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# PATHOLOGY OF SOME PATHOGENIC BACTERIA AMONG CULTRED SHRIMP

(With 4 Tables and 12 Figures)

By

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## دراسات باثولوجية على بعض البكتيريا الضارة في الجمبري المستزرع

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تم تجميع مائة عينة من جمبري المياه العذبة بمستزرع وكذلك ١٠ عينات مياه خلال الفترة من يناير ١٩٩٩ وحتى ديسمبر ٢٠٠٠ . وقد تم الفحص البكتريولوجي والباثولوجي للعينات المجمعة . كما تم تحليل فيزيو كيميائي للمياه. وتم عمل اختبار حساسية للبكتيريا المعزولة. كما تم إجراء عدوى معملية بالبكتيريا المعزولة. وقد أسفرت النتائج عن عزل البكتيريا سالبة الجرام بنسبة ٧٥,٥% وتم معرفة هويتها كالتالي :ميكروب الفبريو (٣٩,١%) – الايروموناس (١٥,٥%) – السيدوموناس (١١.٨%) – الأنتيروباكتر (٥.٥%) والستروباكتر (٣,٦%). وقد تم تصنيف هذه البكتيريا الى الفبريوباراهيموليتيكس ، فبريو الجينوليتيكس ، فبريو فلجارز، ايروموناس هيدروفيلا ، سيدوموناس فلوريسنس ، انتيروباكتر ايروجينس وستروباكتر فرندى. كما تم تحديد مدى إنتشار هذه البكتريا على مدار فصول العام وعلى الأنسجة المختلفة للجمبري المصاب. وقد اتصف الجمبري المصاب إكلينيكيا بنقص في إستهلاك الغذاء والعوم الحلزوني مع نقص في الصبغة الطبيعية للجلد. وبالفحص العيني والهستوباثولوجي فقد اختلفت الصورة باختلاف البكتيريا المعزولة ومع ذلك فقد اتضح احتقان في الأطراف والكبد والبنكرياس والعضلات. كما وضح انحدار ونخر مع إرتشاحات خلوية في الأنسجة المفحوصة وخاصة الكبد والبنكرياس والمبايض. وقد أسفرت اختبارات الحساسية عن حساسية الميكروبات المعزولة للأكسيتتراسيكلين ثم الأرثروميسين والكلورمفينيكول.

## SUMMARY

One hundred naturally infected freshwater cultured shrimp and 10 water samples of its rearing tanks were collected during the period from January 1999 to December 2000. They were subjected to bacteriological and pathological examinations. Water samples were examined for total bacterial count and physiochemical analysis. Antibiotic sensitivity test of isolated bacteria was done in addition to experimental infection of 70 shrimps with isolated bacteria. The bacteria isolated from the collected samples were Gram negative with a total percentage of 75.5%. *Vibrio*

*(V. parahemolyticus, V. alginolyticus & V. Vulgaris). Aeromonas hydrophila, Pseudomonas fluorescens, Enterobacter aerogenes and Citrobacter fermudii* were the isolated genera from the shrimp and water samples with a percentage of 39.1, 15.5, 11.8, 5.5 and 3.6%; respectively. The seasonal prevalence and tissue distribution of the bacteria were reported. The physiochemical analysis of water samples revealed 7.2-8.5 mg/L dissolved oxygen, 22-27°C temperatures, 7.7- 8.3 pH and 14% salinity. Clinically, reduction of food consumption, spiral swimming and loss of normal pigmentation were seen in most bacterial infections. The gross and histopathological findings of infected shrimp were varied with the bacterial isolates, however, congestion of all apppendages, hepatopancreas and muscles and retrogressive and / or necrotic changes as well as cellular infiltration in the examined tissues especially the hepatopancreas, muscles and ovaries were commonly seen. The bacterial isolates were sensitive to oxtetracyclin followed by erythromycin and chloramphenicol.

***Key words:*** *Pathogenic bacteria, cultured shrimp.*

## INTRODUCTION

The commercial culturing of shrimp has a world-wide distribution because of their delicious animal protein. It is reared in many tropical and subtropical countries (New 1982). The rapid growth of shrimp culture industry should accompanied by an increase awareness of the negative impact of disease. The diseases of shrimp may be of infectious or non infectious etiologies, the non infectious disease of shrimp might include extreme environmental stress or nutritional diseases (Turnbull et al., 1994). A number of bacteria especially Gram negative one have been implicated as infectious causes of disease in cultured and wild shrimp, among those *Vibrio species* are by far the most numerous (Lightener, 1988). Bacterial infection of shrimp has brought economic damage to shrimp culturists (Limswan 1988, Tangtrongpairoj et al, 1988), but no information on their pathology or susceptibility to the chemotherapeutic agents is available (Ruangpan and Kitao, 1992). Therefore, it is essential to identify the disease present among shrimp and its pathological effect in order to formulate the useful control measures.

The present study was designed to investigate the pathogenic bacteria in shrimp and their associated pathological lesions along with trails for its treatment via antibiotic sensitivity tests.

## MATERIALS and METHODS

*1- Collection of samples:*

One hundred clinically infected freshwater cultured shrimp and 10 water samples of its rearing tanks were collected from Suez Canal area and Sharkia as well as Karfr El-Sheikh Governorates during the period from January 1999 to December 2000 and used for the laboratory investigations.

*2- Bacteriological examinations:*

A- **Dilution:** Samples from hepatopancreas, hemolymph, intestine and water were prepared and serially dilluted to $10^5$ with 1 ml of sterile water.

B- **Bacterial isolation and identification:** Each dilution was inoculated into TS broth and nutrient broth, then incubated at 18-23°C for 24-48hrs. Then, streaked on RS media, Mac Conkey agar media, Ordal's media and thiosulphate citrate bile salt sucrose agar (TCBS) and incubated at 25-28°C for 48 hrs. The bacterial isolates were identified using the morphological and biochemical tests described by Bergy (1986) and Schäperclaus et al. (1992).

*3- Physiochemical analysis of water:*

Water samples were subjected to physio-chemical analysis to determine the dissolved oxygen, temperature, pH and salinity according to Michael and Somusk (1985).

*4- Experimental infection:*

A total number of 80 adult healthy shrimp (average body weight 30 gm) were obtained and divided into 8 equal groups. The $2^{nd}$ – $8^{th}$ groups were intramuscularly (I/M) inoculated with 0.05 ml of 24 hrs broth culture of each bacteria that has been isolated from the natural infection *(Vibrio parahemolyticus, V. alginolyticus, V. Vulgasris, Aeromonas hydrophila, Pseudomonas fluorescens, Enterobacter aerogenes* and *Citrobacter fernudii)* where 1 ml of broth contained $10^6$ bacterial cell/ml according to Austin and Austin (1989). The $8^{st}$ group served as a control and was injected with sterile broth. The experimentally infected shrimps were observed for 2 weeks and subjected to clinical, gross and histopathological examinations as well as bacterial reisolation.

*5- Histopathgolgical examination:*

Tissues specimens were collected from the gills, skin, muscles and internal organs (hepatopancreas, intestine, heart & ovary) of the naturally and experimentally infected shrimps. They were immediately fixed in 10% neutral buffered formalin, dehydrated in ascending grades

of ethanol, cleared in xylene and blocked in melted soft paraffin. Sections of 5µm were obtained and stained with hematoxylin and Eosin stains (Drury and Willington, 1981).

***6- Antibiotic sensitivity test:***

It was done by disc diffusion method using Muller Hinton agar according to Bauer et al. (1966). The antibiotics used were oxytetracyclin, erythromycin, chloramphenicol, novobiocin, ampicillin, and trimethoprin.

## RESULTS

Table (1) revealed the prevalence of the bacterial isolates from the shrimp and water samples. The bacteria isolated from the collected samples were Gram negative with a total percentage of 75.5% *Vibrio, Aeromonas, Pseudomonas, Enterobacter and Citrobacter* were the isolated genera from the shrimp and water samples with a percentage of 39.1, 15.5, 11.8, 5.5 and 3.6%; respectively. Vibrio species were the most predominant genera from shrimp and water samples and identified as *V. Parahemolyticus, V. alginolyticus and V. vulgaris* with a percentage of 23.6, 10.0 and 5.5%; respectively.

The majority of the bacterial isolates were obtained from the hemolymph followed by the intestine and the liver (Table 2). The seasonal prevalence of the bacterial isolates was also mentioned in Table (2). The morbidity and mortality rates of experimentally infected shrimp with the isolated bacteria from the natural cases were reported in Table (3). *Vibrio parahemolyticus, Aeromonas hydrophila and Pseudomonas fluorescens* represented the most pathogenic bacteria to shrimp with high morbidity and mortality rates. The physicochemical analysis of water samples revealed 7.2-8.5 mg/L dissolved oxygen, 22-27°C temperatures, 7.7-8.3pH and 14% salinity.

Clinically, the naturally and experimentally infected shrimp with the isolated bacteria showed reduction of food consumption, spiral swimming and loss of normal pigmentation. Finally, the infected shrimp revealed slow motion and swim around the pond wall along with loss of escape reflex.

The gross and histopathological findings of experimentally infected (Exp. In.) shrimp with the isolated bacteria were similar to that observed in naturally infected cases.

*1- Vibrio species:*

Grossly, the infected shrimp exhibited enlargement of the hepatopancreas and congestion of internal organs (Fig. 1).

Microscopically, in the gills, the primary filaments were anemic and lacked their secondary lamellae. The gill arch was edematous and infiltrated with some hemocytes (Fig. 2). The muscles were edematous, necrotic and infiltrated with some hemocytes and melanomacrophages (Fig. 3). The hepatopancreas revealed marked vacuolation in their cells and karyolysis of most nuclei. There was mild to marked infiltration of hemocytes in the connective tissue surrounding the hepatopanceas and in the interstitial tissues. The interstitial tissue around the invaded tubules was expanded by edema, enlarged sinuses, eosinophilic granules and hemocyte infiltration. The hemocytes might entirely walled off the necrotic tubules and formed granulomatous lesions (Fig. 4). The ovary was edematous and showed ruptured ova with focal aggregation of some mononuclear cells.

*2- Aeromonas hydrophila:*

Grossly, the infected shrimp revealed congestion in the gills, find, muscles and hepatopancrease along with presence of black spots on the body.

Microscopically, the gills showed congestion and edema in the primary filaments and gill arch. Some hemocytes were seen in the gill arch. The muscles were edematous, vacuolated and exhibited focal areas of necrosis. The necrotic muscles infiltrated with mononuclear cells (Fig.5). The hepatopancreas showed dilated sinuses and vacuolar degeneration in most hepatocytes. Other cells were necrotic and lacked their nuclei or even ruptured. The necrotic hepatocytes were infiltrated with some hemocytes (Fig. 6).

*3- Pseudomonas fluorescens:*

Grossly, the infected shrimp revealed necrotic foci on the hepatopancreasc and intestine along with darkness of the appendages and exoskeleton.

Microscopically, the gills showed cellular vacuolation in the anemic primary filaments and epithelial desquamation in the secondary filaments. The gill arch contained numerous hemocytes. In the muscles, edema, congestion, hemorrhage and hyaline degeneration were evident. Other muscle bundles were necrotic and infiltrated by mononuclear cells (Fig. 7). In the hepatopancreas, the hepatic tubules exhibited either necrotic or ruptures cells and infiltrated by hemocytes, monouclear cells

and melanomacrophages. The intestine revealed marked necrosis in their epithelium with some hemocytes in the submucosa (Fig. 8).

***4- Enterobacter aerogenes:***

Grossly, the infected shrimp revealed congestion in the muscles, hepatopancrease, antenna and walking legs.

Microscopically, the gills showed vacuolation in the cells of the primary filaments along with mononuclear cell infiltration. The gill arch was edematous and contained monouclear cells. The exoskelton was thickened and extended toward the epidermal layer. Scattered dense unclear debris was apparent throughout the melanized layer. Some hemocytes could infiltrate the tissue below the cuticlar melanization (Fig.9). The muscles exhibited intermuscular edema and focal hyaline degeneration. Cytoplasmic vacuolation and zenker's necrosis were evident along with mononuclear cell infiltration. The hepatopancreas showed vacuolar degeneration and necrosis of most hepatocytes along with focal aggregation of hemocytes. The ovary was edematous and exhibited atrophy and degeneration of most ova together with hemocytes infiltration in the ovarian stroma (Fig. 10).

***5- Citrobacter fernudii:***

Grossly, the infected shrimp showed focal erosion to deep ulceration on the body surface with muscular liquefaction.

Microscopically, the muscles revealed focal to diffuse myofibril degeneration and necrosis. Loss of myfibril outline together with a myofibrillar haylininzation and fragmentation were observed. The degenerated bundles showed deep eosinophilic cytoplasm and pynkotic nuclei but the adjacent myofibrils had granular, slightly basophilic cytoplasm and loss their striations. Marked liquefaction in some necrotic areas was evident and surrounded by some hemocytes (Fig. 11). The hepatopancrease revealed vacuolar degeneration in the cells of the hepatic tubules. The ovary was edematous and showed ruptured ova, liquefaction with focal aggregation of some mononuclear cells (Fig. 12).

The results of the antibiotic sensitivity tests for the isolaed bacteria were presented in Table (4) where the bacterial isolated were highly sensitive to oxytetracyclin followed by erythomycin and chloramphenicol.

## DISCUSSION

The outcome of this study is important because the knowledge of whether native shrimp can infect or otherwise transmit diseases to other

shrimp has an influence on the health of both individuals and cultured species.

In the present study, *Vibrio parahemolyticus* represented the most common bacterial isolates of both water and shrimp samples. Similar results were reported by Mohney et al. (1994). A. hydrophila and *Ps. flourescens* were 15.5 and 11.8%; respectively among the examined samples. Nearly similar results were reported by Hung et al. (1991) and Brady and Ernesto (1992). The percentage of other bacterial infections were low similar to those obtained by Brock (1983) as well as Janis and Ronald (1986).

The majority of the bacterial isolates, in the present study, were isolated from the hemolymph followed by the intestine and the hepatopancreas. Since the infection always lead to rapid bacterial multiplication inside the hemocoel (Pitogo et al., 1990) The presence of large number of swarming bacteria inside the hemocoel of moribund shrimp was also observed by Lightner (1988). Pena et al.(1995) domenstrated *Vibrio SP*. In the hemolymph and stomach of infected prawn 3 hrs post-oral inoculation and then in the intestine and hepatopancrease after 6 hrs post- inoculation. The hepatopancrease has a direct access to the stomach chambers via lower cardiac grooves and the primary hepatopancreatic duct (Belland and Lightner, 1988) and the mid gut is just like an extension of the stomach. Thus, the pathogen could have invaded these two organs directly from the stomach.

It was noticed, in the present study that, most bacterial isolates were recorded in the summer season followed by the spring where there was an increase in the water temperature. This high temperature might have stimulated the multiplication of the bacteria. Ulitzur (1974) mentioned that, some strains of Vibrio have a very short generation time at higher temperature. However, Kerthiyani and Iyer (1975) found that, the Vibrio population of 5% increased to 30% in freshly caught wild prawn during the summer. Aly (1994) reported high incidence of Ps. fluorescens infection during the winter season as those obtained in the present study.

The mortality rate that observed among experimentally infected shrimp by the isolated bacteria was varied between 60-100%. This high mortality among the different groups might be due to a variety of factors including the type and dose of bacteria as well as some environmental factors such as temperature, pH, dissolved oxygen and salinity of water (Hameed, 1993). *Vibrio species* were found to be the most pathogenic

isolates in shrimp administered by I/M injection. Similar results were reported in prawn (Hameed, 1989; El sayed et al., 2000). The toxins produced by these species could be incriminated in the death of shrimps (Vera et al., 1992) and this could explain the high mortality observed among the experimentally infected shrimp.

The physiochemical analysis of water samples revealed 7.2-8.5 mg/L dissolved oxygen, 7.7-8.3 pH and 14% salinity. The poor water quality probably supress the host resistance and facilitate the disease progression (Scott and Thune, 1986).

*Vibrio species* were found to constitute the majority of cultural bacteria in wild and cultured shrimp (Yasuda and Kitao, 1980; Sugita et al., 1987). As large number of Vibrio spp. Normally present in the shrimp microflora, it was not surprising that many investigators have found to be frequent in shrimp. *Pseudomonas fluorescens* is a soil and water bacterium and also decaying fishes served as the main source of infection (Aly, 1994). Yasuda and Kitao (1980) observed an abundant Pseudomonas population in the gut of healthy cultured and wild prawn. *Aeromonas hydrophila* is found in water and intestinal tract of fish and establishment of infection is usually associated with stress factors especially variation in water temperature (Yukinori, 1984). Yasuda and Kitao (1980) noted poor growth of the prawn when Aeromonas were dominant in the gut. This observation indicated that Aeromonas species might be harmful to larvae and post-larvae of prawn when present in large quantities.

The clinical signs observed among the naturally and experimentally infected shrimp were nearly similar to that observed by El Sayed et al. (2000) in prawn. Grossly, the hepatopancreas was involved in most bacterial infection. Similar results were mentioned by Song et al. (1993) in shrimp and El Sayed et al. (2000) in prawn. *Citrobacter fernudii* caused skin ulceration and muscular liquefaction.

Histopathologically, there is no enough work on the histopathology of bacterial diseases in shrimp. A systemic infection with hepatopancreatic involvement was common in most bacterial especially *Vibrio* and *Aeromonas*. The intestinal lesions were apparent in Ps. fluorescens infection while exoskelcton involvement was seen with *E. aerogenes*. but myofibril degeneration and liquefaction were noticed in *C. fernudii* infection. This may point to the role of the oral and cuticular routes in the bacterial infection among shrimp. The histophathological findings of shrimp infected by Vibrio similar to that reported by

Jiravanichpaisol and Miyazaki (1994). The shrimp infected by *Ps. Fluorescens* showed enteric lesions and those infected by *E. aerogenes* revealed cuticular lesions, the microscopic pictures of *E. aerogenes* infected shrimp similar to those reported by Anderson et al. (1990) and El- Sayed et al. (2000) in prawn. The muscles of shrimp could be the target tissues for *C. fernudii* were marked liquefaction was noticed. The degenerative changes and inflammatory reactions observed in the hepatopancreas and other organs of naturally and experimentally infected shrimps resulted from the septicemic and/or toxemic picture of these bacteria.

The findings of this study emphasized the monitoring of antibiotic sensitivity testing to the bacterial isolates where most bacterial isolates were sensitive to oxytetracyclin followed by erythromycin and chloramphinicol.

The chemical control of bacterial diseases among shrimp may have limited effectiveness based on the efficacy of the available drugs, the development of resistant strains of the bacteria, the limited tolerance of shrimp to the drug, the very low feeding behavior of shrimp and the very simple digestive system in which the drugs could accumulate in their flesh (Baticados et al., 1991). So, attention should not focus only on the effectiveness of the chemotherapeutints but also on their permeability through the intestinal wall, dissolvablility of drugs and the residue after treatment. However, the best method for the control of these bacteria is prevention with a special care to the water management and sanitation.

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*Table (1): The bacterial isolates and their prevalence from shrimp and water samples.*

| Bacteria | M. shrimp | | D. shrimp | | Water | | Total | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| 1- *V. parahemolyticus* | 12 | 40.0 | 9 | 12.9 | 5 | 50 | 26 | 23.6 |
| 2- *V. alginolyticus* | 3 | 10.0 | 5 | 7.1 | 3 | 30 | 11 | 10.0 |
| 3- *V. vulgaris* | 1 | 03.0 | 3 | 4.3 | 2 | 20 | 6 | 05.5 |
| 4- *A. hydrophila* | 6 | 20.0 | 8 | 11.4 | 3 | 30 | 17 | 15.5 |
| 5- *Ps. fluorescens* | 3 | 10.0 | 6 | 8.6 | 4 | 40 | 13 | 11.8 |
| 6- *E. aerogenes* | 3 | 10.0 | 3 | 4.3 | 1 | 10 | 6 | 5.5 |
| 7- *C. freundii* | 2 | 06.7 | 1 | 1.4 | 1 | 10 | 4 | 3.6 |
| *Total* | 30 | - | 35 | - | 18 | - | 80 | 75.5 |

* M. = Moribund , D. = Dead.

*Table (2): The tissue and seasonal prevalence of bacteria among naturally infected shrimp.*

| Bacteria | Hem. | Hep. | Int. | Season | | | |
|---|---|---|---|---|---|---|---|
| | No. | No. | No. | Sp. | Sum. | Win. | Aut. |
| 1- *V. parahemolyticus* | 12 | 7 | 8 | +++ | +++ | + | + |
| 2- *V. alginolyticus* | 5 | 3 | 6 | +++ | +++ | + | + |
| 3- *V. vulgaris* | 4 | 2 | 4 | +++ | +++ | + | + |
| 4- *A. hydrophila* | 7 | 4 | 9 | ++++ | ++ | +++ | ++++ |
| 5- *Ps. fluorescens* | 7 | 4 | 4 | +++ | - | ++++ | + |
| 6- *E. aerogenes* | 3 | - | 5 | - | +++ | - | + |
| 7- *C. freundii* | 5 | 3 | 2 | - | +++ | - | |
| *Total* | 45 | 23 | 38 | ++ | +++ | + | + |

• *Hem. = hemolymph, Hep.= Hepatopancreas, Int.= Intestine,*

• *Sp.= Spring, Sum.= Summer, Win= Winter, Aut.= Autumn.*

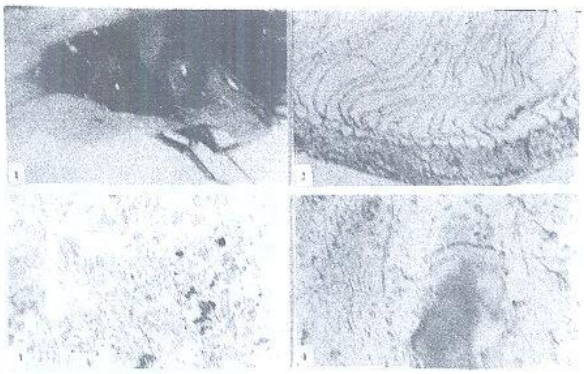
*Table (3): The morbidity and mortality rates of experimentally infected shrimp throughout the post-infection period.*

| Bacteria | No. of Shrimp | Morbidity | | Mortality | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| 1- *V. parahemolyticus* | 10 | 10 | 100 | 10 | 100 |
| 2- *V. alginolyticus* | 10 | 8 | 80 | 8 | 80 |
| 3- *V. vulgaris* | 10 | 8 | 80 | 7 | 70 |
| 4- *A. hydrophila* | 10 | 10 | 100 | 9 | 90 |
| 5- *Ps. fluorescens* | 10 | 10 | 100 | 9 | 90 |
| 6- *E. aerogenes* | 10 | 8 | 80 | 6 | 60 |
| 7- *C. freundii* | 10 | 7 | 70 | 6 | 60 |
| 8- Control | 10 | - | - | - | - |
| Total | 80 | 61 | 76.3 | 55 | 78.6 |

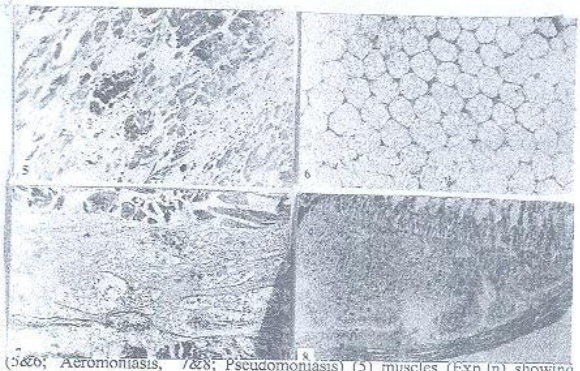
*Table (4): The antibiotic sensitivity to the bacterial isolates (mm).*

| Bacteria | Antibiotics | | | | | |
|---|---|---|---|---|---|---|
| | OTC | EM | CP | NB | ABP | TMP |
| 1- *V. parahemolyticus* | 25 | 23 | 18 | 17 | 14 | 16 |
| 2- *V. alginolyticus* | 25 | 23 | 18 | 16 | 13 | 16 |
| 3- *V. vulgaris* | 25 | 23 | 19 | 16 | 14 | 16 |
| 4- *A. hydrophila* | 24 | 23 | 20 | 18 | 17 | 15 |
| 5- *Ps. fluorescens* | 24 | 23 | 19 | 18 | 16 | 13 |
| 6- *E. aerogenes* | 22 | 21 | 20 | 15 | 15 | 14 |
| 7- *C. freundii* | 23 | 20 | 20 | 15 | 15 | 14 |

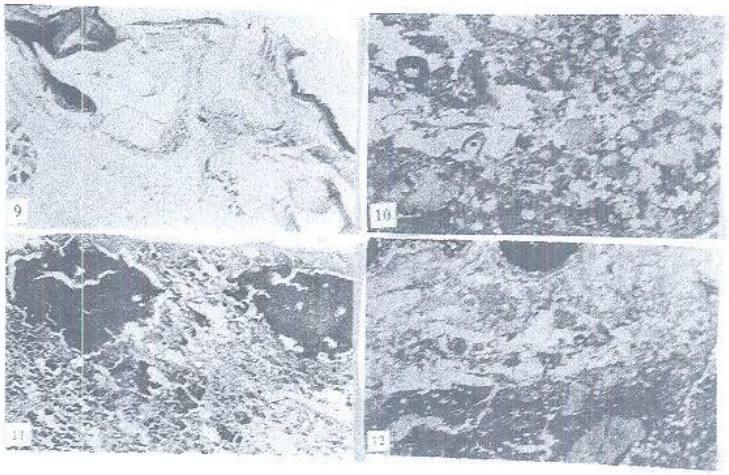
*OTC=Oxytetracyclin, EM=Erythromycin, CP=Chloramphinicol, NB=Novobiocin, ABP=Ampicillin, TMP=Trimethoprin.*



Figs. (1-4; vibriosis). (1) shrimp (Nat.In.) exhibited enlargement of the hepatopanceas and congestion of internal organs. (2) gills (Nat.In.) lacked secondary lamellae with edemata hemocyte infiltration in the gill arch, H&E, x 100.(3) muscles (Exp. In) showing edema, necrosis, hemocytes and melanomacrophages, H&E, x 250. (4) hepatopanceas (Exp. In) showing edema,necrotic tubules and granulomatous lesions, H&E, x 250.



Figs. (5&6; Aeromoniasis, 7&8; Pseudomoniasis) (5) muscles (Exp.In) showing edema, vacuolation, necrosis and mononuclear cells, H&E, x 250. (6) hepatopancreas (Nat.In.) showing dilated sinuses and vacuolar degeneration and necrosis in hepatocytes, H&E, x 250. (7) muscle (Exp.In.) showing edema, congestion, hemorrhage and hyaline degeneration, H&E, x 250. (8) intestine (Nat.In) showing necrosis in their epithelium with some hemocytes in the submucosa, H&E, x 250.



Figs. (9&10;Enterobaceriasis, 11&12 Citrobacteriasis) (9) exoskeleton (Nat.In.) showing thickened epidermal layer and hemocytes infiltration below the cuticular melanization, H&E, x 250. (10). ovary (Exp.In.) showing edema, degenerated ova and hemocytes infiltration in the ovarian stroma, H&E, x 250. (11) muscles (Exp.In.) showing marked liquefaction surrounded by hemocytes, H&E, x 250. (12) ovary (Nat.In.) showing edema, ruptured ova and liquefaction, H&E, x 250.