# Studies on major bacterial diseases affecting fish ; Tilapia *Oreochromis niloticus* , Catfish, *Clarias gariepinus* and mullets in Port Said, Egypt with special references to its pathological alterations

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**Abstracts:** A number of 775 apparently healthy and naturally infected fishes of different species; (Tilapia *Oreochromis niloticus*, catfish, *clarias gariepinus* and mullets) collected from different areas in Port Said governorate. Collected fishes were subjected to full clinical, bacterial and histopathological examinations. The results revealed that the most common clinical signs of the naturally infected fish were darkening of the skin, hemorrhages in skin, fins, oral cavity and muscles (The most common finding), sloughing of scales with superficial and deep ulceration of the epidermis. The isolated bacteria were *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Flexibacter columnaris*., *Streptococcus faecalis* ., *E. coli*, *Y. ruckeri, Citrobacter spp.* and *Edwardsiella tarda.* The isolates were isolated in summer was highest than those of spring . The highest number of isolates were isolated from El-Genka farms (water from Bahr El-Bakar), followed by El-Bashteer farms (water from Lake Manzala) , while isolates from farms inside the lake Manzala were the lowest the results of the histopathological examination of naturally bacterial infected fishes were also discussed.
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**Key words:** Port Said governorate; bacterial examinations; histopathological examinations; bacterial isolates ; Bahr El-Bakar ; Lake Manzala

## 1.Introduction

Bacteria are simply everywhere. (**Trust, 1974**). The world is full of bacteria; in fact, our world would not exist as we know it without them. In 1884 a Danish physician, Christian Gram, discovered that bacteria could be separated into two groups, gram-positive and gram-negative. Using a particular staining process the bacteria could be determined either gram-positive or negative, depending on whether they retained (positive) or lost (negative) a violet color during this process. Most bacteria that cause disease in marine fish are gram-negative. Ones most commonly associated with these infections are of the genus Pseudomonas and Vibrio, as well as Myxobacteria **(Toranzo *et al*., 2005)** .

Infectious diseases of cultured fish are the most notable constraints on the expansion of aquaculture and the realization of its full potential **(Plumb, 1994; Woo and Bruno, 1999 ;Klesius *et al*., 2000; Robert and Moeller, 2012)** . Bacterial pathogens are the most serious disease problem in fish production causing 80% of mortalities **(Austin and Austin, 1993).**

Bacterial disease is the most common infectious problem of fishes. Collectively, only water quality problems exceed bacterial diseases in the area of fish morbidity and mortality. The majority of bacterial infections are caused by Gram-negative organisms including the following pathogenic genera: *Aeromonas, Citrobacter, Edwardsiella, Flavobacterium (Flexibacter), Mycobacterium, Pseudomonas, and Vibrio. Streptococcus,* a Gram-positive genus, has been shown to cause disease in fishes. Bacterial organisms may be the primary cause of disease, or they may be secondary invaders, taking advantage of a breach in the fish's integument or compromise of its immune system. The majority of bacterial fish pathogens are natural inhabitants of the aquatic environment, whether it be freshwater or marine. (**Inglis *et al.*, 1993**; **Robert and Moeller, 2012)**.

The Port Said Governorate is fortunate to have the greatest water surface compared with other governorates in Egypt. It is bounded from the north by the Mediterranean Sea and in the south-southwest by Lake Manzala, which is the largest brackish water body in the country. In the circumstances, fishing activities sustain a large number of people in Port Said Governorate, and fish is a most favored dish to people in urban as well as rural communities. So, there is high ability to occurrence of fish disease in this area especially bacterial one and its ability to affects the human being. So present study was carried out to identify the major bacterial diseases which infects fishes and bacterial isolates which can be isolated from fishes at this governorate causing problems to aquaculture in Port-Said governorate.

**2.Materials and methods :**

A total number of 775 Fishes from different areas in Port-Said governorate were collected and subjected to full clinical examination and transferred alive to the laboratories for performance of complete postmortem, and microbiological examinations.

**2.1.Clinical and Postmortem examinations:**

All collected samples of fish of different species were subjected to clinical and postmortem examination. Abnormalities in the behavior of fish and clinical alterations indicating disease conditions were recorded . samples were examined for detection of abnormal discolorations of skin and fins, swellings, hemorrhages, ulcerations, and other abnormalities. (**Schäperclaus, *et al.*, 1992; Plumb 1994** and **Noga 2000)**.

**2.2. bacteriological examination :**

Sampling and Primary isolation were done according to **(Noga 1996)** under complete aseptic conditions from the kidney, liver, spleen and ascitic fluid, inoculated on tryptic soy broth, and incubated at 25 C for 24 hours. A loopfull of incubated broth was streaked on the different laboratory medium: tryptic soy agar, the isolated bacteria were identified according to **Bergey, *et al.*, (1994) and Elmer *et al*., (1998)** and **(FAO, 2003).**

**2.3.Histopathological examination**:

The naturally bacterial infected fish tilapia, catfish and mullet subjected to histopathological examination using the method described by **Roberts (2001).**

**3. Results :**

**3.1.Results of clinical and post mortem sings of infected fish with bacterial pathogens :**

The results of the clinical signs and postmortem examinations of the naturally infected fish were darkening of the skin, and the most common finding is hemorrhage in skin, fins. Superficial ulceration of the epidermis in the oral cavity and muscles , exophthalmia and ascetis are commonly observed**.** Internally the liver was dark, inflamed with enlarged gall bladder, enlarged kidney and congested, enlarged spleen, Haemorrhage on the skin of catfish and enteritis (figure 1 and 2).

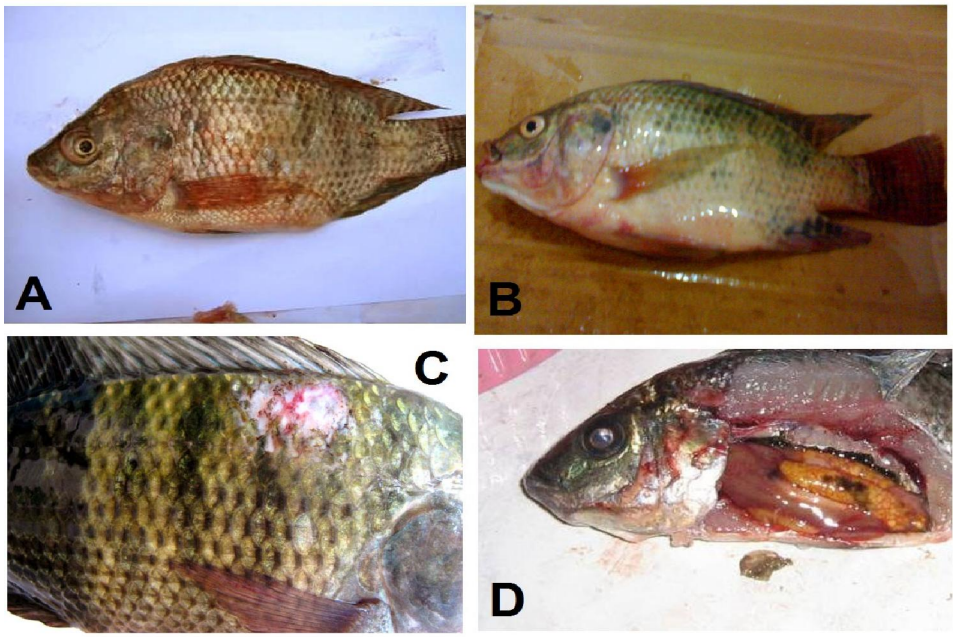


**Figure 1: (A) Tilapia species showing exophthalmia and haemorrhage on skin (B) distended abdomen (ascetic fluids) (C) loose of scales an ulceration of the skin (D) congestion and of inflammation liver and dark pigmentation of ovary**

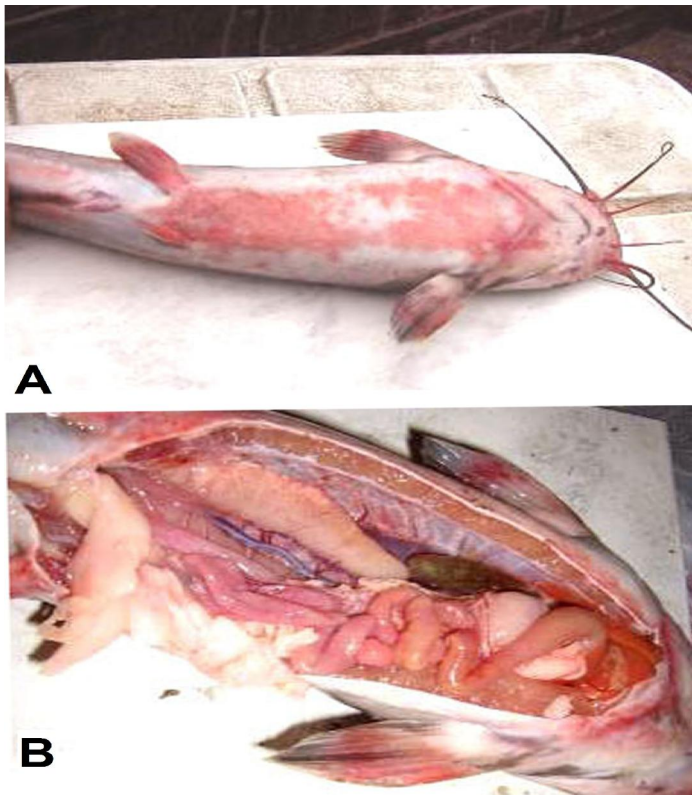


**Figure 2: (A) African catfish (*Claris gariepinus*) revealed inflammation and haemorrhages in the skin of abdomin and fins, (B) African Catfish affected by *Aeromonas hydrophila* showing yellow liver, enlarged gall bladder and kidney and inflamed intestine.**

**3.2.Bacterial examination of fresh water fish :**

Bacteriological examination revealed isolation of 133 bacterial, and isolates were used in the experiments of which isolates related to 98 *fluorescens Aeromonas hydrophila*, 16 *Pseudomonas*, 5 *Flexibacter columnaris*., 5- *Streptococcus faecalis* ., 3 *E. coli*, 3 *Y. ruckeri,* 2 *Citrobacter spp.* and 1 *Edwardsiella tarda*

**Table 1: Bacterial isolates from different fish species**

| No. / Bacteria | Fresh water Fish species | | |
|---|---|---|---|
| | Tilapia | Catfish | Mullet |
| Number of fish | 300 | 250 | 125 |
| *A. hydrophila* | 64 | 28 | 6 |
| *P. fluorescens* | 9 | 6 | 1 |
| *F. columnaris* | 1 | 4 | - |
| *Y. ruckeri* | 3 | - | - |
| *St. faecalis* | 4 | - | 1 |
| *Ed. tarda* | - | 1 | - |
| *E. coli* | 1 | 2 | - |
| *Citrobacter spp.* | 2 | - | - |
| Total | 84 | 41 | 8 |

**3.3. Results number of bacterial isolates in relation to season:**

Thirty seven isolates of bacteria were isolated from different organs from catfish and tilapia in spring season from fresh water fish while no bacterial isolates were isolated from mullet in spring . On the other hand a total number of 96 bacterial isolates were isolated from investigated fish in summer, 76 *Aeromonas hydrophila*, 8 *Pseudomonas fluorescens*, 5 *Flexibacter Colomnaris*., 2 *Streptococcus faecalis ., 3*

*E. coli*, and 2 *Citrobacter spp*. The fish displayed different clinical signs and postmortem lesions with different pathological changes (Table 2)...

**N.B.** *E. coli*, and *Citrobacter spp.* not cause disease for fish.

**Table 2: Isolates number of bacteria in summer and spring season in Port Said governorate.**

| Fish spp. | *A. hydrophila* | | *P. fluorescens* | | *F. colaminaris* | | *St. faecalis* | | *E. coli* | | *Citrobacter spp.* | | *Y. ruckeri* | | *Ed. tarda* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | spring | summer | spring | summer | spring | summer | spring | summer | spring | summer | spring | summer | spring | summer | spring | summer |
| Tilapia | 13 | 51 | 5 | 4 | - | 1 | 3 | 1 | - | 1 | - | 2 | 3 | - | - | - |
| Catfish | 9 | 19 | 3 | 3 | - | 4 | - | - | - | 2 | - | - | - | - | 1 | - |
| Mullet | - | 6 | - | 1 | - | - | - | 1 | - | - | - | - | - | - | - | - |
| Total | 22 | 76 | 8 | 8 | | 5 | 3 | 2 | | 3 | | 2 | 3 | | 1 | |

**3.4.Bacterial isolates from different areas.**

The isolates were isolated in summer (96) was highest than those of spring (36) The highest number of isolates were isolated from El-Genka farms (water from Bahr El-Bakar) (71) followed by El-Bashteer farms (water from Lake Manzala) (16) while isolates from Inside the lake Manzala, from Farm on the lake were the same (14) Table 3.

**Table 3: Bacterial isolates from different areas.**

| Area | Type of bacteria | No. of isolates during spring | Total | No. of isolates during summer | Total |
|---|---|---|---|---|---|
| El-Genka farms (water from Bahr El-Bakar) | ***A. hydrophila*** | 13 | 19 | 37 | 52 |
| | ***Yersinia ruckeri*** | 2 | | | |
| | ***Edwarsiella tarda*** | 1 | | | |
| | ***S. faecalis*** | 1 | | 2 | |
| | ***P. fluorescens*** | 3 | | 5 | |
| | ***F. colaminaris*** | | | 4 | |
| | ***Citrobacter spp.*** | | | 1 | |
| | ***E. coli*** | | | 3 | |
| El-Bashteer farms (water from Lake Manzala) | ***A. hydrophila*** | 3 | 4 | 14 | 16 |
| | ***Yersinia ruckeri*** | 1 | | | |
| | ***Edwarsiella tarda*** | | | | |
| | ***S. faecalis*** | | | | |
| | ***P. fluorescens*** | | | | |
| | ***F. colaminaris*** | | | 1 | |
| | ***Citrobacter spp.*** | | | 1 | |
| | ***E. coli*** | | | | |
| Inside the lake Manzala | ***A. hydrophila*** | | 7 | 12 | 14 |
| | ***Yersinia ruckeri*** | | | | |
| | ***Edwarsiella tarda*** | | | | |
| | ***S. faecalis*** | 2 | | | |
| | ***P. fluorescens*** | 5 | | 2 | |
| | ***F. colaminaris*** | | | | |
| | ***Citrobacter spp.*** | | | | |
| | ***E. coli*** | | | | |
| Farm on the lake | ***A. hydrophila*** | 6 | 6 | 13 | 14 |
| | ***Yersinia ruckeri*** | | | | |
| | ***Edwarsiella tarda*** | | | | |
| | ***S. faecalis*** | | | | |
| | ***P. fluorescens*** | | | 1 | |
| | ***F. colaminaris*** | | | | |
| | ***Citrobacter spp.*** | | | | |
| | ***E. coli*** | | | | |
| **Total** | **------** | **36** | | **96** | |

**3.5. Ratio of bacterial isolates among various organs of fish samples :**

The highest number of isolates were isolated from liver (50), followed by Kidney (35), spleen (31) while the lowest number of isolates were isolated from skin (17) Table 5.

**Table 5: Ratio of bacterial isolates among various organs of fish samples**

| Organs / Type of bacteria | Liver | Spleen | Kidney | Skin |
|---|---|---|---|---|
| ***Aeromonas hydrophila*** | 39 | 23 | 29 | 7 |
| ***Pseudomonas fluorescens*** | 7 | 4 | 4 | 1 |
| ***Yersinia ruckeri*** | 2 | 1 | - | |
| ***Streptococcus faecalis*** | 1 | 2 | 1 | 1 |
| ***Edwarsiella tarda*** | - | - | 1 | |
| ***Flexibacter colaminaris*** | | | | 5 |
| ***Citrobacter spp.*** | 1 | 1 | | |
| ***E. coli*** | | | | 3 |
| Total | 50 | 31 | 35 | 17 |

**Table 6: Cultural characteristics and biochemical reaction of *Aeromonas hydrophila* and *Pseudomonas fluorescenes***

| **Test** | ***Aeromonas hydrophila*** | ***Pseudomonas fluorescens*** |
|---|---|---|
| **Characteristics of colony on TSA media** | Circular convex white colored colonies | Convex, glissining |
| **Characteristics of colony on R.S. media** | Small, smooth and yellow colonies | Dark green colored colonies |
| **Gram stain** | Gram –ve short rods | Gram –ve short rods |
| **Motility** | + | + |
| **Gram staining** | - | - |
| **Gelatin l liquefaction** | + | + |
| **Oxidase** | + | + |
| **O/F** | F | O |
| **Growth on 5% NaCl** | - | + |
| **Indol** | + | - |
| **V.P** | + | - |
| **Methyl red** | + | - |
| **H2S production** | - | - |
| **Catalase** | + | + |
| **Nitrate reduction** | + | + |
| **Citrate utilization** | + | + |
| **Arginin hydrolysis** | + | + |
| **Fermentation of** | | |
| **Glucose** | + | + |
| **Sucrose** | + | _ |
| **Maltose** | + | _ |
| **Lactose** | - | _ |
| **Galactose** | + | + |
| **Trehalose** | + | - |
| **Fructose** | + | - |

**Table 7: Cultural characteristics and Biochemical reaction of *Yersinia ruckeri***

| Test | Result |
|---|---|
| **R.S.** | Yellow colonies |
| **Motility** | |
| **At 37C** | - |
| **25C** | + |
| **Gram stain** | G –ve short rods |
| **Oxidase** | - |
| **Indol** | - |
| **Citrate utilization** | + |
| **V.P** | - |
| **Methyl red** | + |
| **H2S production** | - |
| **Urea hydrolysis** | - |
| **Lysin decarboxylase** | + |
| **Arginin dihydrolase** | - |
| **D-glucose/acid/** | + |
| **Fermentation of** | |
| **Sucrose** | - |
| **Lactose** | - |
| **Manitol** | + |

**Table 8: cultural characteristics and Biochemical reaction of *Edwarsiella tarda***

| Test | Result |
|---|---|
| **TSA** | Round, smooth, glossy, grey white with black center. |
| **Gram stain** | g-ve, short bacilli |
| **Motility** | Highly motile |
| **Oxidase** | - |
| **Lysine decarboxylase** | + |
| **H2S production** | + |
| **Indol** | + |
| **Citrate utilization** | - |
| **V.P** | - |
| **Methyl red** | + |
| **Fermentation of** | |
| **Manitol** | - |
| **Sucrose** | - |
| **Trehelose** | - |
| **Arabinose** | - |
| **Tetrathionat reduction** | + |

**Table9: cultural characteristics and Biochemical reaction of *Streptococcus faecalis***

| Test | *S. faecalis* |
|---|---|
| **Gram stain** | + cocci pairs & short chain |
| **Motility** | - |
| **Growth on tryptic soy broth** | + |
| **Growth on tryptic soy agar** | Pen headed colony, white opaque colour circular, entire, raised edges and glistening |
| **Growth on MacConky agar** | Pink colony |
| **Catalase** | - |
| **Oxidase** | - |
| **Growth at 10 ˚C** | + |
| **Growth at 45 ˚C** | + |
| **Growth at 6.5% NaCl** | + |
| **Haemolysis on blood agar** | α – or non* |

| | |
|---|---|
| **Bile esculin** | + |
| **O/F** | F |
| **Hydrolysis of** | |
| **Argenin dihydrolase** | + |
| **Esculin hydrolysis** | + |
| **Hippurate hydrolysis** | + |
| **Production of** | |
| **Acetoin (Voges-Perskauer)** | + |
| **Indole production test** | - |
| **Acid produced from** | |
| **Ribose fermentation** | + |
| **Arabinose fermentation** | - |
| **Mannitol fermentation** | + |
| **Sorbitole fermentation** | + |
| **Sucrose fermentation** | + |
| **Lactose fermentation** | + |
| **Trehelose fermentation** | + |
| **Inulin fermentation** | - |
| **Raffinose fermentation** | - |
| **Gelatin liquefaction** | - |
| **Citrate utilization** | + |
| **Leucin** | + |

**3.6.Results of histopathological examination :**

The histopathological examination of naturally bacterial infected fishes revealed that the liver of tilapia sp. show congestion of the hepatic sinusoids and vacuolar degeneration of the hepatic cells lyses of hepatocytes were noticed and hepatic cells were necrotic. The spleen of catfish show area of depletion of haemobiotic element appear as honey comb and kidney of tilapia showing collapse of renal capsule and edema in bowman capsule, some renal tubules showing hyaline droplet degenerations while, intestine of catfish show increase numbers of goblet cells with sever congestion of submucosal blood vessels and haemorhgie with heavy leucocytic cells infiltrations (Figure 3).

**4. Discussion:**

Aquaculture is one of the fastest growing food-producing sectors, providing an acceptable supplement to and substitute for wild fish. During the past years the total production of cultured finfish and shellfish have increased in production quantity and value. **(Pérez and Rodríguez, 1997).**

The appearance and development of a fish disease is the result of the interaction among pathogen, host and environment. Therefore, only multidisciplinary studies involving the characteristics of potential pathogenic microorganisms for fish, aspects of the biology of the fish hosts as well as a better understanding of the environmental factors affecting such cultures, will allow the application of adequate measures to prevent and control the main diseases limiting the production of fishes (**Toranzo *et al*.,2005).**

Fish are susceptible to a wide variety of bacterial pathogens. Many of these bacteria capable of causing disease are considered by some to be saprophytic in nature. This bacteria only become pathogens when fishes are physiologically unbalanced, nutritionally deficient, or there are other stressors, i.e., poor water quality, overstocking, which allow opportunistic bacterial infections to proceed. Some of these bacterial pathogens of fishes are fastidious and require special growth media for laboratory culture. Others grow at different temperatures, dependent upon the aquatic environmental temperature of the fish **(Noga 2010).** The disease problem in fish culture usually arises from interaction of the host, pathogen and environment; the last one is the more critical factor (**Austin and Austin, 1993**). The bacteria are transmitted by fish having made contact with other diseased fish. Bacterial fish diseases and infections are very common and are one of the most difficult health problems, to deal with bacteria can enter the fish's body through the gills or skin or it can stay on the surface of the fish's body (**Douglas DuHamel, 2007) .**

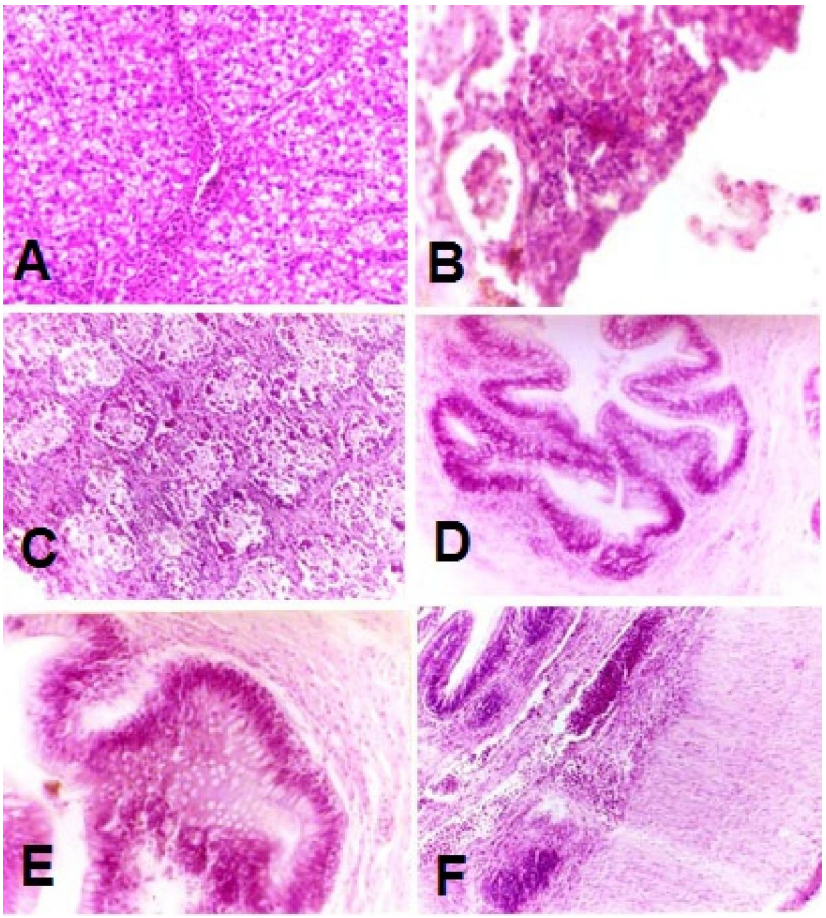


**Figure 3 : (A)** Liver of tilapia sp. Showing congestion of the hepatic sinusoids and vacuolar degeneration of the hepatic cells lysis of hepatocytes were noticed and other hepatic cells were necrotic H&E x300 **(B)** kidney of tilapia showing collapse of renal capsule and edema in bowman capsule, some renal tubules showing hyaline droplet degenerations H&E x300 **(C)** Spleen of catfish showing area of depletion of haemobiotic element appear as honey comb H&E x300 **(D)** intestine of catfish showing increase numbers of goblet cells with leucocytic cells infiltrations. H&E x150 **(E)** intestine of catfish showing increase numbers of goblet cells with leucocytic infiltrations H&E x300 **(F) :** intestine of catfish showing sever congestion of submucosal blood vessel and haemorhgie with heavy leucocytic infiltrations H&E x150

Concerning the clinical sings and postmortem examinations of naturally bacterial affected fish, present study revealed that , the clinical signs were darkening of the skin, and the most common finding is hemorrhage in skin, fins . Superficial ulceration of the epidermis in the oral cavity and muscles, exophthalmia and ascites are commonly observed**.** Internally the liver was dark, inflamed with enlarged gall bladder, enlarged kidney and congested, enlarged spleen, Haemorrhage on the skin of catfish and enteritis. The results nearly agree with that obtained by **Austin, and Austin, 1993**; **Toranzo *et al*.,(2005) and Robert and Moeller (2012).**

Regarding Bacterial examination of fresh water fish, present study displayed that tilapia sp. was the highest sp burden with bacterial isolates and the mullets was the lowest one these may be due to that tilapia sp. was genetically suitable to be infected by investigated bacterial isolates also mullet sp. May be more immunologically protected from that infection.

Regarding the seasonal variation of number of bacterial isolates present study revealed that bacterial isolates were highest in number in summer season than those isolated in spring, this may be due that in summer the temperature of water was high and more suitable to make infection in addition to that in summer with high temperature and low dissolved oxygen there is more stress on fish compromising the immune response make the fish more susceptible to bacterial infection.

Concerning to bacterial isolates from different areas, present study recorded that the highest number of isolates were isolated from El-Genka farms (water from Bahr El-Bakar) (71) followed by El-Bashteer farms (water from Lake Manzala) (16) while isolates from Inside the lake Manzala and from Farm on the lake were the same (14) . these may be due to that El-Genka farms (water from Bahr El-Bakar) more polluted with sewage and heavy metals that make the immune response of investigated fish suppressed leading to more bacterial infection for fish.

Regarding. samples bacterial isolates among various organs of fish the highest number of isolates were isolated from liver , followed by Kidney , spleen while the lowest number of isolates were isolated from skin , this may be due to most of bacterial infections affect haemobiotic system mainly liver, kidney and spleen.

Regarding the histopathological examination present study revealed that naturally bacterial infected fishes revealed that the spleen of catfish showing area of depletion of haemopiotic element appear as honey comb and kidney of tilapia showing collapse of renal capsule and edema in bowman capsule, some renal tubules showing hyaline droplet degenerations while, intestine of catfish show increase numbers of goblet cells with sever congestion of submucosal blood vessels and haemorhgie with heavy leucocytic cells infiltrations the results nearly agree with the results of **Austin, and Austin, 1993** ; **Toranzo *et al*.,(2005) Robert and Moeller 2012** .

From present study, it was concluded that tilapia sp. was the highest sp burden with bacterial isolates and the mullets was the lowest one, number of bacterial isolates present study revealed that bacterial isolates were highest in number in summer season than spring, it was added that the highest number of isolates were isolated from El-Genka farms (water from Bahr El-Bakar) followed by El-Bashteer farms (water from Lake Manzala) while isolates from farms Inside the lake Manzala were the lowest, it was mainly related to the pollution of water in Bahr El-Bakar.

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