Global Veterinaria 9 (5): 617-629, 2012
ISSN 1992-6197
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DOI: 10.5829/idosi.gv.2012.9.5.66201

Further Characterization of *Enterobacteriaceae* Isolated from Cultured Freshwater Fish in Kafr El Shiek Governorate: Clinical, Biochemical and Histopathological Study with Emphasis on Treatment Trials

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Abstract: The present study was carried out to isolate various members of *Enterobacteriaceae* from cultured freshwater fishes (*Oreochromis niloticus" O. niloticus"* and *Mugil capito*) and identifying them by biochemical tests (Traditional and API 20 E kit) and relating the results of isolation to the clinical signs, postmortem and histopathological changes. Antibacterial activity of garlic extract was investigated against the isolated *Enterobacteraceae* strains. The results obtained in the present study showed that 37 *Enterobacteriaceae* strains were isolated from 40 *O. niloticus* fish samples with percentage of 92.5%. The incidence of *Escherichia coli, Salmonella arizonae, Citrobacter braakii, Enterobacter sakazakii, Citrobacter frundi, Raoutella ornithinolytica, Enterobacter cloacae, Klebsiella ozaenae* and *Proteus vulgaris* isolation were 27 %, 21.6%,19%,10.8 %,8.1%,5.4 %, 2.7%, 2.7% and 2.7% respectively. The results showed that the highest number of isolates was recovered from liver and the lowest number of isolates was obtained from the muscles. On the other side 14 isolates of *Enterobacteriaceae* strains were isolated from 20 *Mugil capito* samples with percentage of 70%. The incidence of *Escherichia coli, Enterobacter cloacae, Salmonella arizonae, Klebsiella pneumoniae, Citrobacter braakii* and *Proteus mirabilis* isolation were 42.8 %, 21.4%, 14.2%, 7.2%, 7.2 and 7.2% respectively. The results showed that the highest number of isolates was recovered from liver and intestine while the lowest number of isolates was obtained from the kidney. Histopathological changes were recorded and discussed. Garlic extract was suggested that it can be used in treatment of pathogenic *Enterobacteriaceae* in cultured *O. niloticus.*

Key words: *Enterobacteraceae* · *O. niloticus* · *Mugil capito* · Garlic extract · Histopathological

INTRODUCTION

Bacterial diseases in fish are a serious threat to aquaculture systems that cause severe damage and mortality in Egypt [1]. *Enterobacteraceae* in fish are considered as an indicator to sewage pollution and has been reported as opportunistic pathogen in fish [2]. The pathogenic strains of *Enterobacteraceae* may cause diarrhea in fish [3]. *Enterobacteriaceae* are widely distributed in nature and found in feces of human, poultry and animals [4] and have been recognized to develop resistance to antibiotics [5] as *Enterobacteriaceae* strains which isolated from four representative integrated fish farms in China, were highly resistant to antibiotic [6]. In Egypt, poultry waste, sewage, cow dung are mostly used to fertilize fish ponds and are considered superior to inorganic fertilizers in producing planktonic organisms in freshwater ponds. El Ghazaly *et al*. [7] demonstrated columnar epithelial layer in between the intestinal villi, carrying long hair like extensions and heavily infiltrated with mononuclear cells and they supposed that, these are important in up taking the antigen and presenting it into macrophage and lymphocytes underlying macrophages and lymphocytes to activate immune response against antigen [8].

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Today plant materials are present in, or have provided the models for 50% of western therapeutic drugs in fish [9]. Garlic is one of the edible plants that had a strong interest to scientists and recognized as an important medicinal plant which has a wide spectrum of actions; not only against the variety of Gram-negative and Gram-positive were and continue to be extensively investigated, but also has beneficial effects on the immune systems [10].

MATERIALS AND METHODS

Clinical Examination: The collected fish were examined clinically according to the methods described by Noga [11] Attention to fish behavior in rearing and growing ponds, changes in color, respiratory manifestations and external lesions must be taken in consideration.

Sample Collection and Preparation: Cultured sampled fishes *Oreochromis niloticus* (*O. niloticus*) and *Mugil capito* were collected randomly from the farm's ponds of a private fish farm in Kafr El Sheikh Governorate, Egypt and transported in tanks partially filled with the same water of the pond then transported to the laboratory. In the laboratory each fish was rinsed with de-ionized water and the surface of the fish was decontaminated by dipping it in ethyl alcohol and lightly flamed. After opening the body wall of fish, the surfaces of organs (liver, spleen and kidney) were sterilized by swabbing with 70% ethanol before bacterial isolation. Blood was collected using sterile syringe by direct penetration of caudal vein and then transferred into sterile tubes containing EDTA as anticoagulant. Intestine and muscles were prepared by cutting a part after sterilizing with red hot scalped, however gills' samples were taken by sterilized cotton swab which wiped against the gill filaments by lifting the operculum with the help of a pair forceps. A part of the gills' filament was removed aseptically. All previous samples were inoculated into nutrient and selinite F broth tubes and incubated at 37°C for 24 hours. Total numbers of collected samples from *O. niloticus* and *Mugil capito* were described in table (1).

Bacterial Isolation and Identification: A loop full from each broth tube was streaked onto the following media: Nutrient agar (Oxoid), MacConkey's agar (Oxoid), Salmonella-Shigella agar (SS agar) (Oxoid), Eosin methylene blue agar (EMB) (Oxoid) and blood agar.

Purified isolates were used as stocks for further morphological and biochemical identifications.

Table 1: Numbers of collected samples

Sample	*O. niloticus*	*Mugil capito*
Liver	14	4
Gills	8	4
Blood	5	4
Kidney	3	4
Intestine	2	4
Stomach	2	-
Ascitic fluid	1	-
Muscle	5	-
Total	40	20

Identification of the Bacterial Isolates

Morphological Characterization: Bacterial film was prepared from each suspected purified isolate and stained with Gram's stain [12] then examined under the bright field microscope with the oil immersion lens.

Biochemical Characterization

Traditional Methods: The separate colonies were subjected to biochemical identification by the following tests: oxidase "Biomerieux", triple sugar iron agar, indol, Voges Proskauer, urea utilization, Simmon`s citrate agar and methyle red according to the biochemical identification keys of Practical Medical Microbiology, 14th ed., edited by Collee, *et al*. [13].

API 20 E kits: API 20E kit (BioMerieux) [14] biochemical profiling test was performed according to manufacturer's instructions.

Finally isolates were stabbed into tubes containing semi-solid nutrient agar medium and then incubated at 37°C for 24 hrs. The incubated tubes were examined for detecting motility of inoculated isolates then preserved in the refrigerator at 4°C.

Histopathological Examination: Tissue specimens from intestine, gills, liver, spleen and kidney were collected for histopathological examination. The tissue specimens were fixed in 10% neutral buffered formalin for 24h. The fixed tissue were rinsed in tap water, dehydrated through graded series of alcohols, cleared in xylene and embedded in paraffin wax [15]. 5 µm thick sections were cut and stained with hematoxylin and eosin (H and E) and then examined by light microscopy.

Fish Used for Treatment Trials: A total of 75 *O. niloticus* with an average weight of 60-70 g and length of 15-18 cm and 30 *Mugil capito* with an average weight of 80-100 g and length of 20-30 cm were collected from the same fish

farms. Fish of both types were divided equally to three groups. The first group treated with oxytetracycline, the second group treated with garlic extract and the last one was the control. The fish at the end of the trial were counted to detect the percentage of survivability, then dissected and record infection.

Diet Preparation: The diet was formulated from fish meal, meat meal, soybean meal, corn flour, wheat bran, beside vitamins and mineral mixtures. Diet contained 42% crude protein and 3150 kcal/kg metabolizable energy and all ingredients were finally ground to a size less than 1mm that could apprehended by fish easily, the ingredient were then weighted out according to the formulation, thoroughly mixed then water and oil were added to the dry ingredients mixture shortly before feeding to form a paste, then the feed used quickly after preparation The diet was placed in a feeding basket that hanged close to the water surface at a fixed feeding place with two fixed times daily at a rate of 5% of body weight according to Dosoky [16].

Drugs Used for Treatment

Garlin: In the form of Biofarm dry (one kg), produced by Pharmavet Company, Turkey. It was used as1g/kg ration according to Dosoky [16].

Oxytetracycline HCl: In the form of powder (one kg), produced by ADWIA Company, Egypt. It was used as 1g/10 kg ration according to Chechregani *et al*. [17].

Statistical Analysis: The results of infections were statistically analyzed using method of Maddison [18].

RESULTS

Clinical Examination: The clinical signs of infected fish were loss of appetite, scale loss, ascites, darkness coloration and hemorrhagic patches all over the body. In addition to congestion of all internal organs especially kidney, liver and spleen, these results were appeared in (Figs.1, 2 and 3).

Bacteriological Examination: The results of the traditional biochemical identification of all bacterial isolates match those obtained by the API 20 E system. In this study the result of API 20E accuracy was more than 98%, this results indicated that API 20E was found to be reliable for the identification of members of the family *Enterobacteriacea*e. The number and percentage of bacterial species which were isolated from different tissues of collected *O. niloticus* fish were illustrated in Table 2. Out of 37 *Enterobacteriaceae* strains isolated from 40 *O. niloticus* fish tissues samples with percentage of 92.5%, the incidence of *Escherichia coli, Salmonella arizonae, Citrobacter braakii, Enterobacter sakazakii, Citrobacter frundi, Raoutella ornithinolytica, Klebsiella ozaenae, Enterobacter cloacae* and *Proteus vulgaris* isolation was 27 %, 21.6%, 19%, 10.8 %, 8.1%, 5.4 %, 2.7%, 2.7% and 2.7% respectively. The results showed that

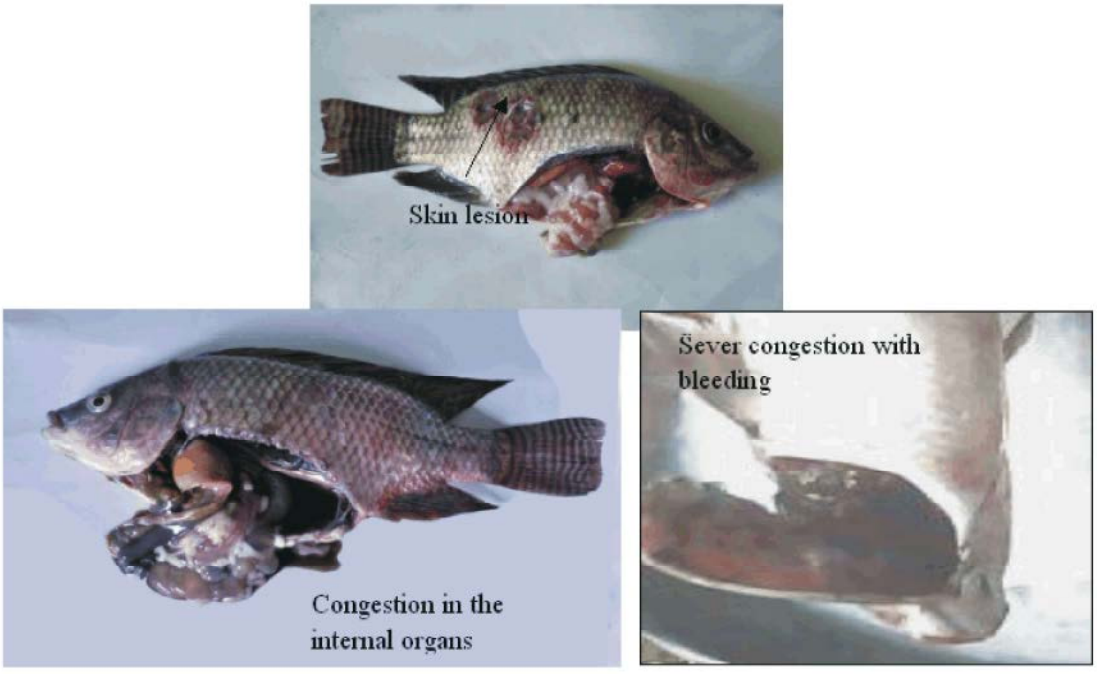

Table 2: Number and percentage of *Enterobacteriaceae* isolated from *O. niloticus* fish

Bacterial strains	Liver	Gills	Blood	Kidney	Intestine	Stomach	Ascetic fluid	Muscle	Total No. and
	No %	No %	No %	No %	No %	No %	No %	No %	ratio of strains
Escherichia coli	5(29.4%)	1 (14.3%)	1 (25%)	_	1 (25%)	2 (100%)	_	_	10 (27.0%)
Salmonella arizonae	4 (23.5%)	3 (42.8%)	_	_	1 (25%)	_	_	_	8 (21.6%)
Citrobactr braakii	5 (29.4%)	1 (14.3%)	_	1 (50%)	_	_	_	_	7 (19%)
Enterobacter sakazakii	1 (5.8 %)	_	3 (75%)	_	_	_	_	_	4 (10.8%)
Citrobacter frundi	_	1 (14.3%)	_	_	1 (25%)	_	1(100%)	_	3 (8.1%)
Raoutella ornithinolytica	2 (11.9%)	_	_	_	_	_	_	_	2 (5.4%)
Klebsiella ozaenae	_	_	_	1 (50%)	_	_	_	_	1 (2.7%)
Enterobacter cloacae	_	1 (14.3%)	_	_	_	_	_	_	1 (2.7%)
Proteus vulgaris	_	_	_	_	1 (25%)	_	_	_	1 (2.7%)
Total	17	7	4	2	4	2	1	0	37

Table 3: Number and percentage of *Enterobacteriaceae* isolated from *Mugil capito* fish

	Liver	Intestine	Gills	Blood	Kidney	Total No. and ratio of strains
Bacterial Strains	No %	No %	No %	No %	No %	
Escherichia coli	2 (50%)	2 (50%)	2 (66.7%)	_	_	6 (42.8 %)
Enterobacter cloacae	1 (25%)	1(25%)	_	1 (50%)	_	3 (21.4 %)
Salmonella arizonae	_	1(25%)	1 (33.3%)	_	_	2(14.2 %)
Citrobacter braakii	1 (25%)		_	_	_	1(7.2 %)
Klebsiella pneumonae	_		_	_	1 (100%)	1(7.2%)
Proteus mirabilis	_		_	1 (50%)	_	1(7.2 %)
Total	4	4	3	2	1	14

the highest number of isolates was recovered from liver and the lowest isolation number was obtained from the muscles. *E. coli* was the dominant isolates as 10 isolates (27.0%) and the lowest isolates were *Klebsiella ozaenae, Enterobacter cloacae* and *Proteus vulgaris* as 1 isolate with percentage 2.7%.

Table (3) showed that out of 14 *Enterobacteriaceae* strains isolated from 20 *Mugil capito* fish tissues samples with percentage of 70% *Escherichia coli, Enterobacter cloacae, Salmonella arizonae, Klebsiella pneumonae, Citrobacter braakii* and *Proteus mirabilis* isolation were 42.8 %, 21.4%, 14.2%, 7.2%, 7.2 and 7.2% respectively. The results showed that the highest number of isolates was recovered from liver and intestine. On the other side the lowest isolation was obtained from the kidney. *E. coli* was the dominant isolates as 6 isolates (42.8%) and the lowest isolates were *Citrobacter braakii*, *Klebsiella pneumonae* and *Proteus mirabilis* as 1 isolate with percentage 7.2%.

Histopathological Changes

Intestine: Intestine of *O. niloticus* revealed, catarrhal enteritis, with necrosis of enterocytes and intra luminal aggregation of mucous exudates mixed with bacterial bacilli (Fig.1a), mucosal edema and intense proprial infiltration with mononuclear cells and eosinophilic granular cells (EGCs) (Fig.1b) and activation of mucous secreting cells (Fig.1c).

Intestine of *M. capito* revealed intense infiltration of lamina propria with mononuclear cells mixed with EGCs and congestion of mucosal and submucosal blood capillaries (Fig.1d).

Gills: The pathological alterations observed in gills of both *O. niloticus* and *M. capito* were nearly similar. The most common ones are vasodilation (Fig. 2a), epithelial hypertrophy and hyperplasia with lamellar fusion as well as epithelial lifting, lamellar telangiectasis and secondary lamellar clubbing (Fig. 2b) and diffuse infiltration of the branchial tissue with inflammatory cells (Fig. 2c).

Liver: Liver of *O. niloticus* showed dilatation and congestion of hepatoportal blood vessel with perivascular aggregation of with leucocytes and EGCs (Fig. 3a), diffuse vacuolar degeneration of hepatocytes that swollen with large intracytoplasmic vacuoles and eccentrically located nucleus (Fig. 3b), and small focal area of hepatocellular necrosis infiltrated with mononuclear cells (Fig. 3c) as well as large area of hemorrhage. Hepatopancreas was infiltrated with melanophores and EGCs (Fig.4d) and degranulated (Fig.4e),

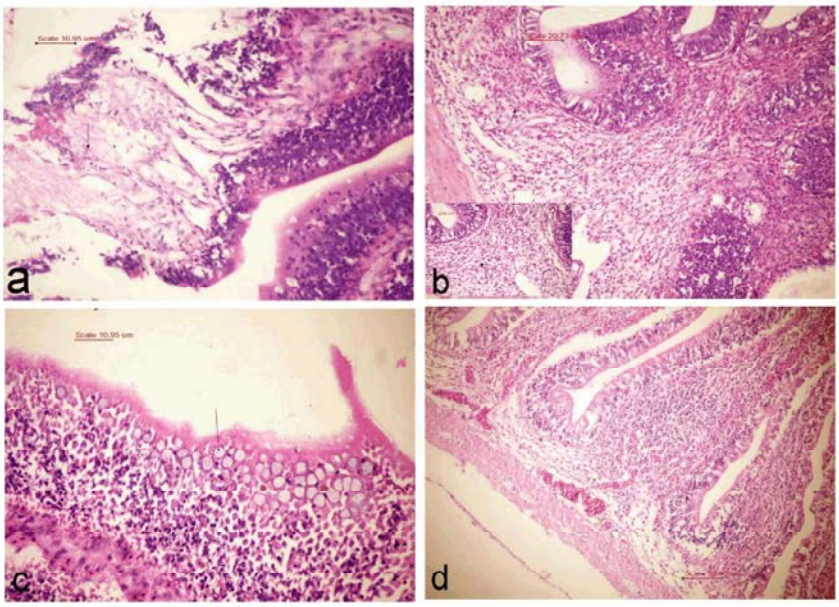

Fig. 1: Light micrograph of intestine of *O.niloticus* showing (a) aggregation of mucous exudates mixed with bacterial bacilli (arrow), (b) proprial infiltration with mononuclear cells and eosinophilic granular cells (EGCs) (arrow) and (c) activation of mucous secreting cells (arrow). Fig.1 (d) Intestine of *M. capito* showing aggregation of mononuclear cells mixed with EGCs and congestion of mucosal and submucosal blood capillaries (H and E). Scale bars: 10.95µm (a, c, d) and 20.73µm (b) H and E stain.

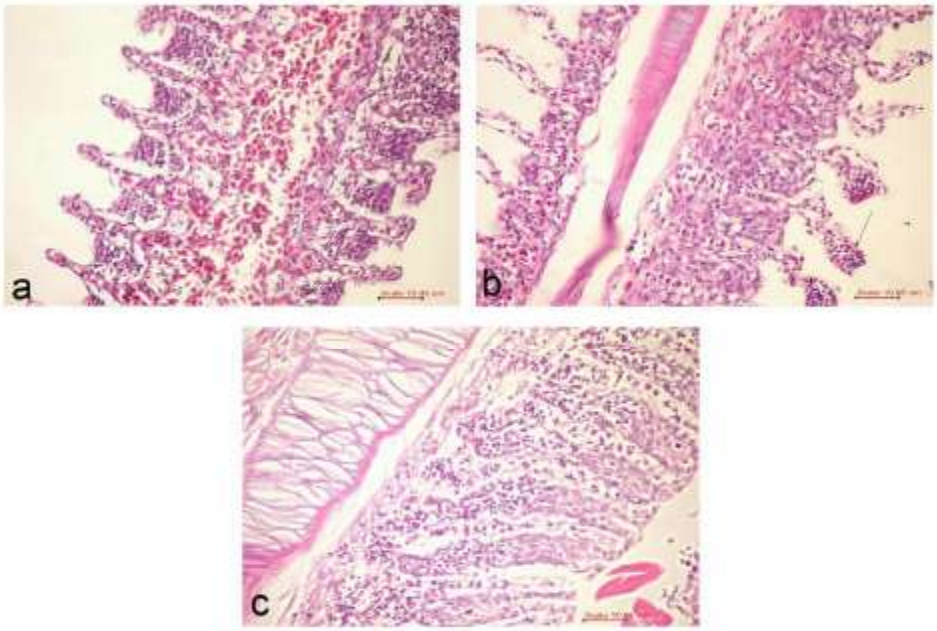

Fig. 2: Light micrograph of gills of *O. niloticus* exhibited, (a) vasodilation, (b) hyperplasia with lamellar fusion (short arrow), epithelial lifting (e), lamellar telangiectasis (long arrow) and secondary lamellar clubbing (H and E) and (c) infiltration with inflammatory cells. Scale bars: 10.95 µm, H and E stain.

Liver of *M. capito* showed the same histopathological alterations recorded in that of *O. niloticus* in addition to portal edema with portal infiltration with EGCs and melanomacrophag cells.

Kidney: Kidney of *O. niloticus* showed vacuolar and\or hyaline droplet degeneration of renal tubular epithelial cells (Fig.4a) and peritubular mononuclear cell aggregation as well as aggregation of melanomacrophage

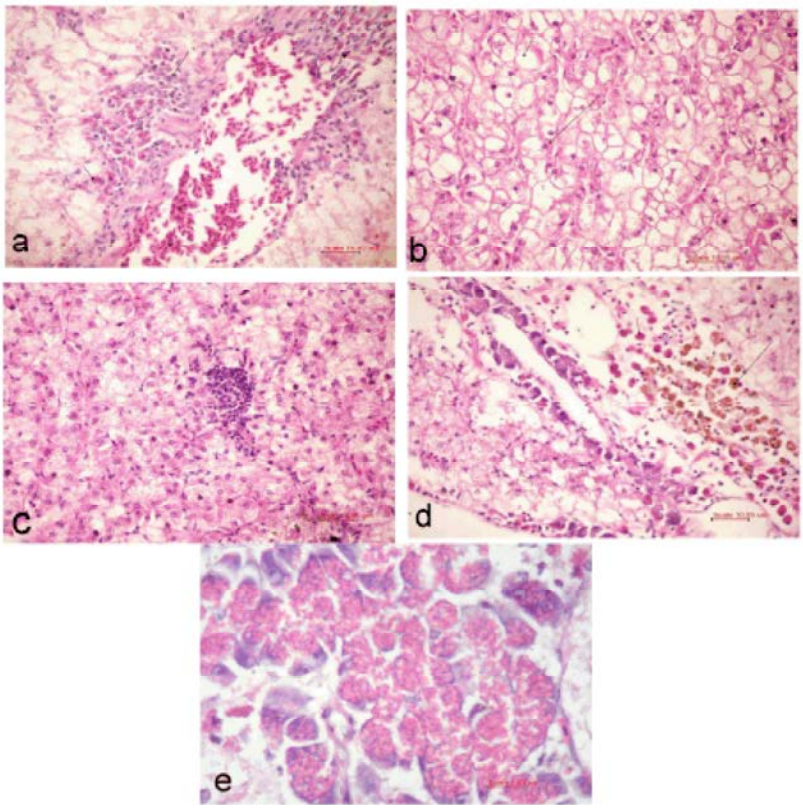

Fig. 3: Light micrograph of liver of *O. niloticus* showing (a) perivascular aggregation of with leucocytes (long arrow) and EGCs (short arrow), (b) large intracytoplasmic vacuoles (short arrow) and eccentrically located nucleus (long arrow), (c) focal hepatocellular necrosis infiltrated with mononuclear cells,(d) infiltration of hepatopancreas with melanophores (long arrow) and EGCs (short arrow) and (e) degranulation. Scale bars: 10.95µm (a, b, c, d) and 4.43 µm (e) H and E stain.

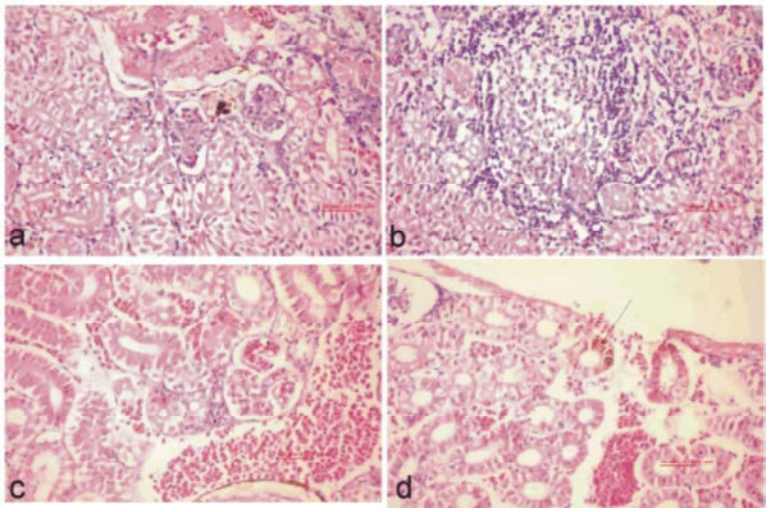

Fig. 4: Light micrograph of kidney of *O. niloticus* showing (a) vacuolar (long arrow) and \or hyaline droplet degeneration (short arrow), (b) necrosis with intense infiltration of mononuclear cells and (c) glomerular congestion (long arrow) and necrosis of some renal tubular epithelium with pyknotic nuclei (n). Fig. 4 (d) kidney of *M. capito* showing intracytoplasmic granules of hemosiderin pigment (arrow). Scale bars: 20.73 µm (a) and 10.95 µm (b, c, d) H and E stain.

Table 4: Survivability after garlic extract (10 mg/kg ration) and Oxytetracycline HCl (150 mg/kg ration) treated groups in relation to control group

Fish	No. at start	After 5 days	After 10 days	After 15 days	After 30 days
Control	25 O	17	10	8	8
	10 M	7	4	3	3
Fish treated with Garlic extract	25 O	23	23	22	22
	10 M	9	9	9	9
Fish treated with oxytetracycline Hcl	25 O	20	20	15	15
	10 M	8	8	6	6
			$Chi^2 = 6.55^*$		

* = Significant at (P < 0.05) O= *O. niloticus* M= *Mugil capito*

cells and thickening of the blood vessel wall. Renal interstitium revealed large area of necrosis with intense infiltration of mononuclear cells (Fig.4b) and interstitial hemorrhage.

The most relevant histopathological changes recorded in renal tissue of *M. capito* were glomerular congestion with periglomerular mononuclear cell aggregation and interstitial hemorrhage as well as necrosis of some renal tubular epithelium with pyknotic nuclei (Fig.4c), peritubular hemorrhage, vacuolar degeneration of renal tubular epithelium as well as presence of intracytoplasmic granules of hemosiderin pigment (Fig.4d).

Treatment Trails: As shown in table (4) the survivability of garlic extract and oxytetracycline Hcl treated groups at the end of the treatment were 98% and 60 % respectively, while control group survivability was 33.3 %.

DISCUSSION

In our study, the main clinical pictures in naturally infected fish were revealed some fish aggregation on the water surface, accumulation at the water inlet of the pond and air pump of aquaria. Others appeared dull with loss of escape reflex, haemorrhage, wounds and ulcers. These may be nearly similar to that recorded by Noor El-Deen, *et al*. [1]. The internal organs of naturally infected fish appeared pale, anemic with enlargement and congestion of spleen, liver with distended gallbladder. Signs of emaciation with petechial haemorrhage on the surface of abdomen, while intestinal wall was congested with the presence of ulcer and protruded from anus accompanied with large amount of catarrhal mucoid secretion, the presence of *Enterobacteriaceae* may be explained these results also our results nearly similar to that recorded by Olurin, *et al*. [19].

Fish is susceptible to microbial spoilage as it carries high microbial load on skin, gills and intestine. One of the greatest risks to human health is the consumption of raw or insufficiently processed fish and fish products. In this study fifty one strains of *Enterobacteriaceae* were isolated from fish samples, the results of bacterial identification indicated high Compatibility between API 20E and traditional methods, these result was agreed with O'Hara, *et al*. [20] who reported that accuracy of API 20E has ranged from 77.0 to 94.6% when performed on 24 hrs old culture, also the results agreed with York *et al*. [21] Who stated that accuracy ranged from 95.2 to 97.5% following the performance of supplemental testing according to the manufacturer's recommendations.

Isolation of *Enterobacteriaceae* with percentage of 92.5% and 70% from collected *O. niloticus* and *mugil capito* samples respectively was in agreement with those of Yagoub [22] that isolated *Enterobacteriacea*e with percentage of 66% from *O. niloticus*. The *E. coli* isolates were the common among the *Enterobacteriacaeae* isolated from *O. niloticus* and *mugil capito* fish samples wit h percentage of 27% and 42.8% respectively and this result was in harmony with Toroglu *et al*. [23] who isolated *E. coli* with percentage of 55% from *Achanthobrama marmid* fish, also Soliman *et al*. [24] isolated different serotypes of *E. coli* from *Clarias gariepinus* "Catfish", *Oreochromis niloticus,* common carp and silver carp" *and Mugil capito* fish mainly from intestine and liver and identifying by various methods. Baird *et al*. [25] reported that, the total losses attributed to the severe mortality among affected fish especially at marketable fish are due to *E. coli* infection.

In our study *Salmonella arizonae* was isolated with percentage of 21.6% and 14.2% from both studied fish species respectively, these results agreed with Toroglu, *et al*. [23] who isolated *salmonella arizonae* with percentage of 21%, the result confirmed that *Salmonella* spp. are one of the most frequent food-born diseases, representing an important public health problem in almost all industrialized countries. *Salmonella* spp. accounted for the largest number of outbreaks [8-26], on the other side fish usually act as vector for *salmonellae* which demonstrate no clinical disease and can shed *Salmonella* spp. without apparent trouble. [27-30].

Isolation of *Citrobacter* spp. especially *Citrobacter freundii* from different organs of collected fishes was agreed with. Jeremi *et al*. [31] isolated *Citrobacter freundii* from all diseased and dead rainbow trout and cyprinids fish. Moreover, before the 80's of the last century, there were indications that *Citrobacter freundii* can cause disease in fish. However, definite data were not obtained until the papers of Sato *et al.* [32] when the organism was proved to be pathogenic for aquarium fish and in the 90's for farmed fish. *Citrobacter freundii* was isolated from diseased Atlantic salmonids in Spain [33] and the USA [34] and from carp in India [35].

The percentage of *Enterobacter cloacae* isolation from different samples was 2.7% and 21.4% from *O. niloticus* and *Mugil capito* respectively, these findings agreed with Sekar *et al.* [36] who reported that *Enterobacter cloacae* is the causative agent of selective mortality observed in *Mugil capito*.

Raoutella ornithinolytica was isolated from *O. niloticus* liver with percentage of 5.4% and this nearly agreed with the data obtained by Kanki *et al*. [37] who isolated the organism with percentage of 8% from tuna, bonito and sardines fish. *Raoutella ornithinolytica* is the most important histamine producing bacteria (HPB) that cause histamine fish poisoning (HFP), which is a mild illness; however, serious complications such as cardiac and respiratory manifestations may occur in individuals with pre existing conditions [38], as a hazardous level of histamine is produced by the microbial decarboxylation of the free histidine in the muscular tissue of fish, also *Raoutella ornithinolytica* carried hdc genes which encoding histidine decarboxylase [37]. Moreover, *Klebsiella pneumonae* has been considered as important (HPB) isolated from fish even after *K. planticola* was described as new species [38-39].

The study reported that presence of the coliform group of bacteria, mainly *Escherichia, Citrobacter, Enterobacter* and *Klebsiella* in fish presents a health hazard to human [2]. Allen and Hepher [40] have stated that most of the epidemics attributed to waste water sources are from raw sewage gaining access to food eaten directly by man, or from contamination of water supply systems by untreated sewage. Korzeniewska and Harnisz [41] have reported that the presence of fecal coli form in fish intended for human consumption may constitute a potential danger not only in causing disease, but also because of the possible transfer of antibiotic resistance from aquatic bacteria to human infecting bacteria from non-aquatic sources. Some human pathogens such as, *Escherichia, Klebsiella* and *Salmonella* spp. have been found to survive and multiply in the gut, mucus and tissues of fish and thus render fish a potential vector of human disease over long periods [42].

The presence of these bacterial isolates *Citrobacter braackii, Citrobacter freundii, Enterobacter sakazakii, Enterobacter cloacae, Proteus mirabilis, Proteus vulgaris and Klebsiella pneumoniae* indicated the degree of cross contamination from the handlers [43] and their presence represents a potential hazard, especially to the immunocompromised consumers. Finally particular isolation of some highly pathogenic agents such as *Salmonella* spp., *E. coli* and potential pathogenic organisms such as *Klebsiella* spp., *Citrobacter* spp. and *Proteus* spp., which when were isolated from fish and fish products it gives indication of fecal and environmental pollution of fish environment [4, 43, 37, 44]. Most of the pathogenic enteric bacteria produce virulence-associated factors such as somatic antigens, adhesins, serum resistance, lipopolysaccharides, colicins, siderophores and haemolysins [45]. Many of these factors are able to penetrate the epithelial layers of the intestinal mucosa and facilitate pathogenic process. Extra-cellular protein is also implicated in virulence and pathogenicity of enteric bacteria [46]. In addition to that haemolysin and leucotoxins have been isolated from these bacteria, which are associated with the disease process [47, 48].

In the present study, a wide spectrum of histopathological alterations were revealed in the intestine, gills, liver and kidney of sampled *O. niloticus* and *M. capito* collected from different farms in Kafr El Sheikh Governorate,

Necrosis and destruction of enterocytes, demonstrated in this study, could be the direct effect of bacterial toxins. This mucosal necrosis interferes with absorption [49]. Mucosl edema, congestion of mucosal and submucosal blood vessels and intense leucocytic cell infiltration are inflammatory reaction induced by these pathogenic bacteria which was confirmed by demonstration of these bacterial bacilli in the mucous exudates mixed with desquamated epithelium. These results are potentiated by Ferguson [50], who attributed these result to acute bacteremia. Moreover most of the pathogenic enteric bacteria produce virulence-associated factors such as somatic antigen, adhesions, serum resistance, lipopolysaccharide, colicin, siderophores and haemolysins [51]. Many of these factors are able to penetrate lamina epithelialis and fascilitate pathogenic process. Extra cellular protein is also implicated in virulence and pathogenecity of enteric bacteria [46]. Recently, haemolysins and leucotoxins have been isolated from these bacteria, which are associated with the disease process [48].

Demonstration of bacterial bacilli mixed with mucous exudates and desquamated epithelial cells confirm that members of *Enterobacteriace*ae, identified in this study, are pathogenic strains, capable of invasion the intestinal mucosa and producing marked tissue damage.

Concerning gills, histopathological examination revealed epithelial lifting which either induced by edematous changes in gill filaments and secondary lamellae, probably due to vasodilation and increased capillary permeability [19], or related to a decrease in the gill Na^+ and K^+ activated ATPase and/or adecline in blood Na^+ and Cl concentration [52].

Hypertrophy and hyperplasia of lamellar epithelium leading to lamellar fusion would increase the thickness of water-blood barrier and decrease both oxygen uptake and free gas exchange, affecting the general health of fish [53] and can cause capillary hemorrhage [54, 55] and create an anoxic condition that will ultimately harm the fish [56]. Hyperplasia of mucous cells could be considered a protective response to binds toxins transport, also it may to serve to protect the epithelia against both mechanical abrasion and infection as suggested by Olsen and Formm [57], more over fish mucous is considered to be implicated in resistance against disease [58], as it prevents the entry of pollutants and toxin into gills.

Lamellar and capillary aneurysim is related to damage of pillar cells [59 and 60], that lead to loss of supportive properties with subsequent blood stasis in lamellar capillaries with separation of respiratory epithelium [61].

The organ most associated with the detoxification and biotransformation process is the liver [62] and due to its function, position and blood supply it is also one of the organs most affected by contaminants in the water [63].

The liver of both studied fish showed vacuolar degeneration of hepatocytes, congestion of sinusoidal and hepatoportal blood vessel and these changes are early degenerative lesions that could be attributed to both, Oxygen deficiency as a result of gill degeneration being the most common cause of the cellular degeneration in the liver [64] and the toxins produced by enteric bacteria. In most cases, however, infection is caused by the pathogen penetrating the epithelium and then growing in the sub mucosa or spreading even further [65]. Pacheco and Santos [66] described increased vacuolisation of the hepatocytes as a signal of degenerative process that suggests metabolic damage, possibly related to exposure to contaminated water. Jeremi *et al.* [31] demonstrated fatty degeneration of the liver, inflammatory and necrotic changes and weaker bleeding, in rainbow trout fry and cyprinids, indicating typical acute bacterial septicemia, caused by *Citobacter freundii*. Similar alterations were recorded, in fish living in aquatic environment contaminated with sewage [67].

Liver necrosis demonstrated in this study is strongly induced by the toxins of these enteric bacteria because of their extra intestinal spread.

One of the remarkable liver alterations revealed in this study, was the aggregations of melanomacrophage cells in the hepatic parenchyma especially the hepatopancreas. An increase in the intensity of the melanomacrophage aggregates was observed in the liver of *P. lineatus* and it was generally related to important hepatic lesions [66], such as degenerative and necrotic processes. The function of the melanomacrophages in the liver of fishes remains uncertain, but some studies have suggested that it is related to destruction, detoxification or recycling of endogenous and exogenous compounds [68].

The kidney of teleost is a target organ in many diseases, particularly the economically important ones of cultured fish. A reason for this may be the affinity of the organ for circulating particulate antigens which is performed by, the large number of phagocytes lining the renal sinusoids and peritubular capillaries of anterior and posterior portions that trap pathogens [50].

In the present study, the kidney revealed vacuolar degeneration of renal tubular epithelial cells, this change represent initial stage in the degeneration process that progress to hyaline degeneration, which characterized by the presence of large eosinophilic granules inside the cells. These hyaline droplets are product of degenerating renal tubular epithelium induced by the various toxins produced by these pathogenic bacteria. In more severe cases, the degenerative process can lead to tissue necrosis and this is obvious in our study that revealed large area of necrosis with intense infiltration of mononuclear cells [56].

One of the most relevant pathological alterations demonstrated in the kidney of both *O. niloticus* and *M. cephalus* was interstitial hemorrhage, peritubular hemorrhage and presence of intracytoplasmic granules of hemosiderin pigment. These alterations could be related to the effect of bacterial hemolysins [50].

Treatment Trials: Treatment trials of naturally infected *O. niloticus* and *Mugil capito* with *Enterobacteriaceae* were applied using Garlic extract. It was noted that, the best suitable and effective causing a great damage to the bacteria was 10 mg/kg ration for three successive days. These results may be attributed to antibacterial action of allicin in garlic, these results similar to that recorded by Noor El-Deen and Razin [69].

Garlic (*Allium sativum*) has proven to be hypolipidemic [70 and 71] and antimicrobial [72].

Garlic contains sulfur containing compounds; Alliin, which is converted to the antimicrobial active allicin, when the bulb is cut or bruised [73]; so, the fresh bulb contains alliin, allicin and volatile oils. When the garlic clove is crushed, the odorless compound alliin is converted to allicin, via the enzyme allinase. Allicin gives garlic its characteristic pungent smell [74]; also it contains vitamins and minerals [75].

Conclusion and Recommendations: This study concluded that a positive correlation between contaminated water supply of fish ponds with sewage and increase the incidence of *Enterobacteriaceae* infection in cultured fish and recommended increase using of natural products as garlic extract in cultured fish ration to enhance prevention and treatment of various bacterial infections.

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