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Research Article
Treatment Trial of Nile Tilapia (*Oreochromis niloticus*) Experimentally Infected with *Vibrio alginolyticus* Isolated from Sea bass (*Dicentrarchus labrax*)

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Abstract

Background and Objective: In Egypt, Nile tilapia represents the main cultured type due to its economical price, palatability and easy culturing. This study was aimed to elucidate the pathogenicity of *V. alginolyticus* isolated from diseased sea bass and experimentally infected healthy Nile tilapia fish. **Materials and Methods:** Healthy Nile tilapia fish were injected I/P with *V. alginolyticus* isolated from diseased sea bass. Symptoms and mortality rates of infected Nile tilapia fish were recorded during the experimental period. Re-isolation of *V. alginolyticus* was done from infected tilapia fish by bacteriological methods. For confirmation the pathogenicity of *Vibrio* isolated either from marine fish or tilapia fish, PCR test was done using *tdh* and *bla* gens. Liver and kidney function tests with histopathological examinations of some organs were performed. Treatment trial was done according to the antibiotic sensitivity test. **Results:** The isolated *Vibrio* is highly pathogenic to Nile tilapia fish causing deterioration in all parameters which finished by severe mortalities. Treatment with florfenicol, enrofloxacin, or oxytetracycline reduced the mortality rate and improved liver and kidney function parameters of infected Nile tilapia fish. **Conclusion:** *V. alginolyticus* can infect both marine and fresh water fish inducing a high mortality rate. Treatment of infected fish with florfenicol, enrofloxacin, or oxytetracycline reduces the mortality rate.

Key words: Nile tilapia, sea bass, infection, *V. alginolyticus*, mortality, pathogenicity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nile tilapia represents the main cultured type due to its economical price, palatability and easy cultivation in rivers, pond, or dams[1]. As a result of economical highly density cultured Nile tilapia, which leads to an increase in the ammonia level, water pH and physical contact; all these factors may lead to the spread of several bacterial infections, as a result of depression of fish immunity[2]. *Vibrio* spp. were widely detected in both marine environment and brackish waters[3]. Vibrio species were isolated nearly from all fish farms, either fresh, brackish or marine water fish as; sea bream (*Acanthopagrus arabicus*), silver carp (*Hypophthalmichthys molitrix*), green mullet (*Planiliza subviridus*), common carp (*Cyprinus carpio*), molly fish (*Poecilia latipinna*), Blue tilapia (*Oreochromis aureus*) and redbelly Nile tilapia (*Coptodon zilli*), *Mugil cephalus*, catfish (*P. hypothalamus*); especially of wormed water[3-6]. Among fish aquacultures, *Vibrio* spp., considered the most significant bacterial problem leading to severe economic losses worldwide as a result of its high morbidity and mortality rates which may reach more than or equal to 50%[7-9]. *Vibrio* spp. are Gram-negative motile bacteria, rod-shape, oxidase and catalase positive[5,10-12]. Both *V. alginolyticus* and *V. vulnificus* were isolated from apparently healthy Nile tilapia fish (*Oreochromis niloticus*) in a private farm around Quran Lake, Egypt without any fish morbidity or mortality documented, These bacteria were identified as an opportunistic pathogen that may be inadvertently under stress conditions of changing water, *V. alginolyticus* is pathogenic to Nile tilapia[13] with LD50 at 1×10^6. Improper uses of antibiotics may lead to the appearance of resistant strains so, these antibiotics should be given under veterinary supervision, at the full therapeutic doses and for the full number of days (recommended on the label). At sub-therapeutic doses, the bacteria become able to adapt with the antibiotic by mutating and developing the resistance[14,15], bla TEM gene is B-Lactam resistance gene (e.g., Ampicillin, Amoxicillin), which developed recently as a result of uncontrolled or sub-therapeutic use of antimicrobials[16], this gene has found in many pathogenic bacteria[17]. *V. parahaemolyticus* and *V. alginolyticus* associated with the outbreak were found to produce thermostable direct hemolysin (*tdh*) and *tdh*-related hemolysin (*trh*) genes[18].

The pathological lesions of *O. niloticus* infected with *Vibrio* spp. appear as a hemorrhage lesion in all organs, especially liver. The liver also showed areas of degeneration and necrosis among per-vascular hepatocyte[19].

The study was aimed to show the pathological effects of *vibrio* spp. isolated from marine fish on Nile tilapia fish (*Oreochromis niloticus*), in addition to the effects of commonly used antimicrobials on isolated vibrio bacteria for reducing the economic losses.

MATERIAL AND METHODS

Study area: The study was carried out at units of bacteriology and fish diseases Animal Health Research Institute, Kafr El-Sheikh branch, Egypt from March, 2018-December, 2019.

Fish:

- **Sea bass (*Dicentrarchus labrax*) for isolation of *Vibrio* species:** 25 diseased sea bass fish were randomly collected from some marine fish farms at Damietta governorate for isolation of *vibrio* spp. Fish samples were collected and transferred to the lab. in icebox
- **Nile Tilapia fish *(Oreochromis niloticus*) for experimental infection:** A total number of 120 healthy *Oreochromis niloticus* fish (Weighing 60±10 g) were obtained from some private farms at Kafr El-sheikh governorate and transferred to the lab. in aerated plastic bags. Fish have been adapted for 2 weeks and random 10 Nile tilapia fish were examined to ensure its health status before starting the experimental infection as described by Austin and Austin[20]

Bacteriological examination: Isolation of *Vibrio* species from sea bass and re-isolation from Nile tilapia *(Oreochromis niloticus*) fish after the experimental infection were performed according to the protocol recommended by ISO/TS 21872-1[21] and ISO/ TS 21872-2[22]: After sterilization of the skin by flaming, 5 grams of gills and internal organs (liver, spleen and kidney) were taken under aseptic conditions. These organs were incubated in 45 ml of sterile alkaline peptone water (3% NaCl and pH 8) for 24 h at 37°C. Loopfuls from each previously cultured tubes were separately streaked onto Thiosulfate citrate bile and sucrose agar (TCBS), then the medium was incubated at 37°C for 24 h. Bacterial isolates were distinguished morphologically utilizing Gram's stain.

Biochemical identification: Suspected *Vibrio* spp. colonies on TCBS media with positive oxidase test were subjected to further identification by *Microbact 12A, 12B*, (Oxoid, UK).

Molecular identification

DNA extraction: DNA extraction from samples was performed using the QI Aamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations Primers used were supplied from Metabion (Germany) are listed in the Table 1.

Table 1: Primers sequences, target genes, applicant sizes and cycling conditions

Microbial agent	Target gene	Primers sequences	Amplified segment (bp)	Amplification (35 cycles) Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension	References
Vibrio alginolyticus	*blaTEM*	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516	94°C 5 min	94°C 30 sec	54°C 40 sec	72°C 45 sec	72°C 10 min	Colom *et al.*[23]
	tdh	CCATCTGTCCCTTTTCCTGC CCAAATACATTTTACTTGG	373	94°C 5 min	94°C 30 sec	54°C 30 sec	72°C 40 sec	72°C 7 min	Mustapha *et al.*[24]

PCR amplification: primers were utilized in a 25 µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water and 6 µl of DNA template. The reaction was performed in an Applied Biosystem 2720 thermal cycler.

Analysis of the PCR products: The products of PCR were separated by electrophoresis on 1% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V cm^{-1}. For gel analysis, 40 µl of the products were loaded in each gel slot. Gelpilot 100 bp (Qiagen, Germany, GmbH) and GeneRuler 100 bp ladder (Fermentas, Thermo, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Antimicrobial sensitivity test: The antimicrobial sensitivity test of *V. alginolyticus* isolated from sea bass was determined by the disc diffusion method. The diameter of the inhibition zone was estimated by millimeter and expressed as sensitive, intermediate and resistant according to the National Committee for Clinical Laboratory Standard (NCCLS)[25].

Experimental infection of Nile Tilapia fish:

- **Challenge test:** After the end of adaptation time (2 weeks), total No. of 100 healthy Nile tilapia (*Oreochromis niloticus*) fish (weighing 60±10 g) obtained from private farm at Kafr El sheikh governorate, were divided into 5 groups (20 fish in each one). Fish were maintained in glass aquaria supplemented by de-chlorinated water with temperature 25+2°C using thermostatic heaters and aerators. Fish were fed (3% of their body weight/day) a commercial fish diet throughout the experiment time[26]. Bacterial suspension of *V. alginolyticus* was prepared by turbidity matching with McFarland standard number 1 (which is equivalent to 3×10^8 CFU ml) and then 10 fold serial dilution was done reaching a concentration[27,28] 3×10^7 CFU ml then the groups 2, 3, 4 and 5 were I/P injected with 0.5 ml of 10^7 CFU ml *vibrio alginolyticus*. While group 1 was I/P injected with 0.5 ml sterile normal saline (negative control). After 3-7 days the symptoms and mortalities started. Bacteriological samples were taken to clarify the etiology of deaths

Histopathological examination: Tissues (spleen, liver, gills and intestine) of experimentally infected Nile tilapia with vibrio *alginolyticus* were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and eosin for histopathological examination according to Bancroft *et al.*[29].

- **Treatment trials:** By using the effective anti-microbial previously resulted in the sensitivity test as following: group 1 control negative (non-infected non treated group), group 2 control positive (infected non treated group), group 3 was infected and treated with Florfenicol (with dose of 25 mg kg^{-1} fish b.wt.), group 4 was infected and treated with Enrofloxacin (with dose of 50 mg kg^{-1} fish b.wt.), while group 5 was infected and treated with Oxytetracycline (with a dose of 50 mg kg^{-1} b.wt., of fish), for 7 days. The previous antibiotic doses were used according to Aboyadak *et al.*[30]. All groups were observed and mortalities were recorded for 8 days after starting treatment

Collection of blood samples: Blood samples were collected from each group one day after the end of treatment (7 days). Blood samples were taken (1 ml/fish from three fish of each group) from caudal vein using disposable 3-cc syringes and 21-gauge needles[31]. Blood samples were transferred into Eppendorf tubes without anticoagulants for serum separation for biochemical tests[32]. ALT and AST were determined[33]. Urea and creatinine were measured according to Varley *et al.*[34] and Michael and Malcolm[35], respectively.

Statistical analysis: The obtained results were analyzed using SAS[36].

RESULTS

Bacteriological examination: Table 2 shows strains of *Vibrio* spp. isolated from sea bass fish. It was found that *V. alginolyticus* and *V. harveyi* were isolated at 20 and 4% of sea bass, respectively. Table 3 explains re-isolation of *V. alginolyticus* from Nile tilapia after the experimental infection. *V. alginolyticus* was re-isolated from 60% of infected Nile tilapia fish.

Antibiogram (*in vitro*): Table 4 explains the effect of different antibiotics on isolated *V. alginolyticus in-vitro*. The results showed that, *V. alginolyticus* is highly sensitive to florfenicol and enrofloxacin. Meanwhile, it is resistant to many antibacterial agents as erythromycin, ampicillin, cefotaxime, streptomycin and sulfamethoxazole with trimethoprim.

Treatment trials (*in vivo*)

A-mortality rate: Table 5 shows the effects of Florfenicol (25 mg kg^{-1} fish b.wt.), enrofloxacin (50 mg kg^{-1} fish b.wt.) or oxytetracycline (50 mg kg^{-1} fish b.wt.) supplementation for 7 days on the mortality rate in examined fish. It was noticed that the highest rate of mortality was in infected non treated groups followed by groups treated with Oxytetracycline, enrofloxacin and florfenicol. The lowest mortality rate was detected in the non-infected non treated group.

Biochemical analysis after treatment trail: Table 6 shows the liver and kidney function tests of experimentally infected Nile tilapia fish with *V. alginolyticus*. It was found that AST, ALT, urea and creatinine levels of infected Nile tilapia fish were decreased significantly after antibiotics administration.

Table 2: *Vibrio* spp. isolated from sea bass fish

Vibrio spp.	Total samples	Positive	Percentage
V. alginolyticus	25	5	20
V. Harveyi		1	4
Total		6	24

Table 3: *Vibrio alginolyticus* re-isolated from Nile tilapia *(Oreochromis niloticus)* fish after the experimental infection

Vibrio spp.	Total samples	Positive	Percentage
V. alginolyticus	20	12	60

Table 4: Antimicrobial sensitivity test of *Vibrio alginolyticus* isolated from naturally infected sea bass fish

Antibiotic	Disc symbol and concentration (µg/disc)	Interpretation
Florfenicol	FFC (10)	HS
Erythromycin	E (15)	R
Ampicillin	AMP (10)	R
Amoxicillin	AML (10)	MR
Cefotaxime	CTX (30)	R
Oxytetracycline	Ox (30)	S
Streptomycin	S (10)	R
Enrofloxacin	Nor (5)	HS
Sulfamethoxazole+trimethoprim	SXT (25)	R

S: Sensitive (more than 50% and less than 75% of isolates were susceptible to the antimicrobial agents), MS: Moderately susceptible (50% of the isolates were susceptible to the antimicrobial agents), HS: Highly sensitive (75% or more of the isolates were susceptible to the antimicrobial agents), R: Resistant (more than 50% and less than 75% of the isolates were resistant to the antimicrobial agents), HR: Highly resistant (more than 75% of the isolates were resistant to the antimicrobial agent

Table 5: Experimental design and the mortality rate of experimentally infected Nile tilapia fish with *Vibrio alginolyticus*

Group numbers	Antibiotic doses/kg fish B.W (in fish ration)	Number of fish	Number of affected fish	Number of dead fish	Mortality (%)
Non-Infected, non-treated group (control negative)	--------	20	----	1	5
Infected, non treated group (control positive)	--------	20	18	16	80
Infected, florfenicol treated group	25 mg	20	12	6	30
Infected, enrofloxacin treated group	50 mg	20	14	8	40
Infected, oxytetracycline treated group	50 mg	20	13	10	50

Table 6: Effects of different antibacterial treatment on AST, ALT, urea and creatinine levels of *Vibrio alginolyticus* experimentally infected Nile tilapia fish

	Parameters			
Groups	AST (µ ml^{-1})	ALT (µ ml^{-1})	Urea (mg dl^{-1})	Creatinine (mg dl^{-1})
Non Infected non-treated group	141.00±11.3^{a}	34±2.45^{a}	11.37±1.5^{a}	0.24±0.01^{a}
Infected non-treated group	177.00±16.4^{b}	58±4.23^{c}	21.63±1.1^{c}	0.43±0.05^{d}
Florfenicol treated infected group	148.76±15.7^{a}	41±3.18^{a}	13.48±1.9^{a}	0.33±0.03^{b}
Enrofloxacin treated infected group	154.21±13.5^{a}	43±2.79^{a}	14.12±2.0^{a}	0.35±0.04^{b}
Oxytetracycline treated infected group	152.32±16.3^{a}	47±2.85^{ab}	17.41±1.4^{b}	0.36±0.02^{bc}

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, Means within the same column of different superscript digits are significantly different at ($p<0.05$)

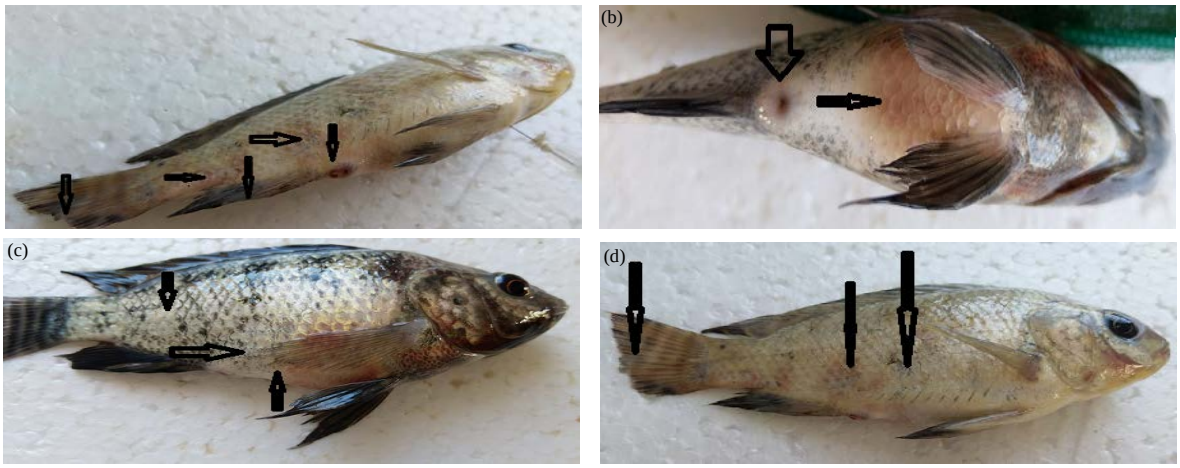

Fig. 1(a-d): Nile tilapia fish (*Oreochromis niloticus*) experimentally infected with *Vibrio alginolyticus* show (a) erosion of fins, skin hemorrhage and inflammation of anal opening (arrows), (b) severe hemorrhage, abdominal dropsy and inflammation of anal opening. (arrows), (c) abdominal dropsy, dark coloration and detached scales. (arrows) and (d) fins erosions and detached scales. (arrows)

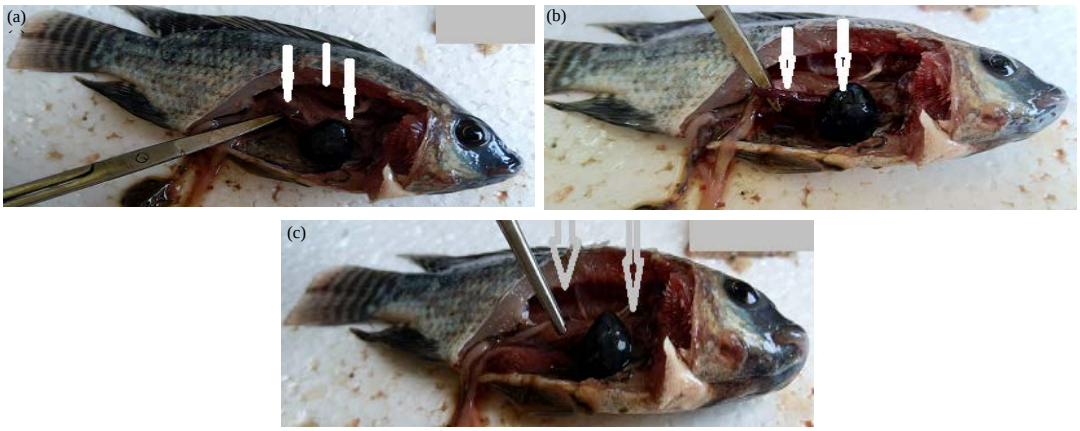

Fig. 2(a-c): Nile tilapia fish (*Oreochromis niloticus*) experimentally infected with *V. alginolyticus* shows, (a) Distended gall bladder, inflamed liver and enlarged spleen. (arrows), (b) Distended gall bladder, enlarged and inflamed spleen. (arrows) and (c) Inflamed kidney, congested gills and the abdominal cavity filled with bloody serious fluid (arrows)

Clinical signs: Figure 1(a-d) explains the clinical signs of *V. alginolyticus* infection in Nile tilapia fish (*Oreochromis niloticus*). It was observed that *V. alginolyticus* induced characteristic signs on skin, fine and abdomen, these signs include fins and tail erosion, skin hemorrhage, inflammation of anal opening, dark coloration, detached scales and abdominal dropsy.

Post mortem lesions: Figure 2(a-c) shows the post mortem lesions of Nile tilapia fish (*Oreochromis niloticus*) experimentally infected with *V. alginolyticus*. It was found that *V. alginolyticus* induced pathognomonic lesions as distended gall bladder, inflammation of liver and kidney, enlarged spleen, congested gills and the abdominal cavity was filled with bloody serious fluid.

Analysis of the PCR products: All isolated *V. alginolyticus* from naturally infected sea bass and experimentally infected Nile tilapia were positive for the presence of *blaTEM* and *trh* virulence genes as shown in Fig. 3. Lane 1-6 was positive, for *bla TEM* (516 pb) and the other Lane 1-6 was positive of *tdh* (373 pb).

Histopatholgical examination of infected fish: The histopathological findings of some internal organs of Nile tilapia (*Oreochromis niloticus*) experimentally infected with *V. alginolyticus* were illustrated in Fig. 4(a-g). It was observed

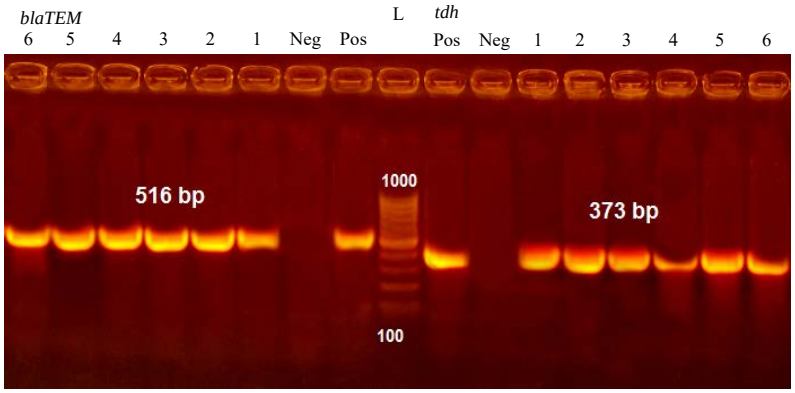

Fig. 3: PCR for *V. alginolyticus* isolated from both naturally infected sea bass and re-isolated from experimentally infected Nile tilapia, Agarose gel electrophoresis of PCR amplification of vibrio alginolyticus extracted DNA
Lane L: 100 bp DNA Ladder. Neg: Negative control, Pos: Positive control, Lane 1,2 and 3 were isolated from sea bass, Lane 4, 5 and 6 isolated from Nile Tilapia, Lane 1-6 (on the left): Positive, *bla TEM* (516), Lane 1-6 (on the right): Positive, *tdh* (373 pb)

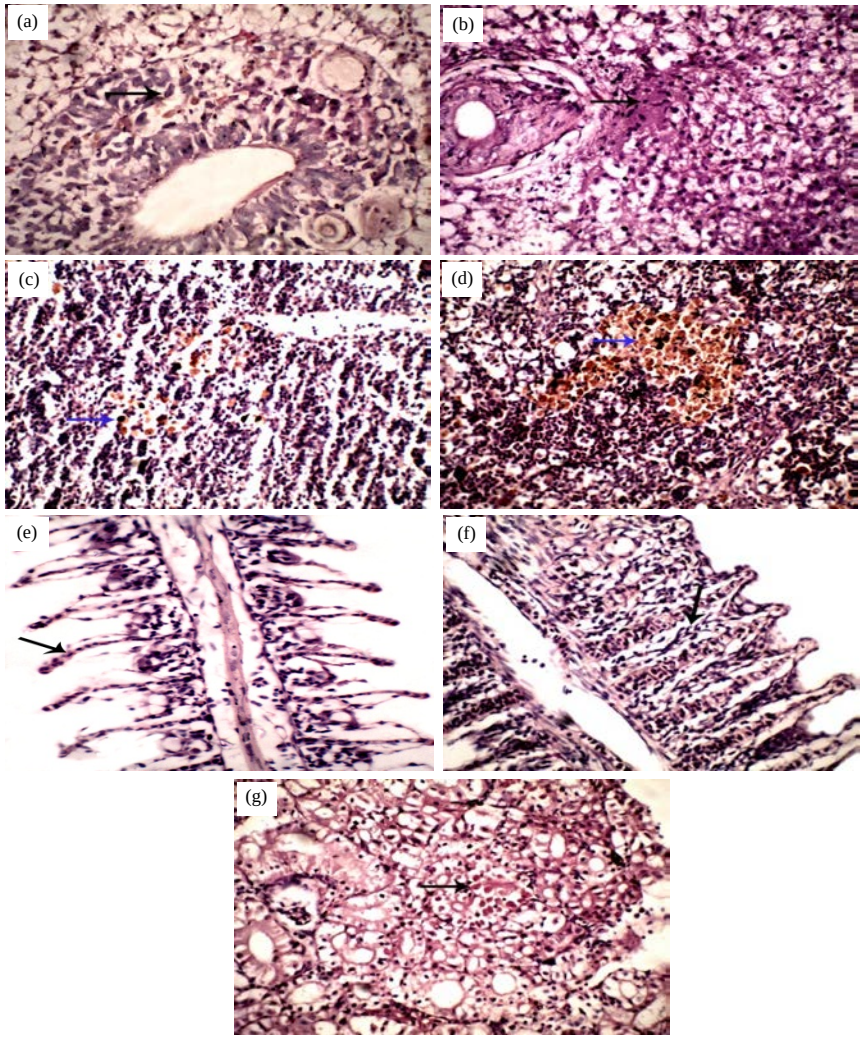

Fig. 4: Histopathological examination of (a-b) liver, (c-d) spleen, (e-f) gills and (g) kidney of healthy and *V. alginolyticus* experimentally infected Nile tilapia (*Oreochromis niloticus*) fish (HE, 400x)

that *V. alginolyticus* is highly pathogenic to Nile tilapia fish causing severe damage of liver, spleen, gills and kidney. Histopathological examination of infected fish revealed coagulative necrosis of hepatocyte, hyperplasia of melanomacrophages in the spleen, proliferation of the secondary lamellae of the gills and coagulative necrosis of the renal tubular epithelium.

Figure 4a-b shows normal hepatocytes and coagulative necrosis of hepatocytes, respectively. Figure 4c-d shows spleen histology as of normal and hyperplasia of melanomacrophages, respectively. Gills display normal secondary lamellae and normal lamellar blood vessels in Fig. 4e, while gills display proliferation of the secondary lamellae in Fig. 4f. Kidney displays coagulative necrosis of the renal tubular epithelium lining renal tubules (Fig. 4g).

DISCUSSION

Fish products became an important source of meat supply. Nile tilapia still ranked on the top of aquaculture fish in Egypt[37]. The aim of this work was studying the pathogenic effects of *Vibrio* spp. isolated from marine fish (sea bass) on Nile tilapia (*O. niloticus*). One hundred healthy Nile Tilapia (*O. niloticus*) fish (weighing 80±10 g) were used for experimental infection, these fish were divided into 5 groups, the first group was control negative while the other 4 groups were I/P injected with 0.5 ml of 10^7 CFU mL *Vibrio alginolyticus*. After 3-7 day of experimental infection, clinical examination of Nile tilapia fish (Fig. 1) showed hemorrhages all over the fish body, tail/fin rot, abdominal dropsy, hemorrhagic ulceration, detachment of scales and skin darkness. These outcomes are consistent with those previously described by Abdel-Aziz *et al.*[2] and Younes *et al.*[13]. Post mortem lesions appeared as congestion of gills, enlargement and congestion of liver with distended gall bladder, enlarged spleen and inflammation of the kidney and in some cases paleness of liver. These results are in agreement with that formerly obtained by Abdel-Aziz *et al.*[2], Younes *et al.*[13], Omaima[37], Rameshkumar *et al.*[38], Stephens *et al.*[39] and EL-Sayed *et al.*[40].

The data obtained from the present study revealed that the bacteria of *Vibrio* spp., isolated from marine fish can cause severe disease problems in fresh water fish, these results are following the results obtained by Younes *et al.*[13] and EL-Sayed *et al.*[40]. Polymerase chain reaction (PCR) as a recent reliable technique used to clarify certain DNA molecules even with low concentration[41]. It was performed using *tdh* and *bla* gens for confirmation of the pathogenicity of *V. alginolyticus* isolated either from marine fish or Nile tilapia fish, (Fig. 3). This test showed that all examined isolates have both genes. Those results provide that *V. alginolyticus* has pathological effects in both marine and fresh water fish, these results match with that recorded by EL-Sayed *et al.*[40].

Histopathological examination of affected tissues from experimentally infected *O. niloticus* revealed that the liver showed coagulative necrosis of hepatocyte, the spleen showed hyperplasia of melano-macrophages, proliferation and thickening of secondary lamellae of gills with congestion of blood vessels and coagulative necrosis of renal tubular epithelium lining with congested glomerular capillaries (Fig. 4), these observations were not completely matched with that recorded by Chen *et al.*[42], who recorded sever histopathological changes of infected *O. niloticus* with *Vibrio vulnificus*; including congested liver with erythrocytes that occupied all sinusoids, but no apparent damage could be observed in either hepatocyte or pancreatic tissues. Also, they noticed splenomegaly with congestion and infiltration of epithelioid cells with extensive necrosis. On the other hand, the histopathological changes obtained from this work are similar to those recorded by Diggles *et al.*[43] and Korun[44].

Agar disc diffusion test of *V. alginolyticus* isolated from sea bass fish show that it was sensitive to enrofloxacin and florfenicol, cefixime and oxytetracycline. On the other hand it resists sulfamethoxazole-trimethoprim, gentamicin, erythromycin, ampicillin, amoxicillin and streptomycin (Table 4). Similar results were reported by Younes *et al.*[13] and EL-Sayed *et al.*[40].

After 7 days from starting the experimental infection of apparently healthy Nile tilapia fish (*Oreochromis niloticus*) with *V. alginolyticus*; mortality rates were 5, 80, 30, 40, 50% in group1 (control negative), group 2 (control positive), group 3, group 4 and group 5, respectively (Table 5). The 5% mortality rate in the first group may be due to injection stress with 0.5 ml normal saline or other noninfectious causes. These results agree with that recorded by Al-Sunaiher *et al.*[8], Frans *et al.*[9] and Austin and Austin[20]. Among the treated groups, the best results in a reduction of mortality rates was recorded in group 3 which treated with florfenicol followed by enrofloxacin and oxytetracycline treated groups, this may be due to the high sensitivity of *vibrio alginolyticus* to florfenicol more than other used antimicrobials Younes *et al.*[13].

The biochemical examination of liver enzymes indicated the presence of a significant elevation in both AST and ALT in the infected non-treated group in comparison with other

groups (Table 6). This could be attributed to hepatic cell destruction induced by *Vibrio alginolyticus.* The hepatic cell destruction leads to the escape of enzymes to serum elevating their levels. Also, the significant increase in serum creatinine and urea in the infected non-treated group may be attributed to the kidney and gill damage induced by *V. alginolyticus*. This explanation coincides with the results described by Martins *et al.*[45]. The authors reported that *V. alginolyticus* induced severe damage to the liver and kidney of fish. The biochemical analysis of the infected antibiotics treated groups revealed improvement of liver and kidney functions. That pointed to the effect of these antibiotics on *V. alginolyticus* and this explained low mortality rate in these groups where the infected non-treated group had the highest rate of mortality as it reached 80% followed by group treated with oxytetracycline (50%), enrofloxacin (40%), florfenicol (20%) and non-infected non treated group (5%) (Table 6). Finally, *Vibrio* spp is highly pathogenic not only for marine but also for fresh water fish inducing a major economic loss. Strict measures should be taken to prevent infection of fish farms. However *V. alginolyticus* is highly sensitive to enrofloxacin and florfenicol (*in vivo* and *in vitro*) but it is not recommended to use many antibacterial agents as erythromycin, ampicillin, cefotaxime, streptomycin and sulfamethoxazole with trimethoprim for treatment of fish infected with *Vibrio* spp.

CONCLUSION

It could be concluded that although *Vibrio* spp. need the salted environment for life, it can induce pathogenic infection in fresh water fish as Nile tilapia. Moreover, *in vitro* and *in vivo* studies revealed that *Vibrio alginolyticus* was sensitive to enrofloxacin, florfenicol, cefixime and oxytetracycline.

SIGNIFICANCE STATEMENT

This study discovers the possibility of *Vibrio* bacteria to induce infection in fresh water fish although it needs salted environment for live that can be beneficial to take all necessary measures to avoid infection with *Vibrio* in tilapia fish. This study will help the researchers to uncover the critical area of antibiotic-resistant genes in *Vibrio* which induces the treatment failure that many researchers were not able to explore. Thus, a new theory on the selection of effective antibiotics in *Vibrio* infected fish based on the absence of these antibiotics resistant genes may be arrived at.

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