Bacteriological and Histopathological Studies on Enterobacteriacea in Nile Tilapia *Oreochromis Niloticus*

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ABSTRACT

Bacterial examinations of Tilapia nilotica cultured at Abassa Fish Farms were conducted. *E coli, Enterobacter colacea, Citrobacter frunidii* and *Yersinia intermediate* were isolated.

The experimental infection of Nile tilapia with the isolated bacteria was done and revealed high mortality rate with enterobacter cloacae (43, 33%). Blood samples were taken from fish for analysis through 3 and 6 days post-infection.

At 3 days post-infection, decrease in RBCs and increase in WBCs was seen in all groups in comparison with the control. At 6 day post-infection, a significant to non-significant increase in WBCs and significant decrease in RBCs were seen in tilapia of all infected groups. The liver albumin and bilirubin and kidney function tests showed significant to non-significant variable values depended on type of infection and period of study.

The histopathological findings of the experimentally infected Nile tilapia by the isolated enterobacteriacea were recorded and showed variable changes depending on the type of bacteria and the period of infection.

It was found in this work that, a great relationship occurs between the bacterial flora of the alimentary tract and that of the environment in which the fish lives, some of these bacteria are pathogenic to fish and may constitute a human risk upon handling or consumption. Therefore, the study of risk assessment or surveillance needs is recommended.

KEY WORDS: Histopathology, heamatology, enterobacteriacea, Nile tilapia

INTRODUCTION

International trade in fishery commodities reached US 58.2 billion dollars in 2002, a 5% improvement relative to 2000 and a 45% increase over 1992 levels. Within this global trade, developing countries registered a net trade surplus of US 17.4 billion dollars in 2002 and accounted for almost 50% by value and 55% of fish exports by volume. This globalization of fish trade, coupled with technological developments in food production, handling, processing and distribution, and the increasing awareness and demand of consumers for safe and high quality food have put food safety and quality assurance high in public awareness and a priority for many governments. Consequently, many countries have tightened food safety controls, imposing additional costs and requirements on imports [1].

Microbial pathogens associated with marine and fresh water aquatic animals, including *Vibrio species, Escherichia coli, Streptococcus iniae, Salmonella species,* and *Edwardsiella tarda.* Historically, cultured fish were not considered important vectors of human pathogens. This situation is changing, partly due to increasing animal densities as a consequence of a rapidly growing industry and partly due to increasing awareness by health care providers of pathogens in aquatic species that may result in human illness [2].

Bacterial diseases in fish generally do not develop simply as the result of exposing a host to an infectious agent [3]. In most instances, disease occurs as the result of complex interactions between pathogen, fish and environmental stress which affect the susceptibility of the host to disease. [4] recently reviewed the role of stress in the susceptibility of fish to disease. Environmental stresses can affect the homeostatic mechanism of fish, thus reducing their resistance to pathogenic organism [5]. Fish reared in intensive culture are exposed to extreme environmental fluctuations, and they may be more sensitive to stress than wild populations.

Enterobacteriaceae is a common water-borne bacterium which may be present in the tissues of apparently normal fish [6]. Whenever fish are exposed to environmental stress, or injury, it causes serious outbreaks of disease with mortalities [7]. Environmental stresses such as high temperature, poor water quality and high organic content primarily contribute to the onset and severity of

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Enterobacteriaceae infections in fish [8]. Juveniles of channel catfish exposed to high carbon dioxide and ammonia, and low dissolved oxygen showed higher rates of *E. tarda* infection than fish that were not environmentally stressed [9]. Specific examples of fish diseases thought to reflect the effects of pollution include surface lesions attributed to *Serratia plymuthica*, fin and tail rot caused by *Aeromonas hydrophila* and *Pseudomonas fluorescens*, gill disease resulting from the actvity of *Flavobacterium* spp., vibriosis as caused by *Vibrio anguillarum*, and enteric redmouth (causal agent, *Yersinia ruckeri*). Research indicated that, some of the diseases caused by Aeromonas, Flavobacterium and Pseudomonas resulted from generally adverse water quality, i.e. higher than usual quantities of organic material, oxygen depletion, changes in pH values and enhanced microbial populations [10].

Fish and shell fish are capable of transmitting many food-borne microbial infections to man who attributed to the direct contamination of fish product with the polluted water, contaminated harvesting area, utensils, equipment, transportation distribution and food preparations [11, 12, 13].

The initial microbial flora of the caught fish depending on the contamination of the water and bottom sediment at the area of catch. The occurrence of faecal coliform, as a representatives of family enterobacteriaceae, on fish was considered as an indication of the pollution level of their water environment [6, 14].Tilapia nilotica sample from Upper Egypt act as vector of enterobacteria, other bacteria and certain pathogens to human beings. Moreover, Nile tilapia within Winam Gulf are infected by human enteric pathogens. *Shigella* spp., Salmonella and *E. coli* were the most frequently isolated, an indication that the beaches may be contaminated by untreated municipal sewage, runoff, and storm-water [15].

The present study was planned to study the bacteriology and pathology of enterobacteriacea in Nile Tilapia (*Oreochromis Niloticus*) and their possible impact on human health.

MATERIALS AND METHODS

1-Sampling:

A total number of 200 Nile Tilapia (*Oreochromis niloticus*) were collected from Abassa fish farms, Sharkia. The collected fish were aseptically transferred to the fish health laboratory for the bacteriological examination within one hour.

2-Bateriological examinations:

The gastro-intestinal tract was carefully dissected out and the intestine was seared by firing with red hot spatula and was opened using a sterile scissor and forceps; a loopful from the contents of each part of intestine was taken and inoculated on plates of blood agar, MacConkey's agar media, nutrient agar media, broth, peptone water as well as selenite F broth.

A- Isolation and identification of enterobacteriacea.

The inoculated nutrient agar plates and tubes of peptone water and nutrient broth were incubated at 25°C, MacConkey's agar plates as well as selenite F broth tubes were incubated at 37°C for 18 hrs. A loopfull from incubated selenite F broth tubes was taken and streaked on plates of MacConkey's agar plates and incubated for 24-48 hours of 37°C. The appeared colony was streaked on a fresh set of plating media, incubated and examined for purity after 24 hours. The pure cultures were then transferred to a sterile nutrient agar slant, incubated at 37°C for 24 hours.

Purified cultures were subjected to microscopical examination and motility. The stock cultures were used for further identification by biochemical procedures [16, 17]. Biochemical tests used for identification of Gram-negative isolates were carried out according to the schemes described by [18]. The tests are oxidase, indole, methyl red, Voges-Proskour-citrate utilization, urease, triple sugar, iron agar and sugar fermentation test.

3-Experimintal infection:

A total of 150 apparently healthy Nile tilapia were divided into 5 equal groups (30 fish/ group). Each group injected intraperitoneal (I/P) with 0.5 ml of sterile suspension of bacteria (conc. 1.5×10^8 /fish), group 1 inoculated with *E. coli*, group 2 with *Citrobacter freundii*, group 3 with *Yersinia intermediate* and group 4 inoculated with *Enterobacter cloacae*. Group 5 with 0.5 ml sterile saline solution and act as control. All experimentally infected fish were daily observed for clinical signs of infection and mortalities along 6 days post-infection. The experimented fish were subjected for bacterial re-isolation and hematology, liver and kidney functions as well as histopathological examinations.

4-Hematological and serum biochemical parameters :

Two blood samples were collected from the fish groups at 3 and 6 days from the beginning of the experiment (FBE) by cutting the tail, each blood sample was divided into two portion. The first portion (1 ml) was collected in vacutainer tubes containing EDTA as anticoagulant for hematological studies. Erythrocytes and leukocytes counts were carried out by standard clinical method [19, 20]. The second portion was collected in a plain centrifuge tubes and serum was separated and used for determine the

activities of albumin (Alb), billurobin (Bil) blood urea nitrogen (BUN) and creatinine (CR) using an Auto Clinical Analyzer, Hitachi Model 7150 (Hitachi Ltd., Tokyo, Japan).

5-Histopathological technique:

Tissue specimens were taken from the gills, liver, kidneys, spleen ,muscle and intestine of experimented fish of all groups at 3 and 6 days of experiment and fixed in 10% phosphate buffer formalin. Processed routinely and blocked in paraffin then, five micron thick paraffin sections were prepared and stained with hematoxylin and eosin, (H&E) [21].

6-Statistical analyses:

One-way ANOVA was used to evaluate the significant difference of the different treatments and duration. A probability at level of 0.05 or less was considered significant. Means and standard errors were also estimated. All statistical analyses were run on the computer, using the SAS program [22].

RESULTS

Bacteriological examinations:
Prevalence of bacteria isolates in all fish samples:

In the present study, out of 200 examined fish (*Oreochromis niloticus*), the positive samples for enterobacteriacae was of a total prevalence of 44.1%. *Citrobacter freundii* was the most predominant bacteria (26.74%) and *E. coli* isolated with a percentage of 10.46% while *Yersinia intermediate* and *Enterobacter cloacae* were the lowest bacteria isolated (2.32%).

Mortality after experimental infection:

The experimental infection of Nile tilapia with the isolated bacteria revealed no remarkable clinical signs of infection but variable mortalities were detected depending on the type of bacterial isolates injected where high mortality rate was seen with *Enterobacter cloacae* (43, 33%). The detailed number and percentage of mortality among experimented tilapia that inoculated with the enteric bacteria along the 3rd and 6th day post-infection were reported in Table (1).

Table (1): Mortality number and percentage in experimented tilapias at 3rd and 6th day post-infection.

Isolated bacteria	3rd day No. (%)	6th day No. (%)
E. coli	2 (6.66)	4 (13.33)
Citrobacter freundii	2 (6.66)	1(3.33)
Yersinia intermediate	4 (13.33)	2 (6.66)
Enterobacter cloacae	10 (33.33)	3 (10.00)

Hematologically:

The experimentally infected Tilapia nilotica (*Oreachromis niloticus*) by the isolated bacteria revealed significant decrease in the total erythrocytic count and significant increase in the total leukocytic count after 3 days of infection. At 6 day post-infection, a significant to non-significant increase in the total leukocytic count was seen in comparison to the control group while the RBCs showed significant decrease values in tilapias of all infected group in comparison to the control group. The detailed erythrocytic and leukocytic paramters among the infected tilapia along 3rd and 6th days of infection are recorded in Tables (2).

Serum biochemistry :

The liver (ALB and BIL) and kidney (urea and creatinine) tests showed significant to non-significant variable values that increased in same groups and decreased in others. These findings were dependent on type of infection and period of study (Tables 3 & 4).

Table (2): Total leukocytic and erythrocytic counts in experimented tilapias after 3 and 6 days of infection in comparison to the control (Mean values ± SE).

Groups	3 days		6 days	
	WBCs	RBCs	WBCs	RBCs
E. coli	$18.67^{AB} \pm 0.88$	$34.67^{B} \pm 0.88$	$11.67^{CD} \pm 0.88$	$16.67^{C} \pm 0.88$
Citrobacter freundii	$20^{AA} \pm 1.2$	$26.67^{CD} \pm 1.2$	$19.33^{A} \pm 1.2$	$26.67^{B} \pm 1.2$
Yersinia intermediate	$17.33^{AB} \pm 1.2$	$33.33^{BC} \pm 0.88$	$12.7^{BCD} \pm 1.45$	$14.67^{C} \pm 0.88$
Enterobacter cloacae	$14.67^{B} \pm 0.88$	$27.67^{BCD} \pm 0.66$	$14^{BCD} \pm 0.58$	$27.33^{B} \pm 0.88$
Control	$10.33^{C} \pm 0.88$	$54.67^{A} \pm 5.2$	$10.33^{D} \pm 0.88$	$54.67^{A} \pm 5.2$

Table (3): Total albumin and billirubin in experimented tilapias after 3 and 6 days of infection in comparison to the control (Mean values ± SE).

Groups	3 days		6 days	
	ALB	BIL	ALB	BIL
E. coli	$0.05^A \pm 0.01$	$0.5^B \pm 0.06$	$0.02^B \pm 0.003$	$0.23^{CD} \pm 0.03$
Citrobacter freundii	$0.02^B \pm 0.01$	$0.37^B \pm 0.03$	$0.02^B \pm 0.01$	$0.17^{CD} \pm 0.03$
Yersinia intermediate	$0.4^A \pm 0.01$	$0.4^B \pm 0.01$	$0.02^B \pm 0.003$	$0.3^{BC} \pm 0.06$
Enterobacter cloacae	$0.06^A \pm 0.01$	$0.67^A \pm 0.03$	$0.57^A \pm 0.088$	$0.57^A \pm 0.03$
Control	$0.05^A \pm 0.01$	$0.4^B \pm 0.06$	$0.05^B \pm 0.01$	$0.4^B \pm 0.06$

Table (4): Kidney functions in experimental tilapias after 3, 6 days of infection in comparison to the control (Mean values ± SE).

Groups	3 days		6 days	
	Urea	Creatinine	Urea	Creatinine
E. coli	17.67 ± 0.67	$0.03^B \pm 0.01$	$19.67^D \pm 1.45$	$0.03^B \pm 0.001$
Citrobacter freundii	$20^D \pm 0.12$	$0.03^B \pm 0.001$	$20.33^{CD} \pm 0.33$	$0.034^B \pm 0.001$
Yersinia intermediate	$22.67^B \pm 0.33$	$0.03^B \pm 0.003$	$21.33^{BCD} \pm 0.88$	$0.032^B \pm 0.0003$
Enterobacter cloacae	$22^{BC} \pm 0.58$	$0.04^B \pm 0.001$	$23^{BC} \pm 1.53$	$0.04^B \pm 0.001$
Control	$29^A \pm 0.58$	$0.42^A \pm 0.02$	$29^A \pm 0.33$	$0.42^A \pm 0.02$

Histopathologically:

Group 1. (*E. coli*):

The experimentally infected Nile tilapia, by *E. coli* at the 3rd day of infection, showed focal hyperplasia in the epithelium of the secondary lamellae while other were desquamated (Fig. 1). Edema, congestion and focal hemorrhages in the gill arch were evident. The hepatopancreas revealed vacuolar degeneration in some hepatocytes with nuclear pycknosis (Fig. 2). At 6 day post-infection, similar histopathological findings were seen.

Group 2. (*Citrobacter freundii*):

The experimentally infected Nile tilapia, by *Citrobacter freundii* at the 3rd day of infection, showed congestion and some leukocytic infiltration in the primary gill lamellae (Fig. 3). The hepatopancreas revealed cloudy swelling of the hepatocytes with vacuolar degeneration in other cells. The intestine exhibited mucinous degeneration in the epithelial lining with mononuclear cell infiltrations in the lamina propria (Fig. 4). At 6 day post-infection, vacuolar degeneration in the hepatocytes was seen with nuclear pycknosis.

Group 3. (*Yersinia intermediate*):

The experimentally infected Nile tilapia, by *Yersinia intermediate* at the 3rd day of infection, revealed focal desquamation in the secondary lamellae epithelium. The musculature showed focal hyaline degeneration and some bundles were necrotic and showed focal infiltration of mononuclear cells (Fig. 5). Focal degeneration and necrosis in the hepatocytes were evident. The intestine revealed mononuclear cell infiltrations in the lamina propria. The spleen exhibited hemosiderosis and focal depletion of lymphoid cells (Fig. 6).The histopathological picture in the experimentally infected Nile tilapia, by *Yersinia intermediate* at 6 day post-infection were more or less similar to those reported at 3 day post-infection.

Group 4. (*Enterobacter cloacae*):

The experimentally infected Nile tilapia is by *Enterobacter cloacae* at the 3rd day showed focal epithelial desquamation in the epithelium of the secondary lamellae with numerous mononuclear leukocytic infiltration in the primary gill lamellae. The liver revealed congestion and coagulative necrosis in the hepatocytes and pancreatic acini cells (Fig. 7). The intestine suffered mucinous degeneration and focal necrosis in the epithelial lining. At 6 post-infection, the histopathological picture in the experimentally infected Nile tilapia were more or less similar where the liver exhibited advanced vacuolar degeneration and focal necrosis while the kidneys showed marked tubular degeneration and atrophy in the glomeruli with depletion of hematopoietic tissue (Fig. 8).
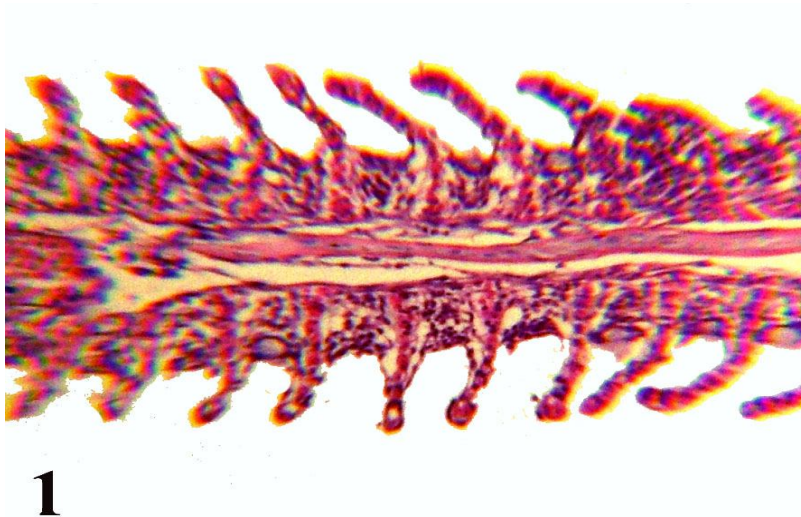
Figure (1): Gills , of tilapia experimentally infected by *E. coli* at 3 days post-infection (PI), showing hyperplasia in the epithelium of the secondary lamella, H&E stain, x 250.
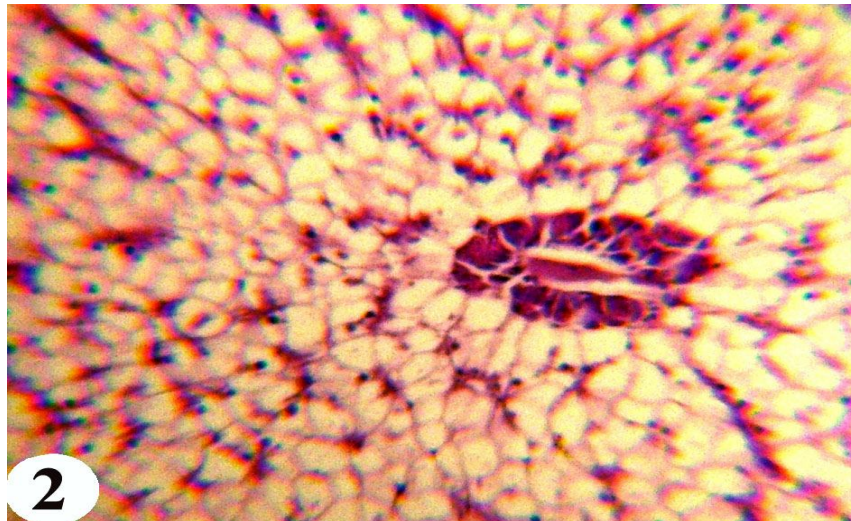

Figure (2): Liver, of tilapia experimentally infected by *E. coli* at 3 days PI, showing advanced vacuolar degeneration and necrosis of hepatopancreas, H&E stain, x 250.
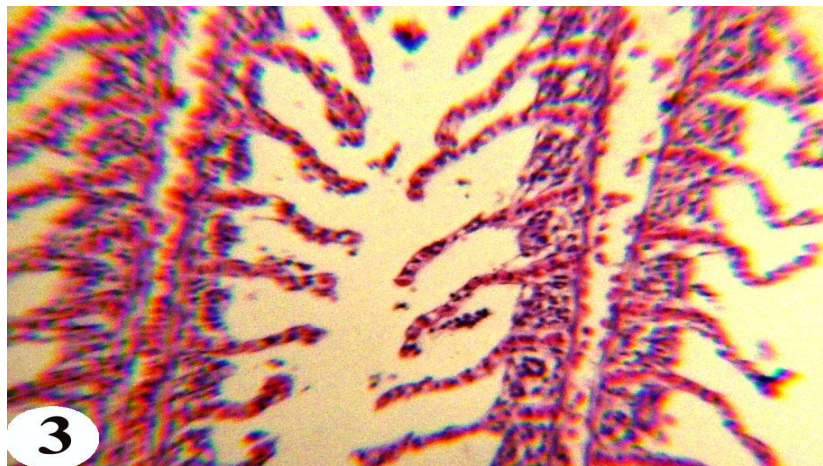

Figure (3) Gills, of tilapia experimentally infected by *Citrobacter freundii* at the 3rd PI, showing congestion and some leukocytic infiltration in the primary gill lamellae. H&E stain, x 250.
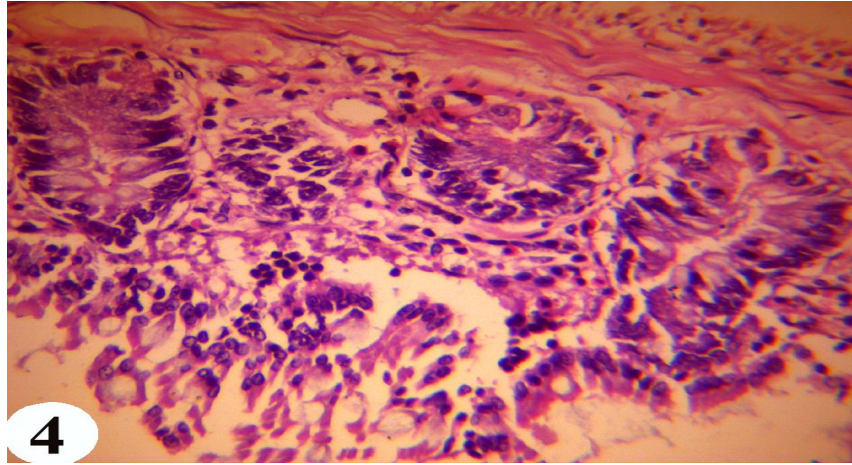
Figure (4): Intestine, of tilapia experimentally infected by *Citrobacter freundii* at 3 days PI, showing mucinous degeneration in the epithelial lining and leukocytic infiltration in the lamina propria, H&E stain, x 250.
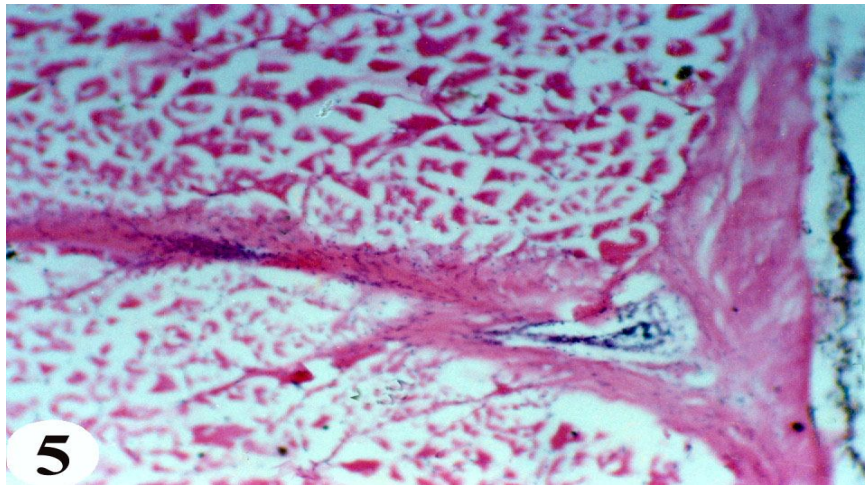

Figure (5): Muscles, of tilapia experimentally infected by *Yersinia intermediate* at 3 days PI, showing hyaline degeneration and necrosis with focal infiltration of mononuclear cells, H&E stain, x 250.
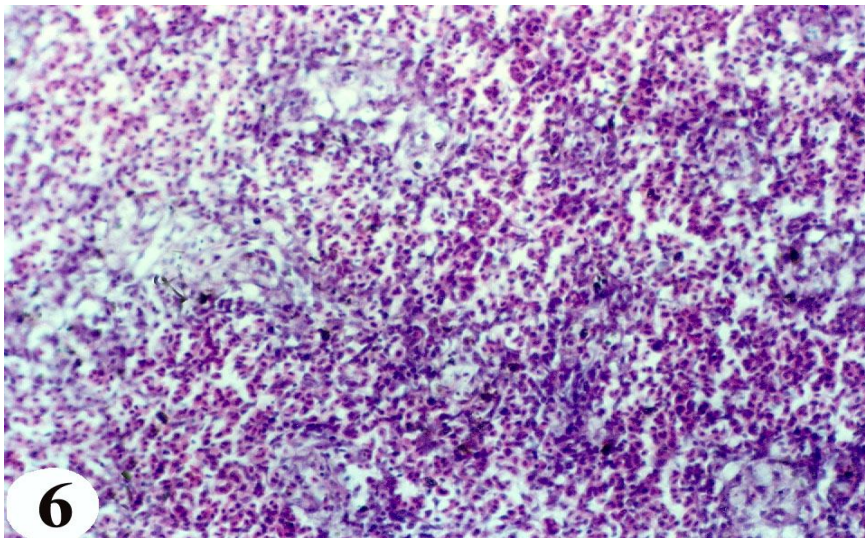

Figure (6): Spleen, of tilapia experimentally infected by *Yersinia intermediate* at 3 days PI, showing focal depletion of lymphoid cells, H&E stain, x 250.
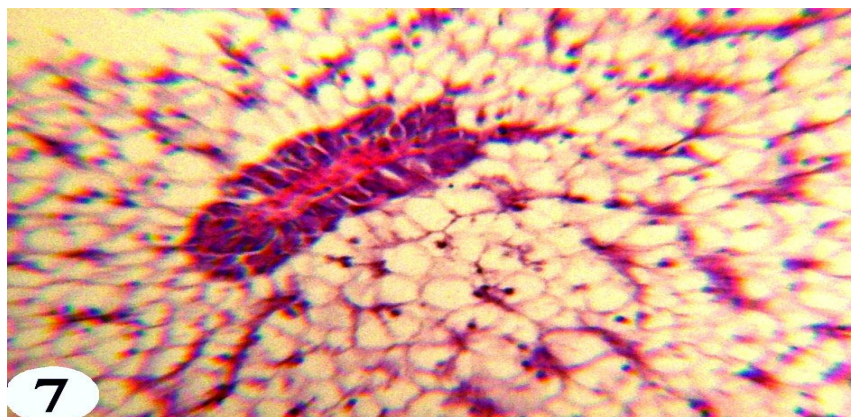
Figure (7): Liver, of tilapia experimentally infected by *Enterobacter cloacae* at 3 days PI, showing congestion and coagulative necrosis in the hepatocytes and pancreatic acini cells, H&E stain, x 250.
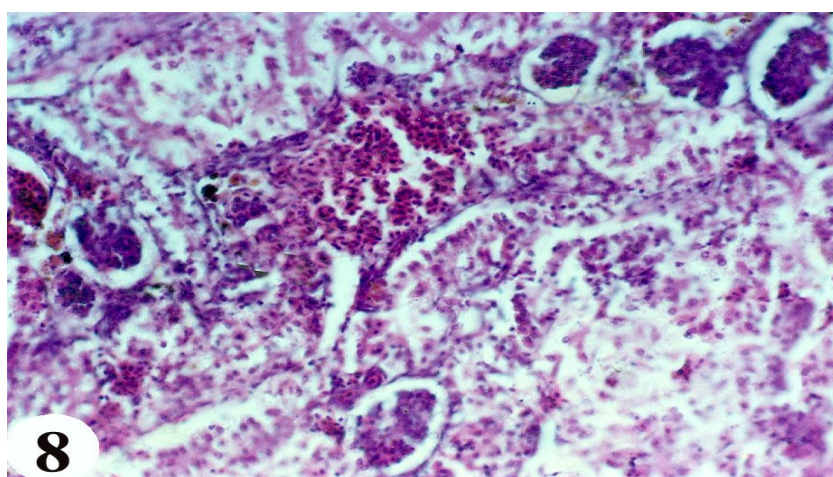

Figure (8): Kidney, of tilapia experimentally infected by *Enterobacter cloacae* at 6 days PI, showing marked tubular degeneration and atrophy in the glomeruli with depletion of hematopoietic tissue. H&E stain, x 250.

DISCUSSION

Although several etiological agents can be transmitted through seafood consumption, *E. coli, Citrobacter freundii, Enterobacter cloacae* and *Yersinia intermediate* are considered among the most important enteric pathogens in terms of public health and disease. In the present study, out of 200 examined fish (*Oreochromis niloticus*) , the positive samples for enterobacteriacae was of a total prevalence of 44.1%. *Citrobacter freundii* was the most predominant bacteria (26.74%) and *E. coli* isolated with a percentage of 10.46% while Yersinia intermediate as well as *Enterobacter cloacae* were the lowest bacteria isolated (2.32%).

In five fish landing beaches within Winam, among 120 Nile tilapia, 63 (52.5%) were infected with Enterobacteriaceae. Out of these, 25 (39.7%) were *Shigella* spp, 9(14.3%) *Salmonella typhimurium*, 7 (11.1%) *S. typhi*, 4 (6.3%) *S. enteritidis*, 16 (25.4%) *Escherichia coli*, 1 (1.6%) *Proteus* spp. and *Enterobacter aerogenes* respectively. Ten fish collected from open-air markets yielded *E coli* (50%), *S. typhimurium* (20%), *S. paratyphi* (10%) and *S. typhi* (20%) [15]. [23] stated that, microbiological quality of fish from the Limbe and Tiko beaches in South West Cameroon, the isolated human pathogenic bacteria from three anatomic sites (skin, gills, intestine) of 50 fish (150 specimens) revealed eleven bacterial species were identified, including *E coli* type 1 (20.8%), *Citrobacter fruendii* (16.4%), *Proteus vulgaris* (13%), *Klebsiella pneumoniae* (12.1%), *Klebsiella ozaenae* (7.7%), *Enterobacter cloacae* (7.2%), *Klebsiella oxytoca* (5.8%), *Serratia marcescens* (4.8%), *Serratia odorifera* (4.8%), *Hafnia alvei* (4.4%) and *Proteus penneri* (2.9%). Enteric bacteria, such as Proteus, Citrobacter and Providencia, were found to be prevalent particularly in the gills and intestines of marketable fishes of the Volga and Caspian Seas. Half of all the enteric bacteria was detected in the fish kidneys, spleen, and liver [24]. [25], reported coliform in Tilapia nilotica from Nasser's lake in

Aswan it nearly 43% of skin or gill samples, 100% of intestine and raw fish flesh samples. Similar finding reported by [26, 27].

The experimental infection of Nile tilapia with the isolated bacteria revealed high mortality rate with *Enterobacter cloacae* (43, 33%). The reported mortalities were varied between the bacteria based on the pathogenesis and virulence as well as the toxins amount and severity among each bacterial isolate. [28], reported that Atlantic salmon and rainbow trout infected with *Yersinia ruckeri* isolates caused mortalities in both rainbow trout and salmon. During the spring of 1996 and autumn of 1997 unusual mortality outbreaks among rainbow trout fry and yearlings occurred at two different trout farms, resulting in mortality of 20 and 10 per cent, respectively. Bacterial isolates from moribund fish from both cases were identified as *Yersinia ruckeri* [29]. [30] recorded that, Zebrafish Danio rerio were injected intramuscularly with *Edwardsiella ictaluri* at different doses. Mortality occurred from 2 to 5 d postinjection at rates of 0, 76.6, and 81.3% for the low, medium, and high doses, respectively. [31] stated that, faecal Streptococcus species belonging to Lancefield group D has been a major cause of mortalities on certain trout farms on the escarpment of the Mpumalanga Province in the Republic of South Africa. An enteric bacterium from the kidneys of moribund fish M. cephalus, was isolated and identified as Enterobacter cloacae. Mugil cephalus was experimentally infected by this isolate revealed high mortality [7].

The experimentally infected Nile tilapia, by *E. coli, Citrobacter freundii, Yersinia intermediate,* and *Enterobacter cloacae* showed focal hyperplasia, desquamation in the secondary lamellae and hemorrhages in the gill arch. The hepatopancreas revealed vacuolar degeneration and necrosis in some hepatocytes with nuclear pycknosis together with mucinous degeneration and leukocytic infiltration in lamina properia of the intestine. On the other hand, the experimentally infected Nile tilapia, by *Enterobacter cloacae* revealed tubular degeneration in the kidney with depletion of heamatopoietic tissue while *Yersinia intermediate* showed hyaline degeneration in muscle with hemosiderosis and focal depletion of lymphoid cells spleen. Our results were in accordance with [32], who observed deep, blood free ulcers at the point of injection of channel catfish with enterobacteriacae. Internal gross lesions showed yellow to sersanguineous ascitic fluid, peritoneal visceral adhesions, splenomegally, enteric hyperemia, yellowish to green liver coloration with occasional petechiae and kidneys of an abnormally soft consistency. Petechiae were present on the intestine and mesentery, the spleen was darkening color and the posterior kidney was very friable. Histopathological findings were compatible with the systemic nature of the disease and the lesions had been reported in the skin, muscle, liver and intestine. On other hand *Citrobacter rodentium* in mice exhibited rapid weight loss and suffered up to 90% mortality with ulceration in the colon [33]. Similar result reported by [34]. [35], reported histological lesions of fishes infected experimentally by enterobacteriacae including necrosis of the endocardium, kidneys and liver. Microscopic lesions were most severe in the intestine and were characterized by mucosal necrosis and cellular influx of the lamina propria and muscularis.

[36] mentioned that, bacteriological and histological examination of infected rainbow trout with Yersinia revealed the presence of high numbers of bacteria in the gills immediately after infection resulting in a rapid spread of *Y. ruckeri* in the internal organs. However, only a virulent strain was able to survive and multiply in the host, causing septicemia and death. Histopathologically, the gill exhibited a lower degree edema mulifocal to coalescing coagulative necrosis of spleen, degeneration and or mild necrosis of proximal tubule, increase cellularity of the glomerular tuft, slight to moderate increase in the number of macrophages and increase in melanomacrophages. This may indicate that, bacteria first adhere to gill mucus and thereafter invade the branchial vasculature leading to septicemia and colonization of the internal organs [37]. For respiration, gills are highly vascularized, with a large number of blood capillaries; therefore, they may provide good entry sites for bacteria to become easily disseminated to the entire body of the fish [38]. The pathogenicity of *Yersinia ruckeri* for carp were studied by intraperitoneal injection of about 5×10^5 cells. Thirteen injected fish were moribund or died within 4 days with septicemic lesions. Two survivors were sampled on Day 28 after infection. *Yersinia ruckeri* was reisolated from the internal organs of all experimental fish. By histopathological examination, moribund fish had generalized bacteremia with inflammation, degeneration and necrotic foci in kidney, liver and spleen. Survivors of challenge on day 28 had a chronic disease characterized by prominent peritonitis and enteritis, exhaustion of the erythroid, granuloid and lymphoid components in hematopoietic kidney tissue as well as focal degeneration and necrosis in organs [39]. Also significant economic losses in salmonid aquaculture worldwide due to *Yersinia ruckeri* infection may result in a septicemic condition with hemorrhages on the body surface and in the internal organs [40]. Six electric eels presented clinically with abdominal distension following prolonged exposure to elevated environmental pH. Postmortem examination revealed marked ascites. Culture of the abdominal fluid from three of the eels yielded either *Aeromonas hydrophila* or *Citrobacter freundii,*

which were most likely secondary invaders. Histopathology showed marked iron accumulation in both hepatocytes and hepatic macrophage aggregates [41].

The degenerative and necrotic changes in the gill, liver, intestine, spleen, muscle and kidneys reflected on the hematological and serum biochemical parameters and resulted from the direct effect of bacterial toxins.These results were in agreement with [42, 43].The proliferation of the hematopoietic tissue and activation of the main phagocytic cells (melanomacrophages) in fish , it parallel with hematological finding which indicate the role of bacteria and its toxins to stimulate the immune response in infected fish. Similar results were reported by [43], who isolated 162 *E coli* from foods (raw meat, fish, and processed foods) in Korea. The virulence characteristics of 32 *Citrobacter freundii* strains of fish, human, and veterinary origin were comparatively analyzed. The isolates displayed a low degree of virulence for trout, inoculated strains were always recovered from the survivors in pure culture [44].

The experimentally infected Tilapia nilotica (*Oreachromis niloticus*) by the isolated bacteria, at 3and 6 days of infection, revealed significant decrease in the total erythrocytic count in comparison with the control. This finding could be due to the toxins produced by some enterobactercae and/or the focal hemorrhages that seen histopathologically, in this study. However, [45] studied the hematological changes in Nile tilapia experimentally infected with 1×10^3 and 1×10^6 Colony-Forming Units (CFU)/ml of Enterococcus species. The red blood cell counts were not significantly different among the treatments. Haemolysin patterns of 175 strains of different Salmonella enterica subspecies enterica serovars could be divided into different hemolysin types based on their inability to produce hemolysis on one or more types of blood agar, [46]. A significant decline in total erythrocyte count, hemoglobin content and packed cell volume in blood was noticed following an intraperitoneal challenge with *Aeromonas hydrophila* at $2.24 \times 10^{(7)}$colony-forming unit (CFU)/fish[47].

The experimentally infected Tilapia nilotica (*Oreachromis niloticus*) by the isolated bacteria, at 3 and 6 days of infection, revealed significant to non significant increase in the total leukocytic count in comparison with the control. The increase in the leukocytic count in the infected groups reflected the body response and defense against these bacteria as it was shown histopathologically, in this study, by the leukocytic infiltrations in the tissues of the examined organs. [45] mentioned that, the lympohcyte numbers increased significantly in fish injected with 1×10^6 CFU/ml of Enterococcus. Similar resulted reported by [47] who found a significant increase in serum myeloperoxidase, ceruloplasmin activities and total leucocyte count in fish intraperitoneally challenge with *Aeromonas hydrophila.*

The liver (ALB and BIL) and kidney (urea and creatinine) tests showed significant to non-significant variable values that increased in same groups and decreased in others depending on type of infection and period of study (3, 6 days). The marked changes in the serum biochemistry parameters could be due to the varied effect of different bacteria in the health status of the infected tilapia where marked degenerative changes were observed in the gills, liver and kidneys, these histopathological lesions that noticed in the present study in the infected tissue affect on the level of urea, creatinine and liver albumin and bilirubin.

Enterobacteracea in seafood has brought to light concerns regarding food safety, it also constitute a negative impact on cultured fish with variable histopathological alterations. However, the studying of information about risk assessment or surveillance needs is recommended.

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