated during this study; the results revealed that the ciprofloxacin is the most susceptible antibiotics to different isolated bacteria. On the other hand the other tested antibiotics appeared as resistant to all tested bacterial isolates. The same results obtained by [51] who found that the resistance of *V. harveyi* and *V. alginolyticus* isolated from sea bream against 15 antimicrobial agents, 96% of the strains showed multiple resistances to the tested drugs, with two strains, Vibrio aestuarianus and Vibrio harveyi resistant to 10 and 9 antibiotics, respectively. Ampicillin, amoxicillin, erythromycin and sulfadiazine showed low efficacy against Vibrio spp.

The results of water quality in shrimp; revealed that the levels of Unionized ammonia (NH3) (mg/L), Nitrite (NO2) (mg/L) and Hydrogen Sulphate (mg/L) in all tested water samples were higher than the Permissible limits. Similar results reported by many researchers [52] who mentioned that the environments of water play an important role for incidence of diseases in cultured fish and shrimp. Concerning the relationship between Fe toxicity and the incidence of bacterial diseases the different pathogens adopt numerous strategies to overcome iron restriction such as siderophores which are excreted, which chelate iron and return to bacteria via specially induced cell-surface protein receptors, after internalization, the siderophores give up their iron under the influence of reductase.

Concerning the relationship between Cu toxicity and the incidence of bacterial diseases; copper concentrations in water were higher than the permissible levels and can increase the infection with Vibriosis especially *V. anguillarum* infection and similar results obtained in eels by [53] where they described the debilitating effects of copper, in terms of concentration and time of exposure with regard to increasing susceptibility to Vibriosis (e.g. *Vibrio anguillarum*). It worthy noted that the relationship between heavy metals and diseases condition in cultured shrimp were mentioned by [54, 55] who recorded that the environmental stress from heavy metal and pollutants seems to be important factor for determining reduction of immunocompetence and is signalled by the appearance or the increased prevalence of diseases in shrimp and crabs.

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