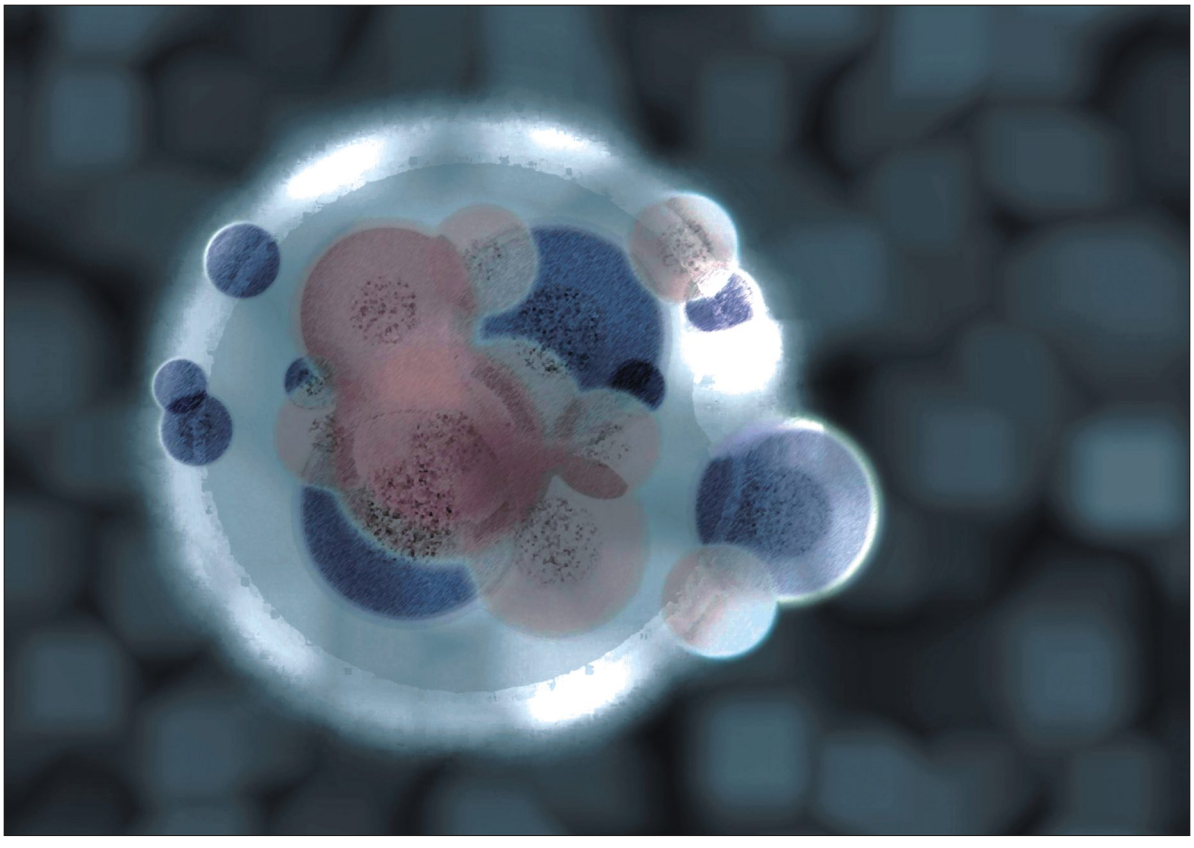

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Research Article
Rapid Detection and Control of Gram-negative Bacterial Pathogens Isolated from Summer Mortality Outbreak Affecting Tilapia Farms

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Abstract

Background and Objective: Summer mortality considered a well-established phenomenon affecting cultured tilapia farms allover Egypt in the past 5 years. The present study was undertaken for determining the pathogenic bacteria incorporated in this phenomenon affecting seven tilapia farms using a rapid diagnostic technique. **Materials and Methods:** Thirty-five clinically diseased Nile tilapia were sampled for determining the clinical, gross internal and pathological lesions followed by isolation and automatic biochemical identification for the recovered isolates using VITEK 2 system. Antibiotic susceptibility test was done for bacterial isolates together with conduction of a treatment trial using oxytetracycline and sulphadiazine-trimethoprim combination. **Results:** Twenty-eight *Enterobacter cloacae*, 15 *Vibrio cholera* and 7 *Plesiomonas shigelloides* isolate were identified using VITEK 2 system. Eighty-six, 52 and 10% of the recovered isolates were sensitive to oxytetracycline, sulphadiazine-trimethoprim and ampicillin, respectively. Various degenerative changes in posterior kidney, hepatopancreas and fundic region of the stomach were observed during the histopathological examination including congestion, hemorrhage, leucocytic infiltration and necrosis. Ammonia level was ranged between 0.33-0.72 mg L^{-1} in farms water. In feed administration of oxytetracycline and sulphadiazine-trimethoprim combination at a dose of 50 and 30 mg kg^{-1} body weight respectively for 10 consecutive days was successful treatment with improving farm water quality. **Conclusion:** *E. cloacae*, *V. cholera* and *P. shigelloides* were the causative agents of the outbreak affecting the studied farms, high ammonia level was act as a predisposing factor, antibiotic treatment with improving farm water parameters was effective in termination of this disease condition.

Key words: *Enterobacter cloacae*, histopathology, oxytetracycline, *Plesiomonas shigelloides*, sulphadiazine-trimethoprim, *Vibrio cholera*

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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

Aquaculture is a fast growing sector in Egypt, it supplies the Egyptian market with 1.175 million t of valuable and cheap source of protein in comparison with other animal protein sources. In last few years, despite the expansion in Nile tilapia production, this sector has suffered from a severe annual economic losses estimated by about one billion Egyptian pound as a direct cause of what is known as summer mortality. During this outbreak, affected fish shows the general signs of septicemia during the clinical and postmortem examination[1].

Gram-negative bacteria including family Vibrionaceae and the members of family enterobacteriaceae considered pathogenic and opportunistic to cultured fish species including tilapia[2,3].

Traditional methods used for biochemical identification of bacterial pathogens are time consuming and laborious, VITEK 2 is a highly accurate automated biochemical bacterial identification system, it provides a rapid identification of clinically important bacterial pathogens which saves a valuable time for fish health manger to give an accurate decision during the early stage of the outbreak when application of treatment is still a good choice in disease control.

Anti-microbials are a valuable tool used in controlling susceptible infectious diseases in farm animals as well as cultured fish[4-6]. Oxytetracycline is a bacteriostatic antibiotic related to tetracycline group, it acts by inhibition of bacterial protein synthesis through binding with 30 subunit of bacterial ribosome[7] it is widely used in aquaculture[8]. Sulfonamides are bacteriostatic anti-microbials, it act by inhibition of dihydropteroate synthase enzyme that incorporate the Para amino benzoic acid (PABA) in folic acid synthesis, sulfonamides turned to bactericidal when potentiated with trimethoprim which is a potent competitive inhibitor of microbial dihydrofolate reductase enzyme, they work together to prevent bacterial nucleic acid synthesis[9], potentiated sulfonamides are successfully used in American and European fish farms for controlling the susceptible bacterial pathogens.

Pond water quality is an essential element for fish culture, water parameters including ammonia, dissolved oxygen and pH can affect fish growth, health and determine the failure or success of the overall culture practices[10]. High organic matter levels and poor quality of the aquatic environment responsible for high mortality rates during outbreaks[11]. Exposure to sub- lethal concentration of un-ionized ammonia increase susceptibility to bacterial fish diseases[12].

The present study was done to determine the bacterial etiological agents responsible for this outbreak in tilapia farms using a rapid technique (VITEK 2 identification system) together with determination of their sensitivity to anti-bacterial agents, followed by conduction of a treatment trial to act as a guide line for dealing with futuristic disease condition.

MATERIALS AND METHODS

Study area: Samples were taken from seven diseased tilapia farms located at El-Kafr EL-Sharky, Alhamoul district, Kafrelsheikh governorate, Egypt. Studied farms located between longitude 31.287389-31.298390 N and latitude 31.070345-31.075491 E. Fish stoking density in studied farms was 4-5 fish per square meter.

Samples: Samples were collected during May and June, 2016, 35 clinically diseased mono-sex (*Oreochromis niloticus*) were collected as five fish were sampled from each farm. Sampled fish weight was 120±25 g, each moribund fish was placed in a separate plastic bag and transported in ice box to fish diseases lab, National Institute of Oceanography and fisheries, Alexandria branch.

Clinical and gross internal examination: The clinical examination was performed as mentioned by Noga[13] for observation of any external abnormality and the gross internal examination was performed as described by Heil[14] to determine any gross internal lesions.

Isolation of bacterial pathogen: It was done according to the method described by Aboyadak *et al.*[1] briefly, two samples were taken from each fish (35 sample from internal organs and 35 from external body lesions), samples each sample was inoculated to tryptic soy broth (Oxoid®) and incubated at 35°C for 12 h then cultured on tryptic soy agar (Oxoid®) with incubation at 35°C for 18-24 h.

Automatic biochemical identification for the recovered isolates using VITEK-2 system: A single colony from each morphologically similar colonies was picked up for gram stain procedures that was performed according to the method described by Collins *et al.*[15] to determine the type of cards used in the identification procedures.

Few morphologically identical colonies were collected with a platinum loop from the agar plate and were subjected to automated biochemical identification as described by Aboyadak *et al.*[4].

Histopathological examination: The histopathological examination was performed according to Robert[16] tissue specimens from hepatopancreas, posterior kidney and stomach wall was taken, fixed in 10% buffered formalin, dehydrated in ascending grade ethyl alcohol and cleared in xylene, sectioned to 4 µm thickness and mounted over a glass slide then stain with hematoxylin and Eosin (H and E). Stained tissue sections were examined & photographed using Optika microscope with digital camera (Optika, Italy).

Determinate of farm water parameters: Dissolved oxygen and pH were determined using portable dissolved oxygen meter for aquaculture and water proof portable pH meter (HANNA, Italy). Ammonia level was determined using portable ammonia photometer (HANNA, Italy) based on the methods described by APHA[17].

Anti-microbial sensitivity test: Agar disk diffusion test was done to determine the sensitivity of recovered bacterial isolates to sulphadiazine-trimethoprim (SXD 25 µg), oxytetracycline (OTC 30 µg) and ampicillin (AM 10 µg) according to the method described by CLSI[18].

Drugs used in the treatment trial: Depending on the sensitivity test results, the majority of recovered bacteria isolates were sensitive to oxytetracycline and sulphadiazine-trimethoprim based on this results both drugs were used in treatment trial at a dose 50 and 30 mg kg^{-1} b.wt., respectively. Oxytetracycline® (oxytetracycline 20%) and Co-Trimazine® (sulphadiazine 10% + trimethoprim 2%), ADWIA, Egypt were used in the treatment trial done in one of the affected fish farms.

Treatment protocol: A daily dose of 470 g from each drug was mixed with 56 kg fish ration using 1 kg fish oil as binder, medicated feed was prepared daily for 10 consecutive days, the given number was calculated based on the stocking density of 16.000 fish/farm (0.4 ha), 1875 kg biomass and 3% daily feeding rate.

Statistical analysis: One sample t-test was used for determine the values of farm water parameters expressed as mean±standard error using MedCalc for windows, version 17.2.2, (MedCalc Software, bvba, Ostend, Belgium, https://www.medcalc.org; 2017).

RESULTS

Results of clinical and gross internal examination: Presence of haemorrhagic skin ulcers of various sizes was the only observed clinical sign while, their were many prominent postmortem lesions as congested internal organs including stomach, intestine, hepatopancreas with enlarged gall bladder filled with thick greenish bile.

Results of automated biochemical identification of bacterial etiological agents: Fifty bacterial isolate were recovered from diseased fish, all of them were Gram-negative. Based on automatic biochemical identification using VITEK 2 system 28, 15 and 7 isolates were identified as *Enterobacter cloaca*, *Vibrio cholera* and *Plesiomonas shigelloides* (Table 1) with a probability estimated by 99, 98 and 99%, respectively. The biochemical characteristics of the recovered isolates was shown in Table 2.

Results of histopathological examination: The histopathological examination of diseased fish posterior kidney tissue revealed the presence of several pathological lesions as darkening and shrinkage of glomerular taught and increase the Bowman's space. Tubular necrosis and detached tubular epithelium with multifocal interstitial hemorrhages were also cleared (Fig. 1a-c).

Hepatopancreas of affected fish was also severely affected, hepatic cell death, vacuolar degeneration, pyknosis and karyorrhexis were clear at the cellular level, at the tissue level inflammation observed by leucocytic infiltration, necrosis and congestion of hepatic parenchyma together with thrombus formation in many blood vessels was also noticed (Fig. 1d-g).

The fundic region of the diseased fish stomach was inflamed manifested in presence of leucocytic infiltration between lamina propria and submucosa, destruction and necrosis of fundic gland was observed in some samples while destruction and detachment of columnar epithelial lining the mucous membrane was dominant in the majority of examined samples (Fig. 1h-j).

Table 1: Recovered bacterial isolates from diseased *Oreochromis niloticus*

Items	Number	Total isolates (%)
Total recovered isolates	50	100
Enterobacter cloacae	28	56
Vibrio cholera	15	30
Plesiomonas shigelloides	7	14

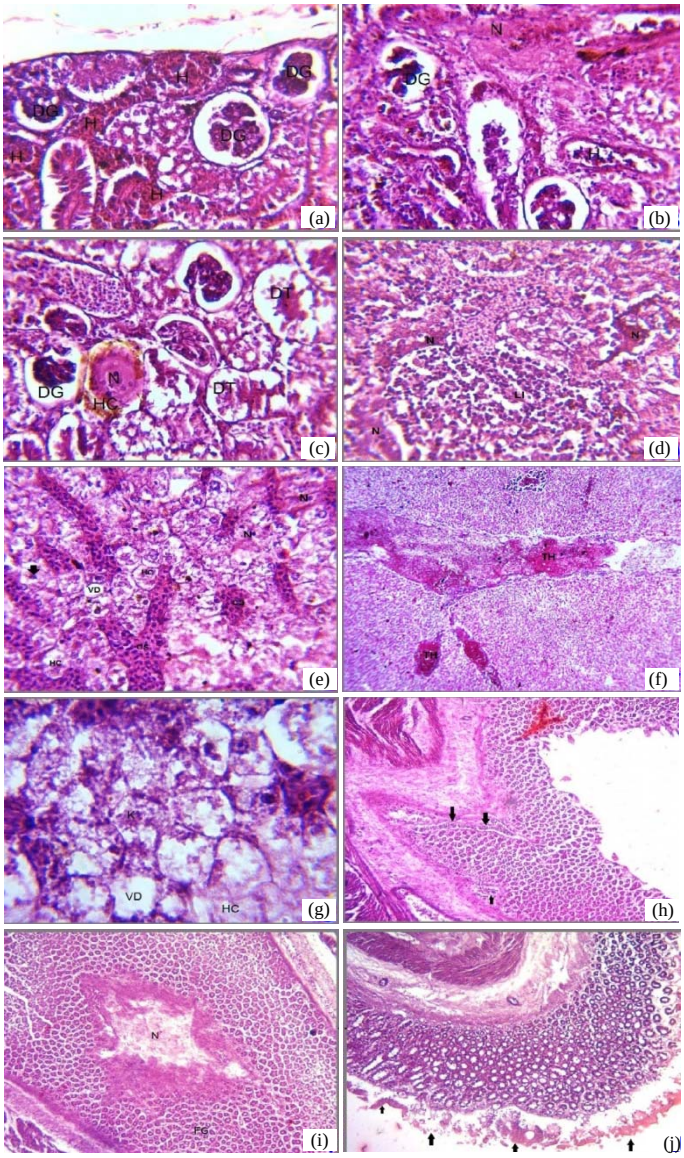

Fig. 1(a-j): Posterior kidney of naturally infected *Oreochromis niloticus* with obvious degenerative changes including, degenerated glomeruli (DG) manifested by darkening, shrinkage of glomerular taught and increasing Bowman's space, presence of necrotic areas (N), multi focal haemorrhage (H) and degenerated renal tubules with detached tubular epithelium and obliterated renal tubules (DT), hemosiderin deposition (HC). X = 400. d-g) hepatopancreas of naturally infected *Oreochromis niloticus* showing loss of tissue architecture with presence of necrotic foci (N) and leucocytic infiltration (LI), X = 400, (d). Severely congested blood sinusoids (CS), necrotic areas (N), vacuolar degeneration (VD), pyknotic nucleus (arrow), swollen hepatocytes with pale cytoplasm in different degrees of vacuolar degeneration (HC), X = 400, (e). Enlarged distended blood vessels with multiple thrombi (TH), X = 40, (f). Ballooning degeneration of hepatocytes, rarefied cytoplasm (HC), Karyorrhexis (K), vacuolar degeneration (VD) X = 1000, (g). h-j) Stomach (fundic region) of naturally infected *Oreochromis niloticus* showing leucocytic infiltration (arrows) between lamina propria and submucosa, X = 100, (H). Large necrotic area (N) with destruction of fundic glands (FG) X = 100, (i). Destruction and detachment of epithelial lining the folded mucosa covering the fundic gland (black arrows) X = 100, (j)

Table 2: Biochemical characters of *Enterobacter cloacae*, *Vibrio cholera* and *Plesiomonas shigelloides* isolated from *Oreochromis niloticus* and identified by VITEK 2 system

Biochemical reactions	Appreciation	*Enterobacter cloacae*	*Vibrio cholera*	*Plesiomonas shigelloides*
Ala-Phe-Pro-ARYLAMIDASE	APPA	-	-	-
ADONITOL	ADO	-	-	-
L- Pyrrolydonyl-ARYLAMIDASE	PyrA	-	-	-
L-ARABITOL	IARL	-	-	-
D-CELLOBIOSE	dCEL	+	-	-
β-GALACTOSIDASE	BGAL	+	+	+
H2S PRODUCTION	H25	-	-	-
β-N-ACETYL-GLUCOSAMINIDASE	BNAG	+	+	+
Glutamyl Arylamidase pNA	AGLTP	-	-	-
D-GLUCOSE	dGLU	+	+	+
GAMMA-GLUTAMYL-TRANSFE RASE	GGT	+	-	-
FERMENTATION/ GLUCOSE	OFF	+	+	+
β-GLUCOSIDASE	BGLU	(-)	-	-
D-MALTOSt	dMAL	+	+	+
D-MANNITOL	dMAN	+	+	-
D-MANNOSE	dMNE	+	+	-
β-XYLOSIDASE	BXYL	+	-	-
β-alanine arylamidase pNA	BAlap	-	-	-
L-proline ARYLAMIDASE	ProA	+	+	+
LIPASE	LIP	-	-	-
PALATINOSE	PLE	+	-	-
Tyrosine ARYLAMIDASE	TyrA	+	+	-
UREASE	URE	-	-	-
D-SORBITOL	dSOR	+	-	-
SACCHAROSE/SUCROSE	SAC	+	+	-
D-TAGATOSE	dTAG	-	-	-
D-TREHALOSE	dTRE	+	+	+
CITRATE (SODTUM)	CIT	+	-	-
MALONATE	MNT	+	-	-
5-KETO-D-GLUCONATE	5KG	-	-	-
L-LACTATE alkalinization	ILATK	+	+	-
ALPHA-GLUCOSIDASE	AGLU	-	-	-
SUCCINATE alkalinization	SUCT	+	+	+
β-N-ACETYL-GALACTOSAMINIDASE	NAGA	+	-	-
ALPHA-GALACTOSIDASE	AGAL	+	-	-
PHOSPHATASE	PHOS	+	-	(-)
Glycine ARYLAMIDASE	GlyA	+	-	-
ORNITHINE DECARBOXYLASE	ODC	+	+	+
LYSINE DECARBOXYLASE	LDC	-	+	+
L-HISTIDINE assimilation	IHISa	-	-	-
COURMARATE	CMT	-	+	+
β-GLUCURONIDASE	BGUR	-	-	-
O/129 RESISTANCE (comp.vibrio.)	o129R	+	-	-
Glu-Gly-Arg-ARYLAMIDASE	GGAA	-	-	-
L-MALATE assimilation	IMLTA	-	-	-
ELLMAN	ELLM	-	+	+
L-LACTATE assimilation	ILATA	-	-	-
Probability		99%	98%	99%

Results of anti-microbial sensitivity test for recovered isolates: Eighty-six, 52 and 10% of recovered isolates were sensitive to oxytetracycline, sulphadiazine-trimethoprim and ampicillin, respectively. Most of recovered *E. cloacae* and all *P. shigelloides* isolates were sensitive to oxytetracycline and sulphadiazine-trimethoprim but resist ampicillin on the other hand nearly all *V. cholera* isolates were sensitive to oxytetracycline but none of them were sensitive to sulphadiazine-trimethoprim and few isolates were sensitive to ampicillin (Table 3).

Results of the treatment trial: Daily mortality was ranged between 55-300 fish/day before treatment, that equals 0.35-1.87% of the stoked fish, then it had been decreased to 0.15% at the 7th day post treatment (Table 4) and ceased 1 day after the last dose.

Table 3: Antibiotic susceptibility of recovered bacterial isolates

	Antibiotic								
	Oxytetracycline			Sulphadiazine-trimethoprim			Ampicillin		
Bacterial strain	S	I	R	S	I	R	S	I	R
Enterobacter cloacae	23	0	5	19	7	2	0	0	28
Vibrio cholera	13	1	1	0	0	15	4	1	10
Plesiomonas shigelloides	7	0	0	7	0	0	1	0	6
Total	43	1	6	26	7	17	5	1	44
Percentage	86	2	12	52	14	34	10	2	88

S: Susceptible, I: Intermediate susceptible, R: Resistant

Table 4: Daily fish mortality before and after treatment

No. of stocked fish/pond	No. of daily dead fish/pond before treatment	Daily mortality (%)	No. of daily dead fish/pond at the 7th day after treatment	Daily mortality (%)
16000	55-300	0.35-1.88	24	0.15

Table 5: Dissolved oxygen, pH and ammonia level in affected farms water

Parameters	Range	Mean±SE
Dissolved oxygen (mg L^{-1})	4.85-6.80	5.45±0.32
pH	7.20-8.13	7.72±0.18
Ammonia (mg L^{-1})		
Before treatment	0.33-0.72	0.52±0.05
After treatment	0.07-0.11	0.08±0.01

Results of tested water parameters: Dissolved oxygen level and pH value of tested water samples were within the acceptable range for tilapia culture as oxygen level was 5.45±0.32 mg L^{-1} and pH was 7.72±0.18 and their range didn't change before and after treatment while the recorded ammonia level was over the permissible limit in all tested water samples as it was ranged between 0.33-0.72 mg L^{-1} (Table 5). During the treatment trial, partial daily change of 30% bond water helped in decreasing ammonia level to 0.08 mg L^{-1} that augmented the treatment trial.

DISCUSSION

Bacterial fish diseases are the major cause of a huge economic losses in aquaculture all over the world, Egypt suffered badly in the past 5 years from summer mortality in *Oreochromis niloticus* farms[1].

In the present study VITEK 2 system helped in the rapid detection of pathogenic bacteria affecting cultured tilapia. Twenty-eight *E. cloacae* isolate were identified, *E. cloacae* considered a potential pathogen infecting many cultured fish species including *Pangasianodon hypophthalmus*, *Mugil cephalus*, tilapia nilotica and red hybrid tilapia[19-22]. Several members of family vibrionaceae are fish pathogens, in the present study 15 *V. cholera* isolate was determined among the causative agents of current outbreak, recently few reports supporting this result and identified *V. cholera* from diseased tilapia[1,23,24]. The present study restricted seven isolates as *P. shigelloides*[25,26] also isolated it from clinically diseased *O. niloticus* and *Clarias gariepinus*, Ozturk and Altinok[27] reported several outbreaks in many fish species infected with this pathogen.

Hemorrhagic skin ulcers was the only clinical sign recorded, while the found gross postmortem lesions were congested internal organs, these observations were in accordance with that recorded by Austin and Austin[2], El-Barbary [28] and Hassan *et al.*[29] regarding bacterial infection of *O. niloticus*. In harmony with this research results[23,25,30] mentioned nearly similar clinical and postmortem signs in *O. niloticus* infected with *E. cloacae*, *V. cholera* and *P. shigelloides*, respectively.

Histopathological examination is an excellent tool in understanding the diseases pathogenesis as well as the development of clinical signs and postmortem lesions that induced by bacterial pathogens. Posterior kidney tissues of naturally infected fish showed degenerated glomeruli and renal tubules, presence of necrotic areas and multi focal haemorrhage similar finding were mentioned, demonstrating the destructive effect of different bacterial infections on fish renal system. Aly *et al.*[31] observed marked tubular degeneration in kidneys and glomerular atrophy with hematopoietic tissue depletion in *O. niloticus* experimentally infected with *E. cloacae*, El-Sharaby *et al.*[32] described congested glomerular capillaries, necrosis in renal tubular epithelium in kidney of *O. niloticus* naturally infected with *Vibrio* species. In agreement with current research results Liu *et al.*[26] observed kidney injury during the histological examination of *O. niloticus* challenged with *P. shigelloides* previously isolated from diseased cultured tilapia[33] and also found necrotic lesions in the kidney of naturally infected farmed *O. niloticus*.

Hepatopancreas of affected fish showed loss of tissue architecture with presence of necrotic foci and congestion, hepatocytes showed vacuolar degeneration, nuclear pyknosis and karyorrhexis that indicating the severe affection of hepatic tissue at the cellular level, liver is the most important organ in metabolism and detoxification mechanism, liver dysfunction is predisposing to increase the susceptibility to environmental pollutant and bacterial toxins that makes fish more suffering under bacterial infection. In accordance with our findings, Aly *et al.*[31] recorded presence of coagulative necrosis in hepatocytes and vacuolar degeneration with focal necrosis associated with *E. cloacae* infection. El-Sharaby *et al.*[32] elucidated congested hepatic sinusoids, mononuclear cell infiltrations, associated with Vibrio species infected. In harmony with current research results[26] as they observed liver injury during the histological examination of diseased cultured tilapia infected with *P. shigelloides*.

Destruction and detachment of epithelial lining the fundic mucosa together with destruction of fundic glands and mononuclear cell infiltration, indicating the destructive effect of the bacterial infection on fish digestive system, it may also pointed to the probability of involvement in this disease pathogenesis as gastric mucosa may act as a porter of entry of pathogenic bacteria.

The recorded clinical signs, gross internal lesions and histopathological alterations were occur as a sequel of the inflammatory reaction which started with leucocytic infiltration after that congestion and edema followed by necrosis, this inflammatory reaction was initiated by bacterial invasion together with production of their virulence factors including enzymes, toxins, adhesins and anti-phagocytic factors.

E. cloacae possess many virulence factors contributing to its pathogenicity, Albesa *et al.*[34] identified leukotoxic toxin and hemolytic toxin from *E. cloacae*, they described their roles in induction of oxaditive stress. Michalska and Gospodarek[35] reported that *E. cloacae* has many virulence factors including lipopolysaccharide endotoxin, type1 fimbriae, capsula and they also recorded the ability of *E. cloacae* to produce enterobactin, yersiniabactin, aerobactin and bacteriocins. Enterobacter species has adhesin that responsible for adhesions to cells and tissues[36].

Austin and Austin[2] mentioned that *V. cholerae* among highly virulent bacterial fish pathogens, the extracellular enzymes of *V. cholera* including proteases and purified vibrio proteases are toxic to fish[37]. Laviad-Shitrit *et al.*[38] recorded the presence of zonula occludens toxin, hemolysin haemagglutinin/protease and outer membrane protein producing gens from *V. cholera* isolates recovered from tilapia. The *V. cholera* has specific surface receptors used for binding to connective tissues as collagen, fibrinogen and fibronectin[39].

The *P. shigelloides* is normal inhabitant micro-organism in the aquatic environment[40], many virulence factors have been incorporated in pathogenicity including enterotoxin[41], cytotoxin lipopolysaccharides[42] and cholera-like toxins[43], Gonzalez [44] enumerated the virulence factors associated with *P. shigelloides*, of them adhesion, invasiveness, enterotoxin, cytolysins, haemolysin and elastin.

Sensitivity test cleared that most *E. cloacae* strains were sensitive to oxytetracycline and sulphadiazine-trimethoprim which is coincided with observation of previous study[45,46], who recorded the sensitivity of *E. cloacae* isolated from several fish species to tetracyclines and sulfa-trimethoprim and resistance to ampicillin, Davin-Regli and Pages[47] mentioned that *E. cloacae* is naturally resistant to ampicillin and Conceicao *et al.*[48] related the natural resistance of *E. cloacae* to ampicillin to β-lactamase production and proved it through identification of new chromosomal AmpC β-lactamase, these observations were in harmony with current research results.

All the recovered *P. shigelloides* strains were sensitive to both oxytetracycline and sulphadiazine-trimethoprim and resistant to ampicillin which come in harmony with the findings described by Matsuyama *et al.*[49] that indicated its sensitivity to tetracycline and trimethoprim and resistance to ampicillin, Stock and Wiedemann[50] also recorded susceptibility of *P. shigelloides* from aquatic environment to tetracycline, trimethoprim-sulfamethoxazole while it resist amoxicillin.

On the other hand, nearly all *V. cholera* isolates were sensitive to oxytetracycline but they were resistant to sulphadiazine-trimethoprim and ampicillin, in accordance with the pervious stusy[51], who recorded the sensitivity of *V. cholera* isolated from tilapia to tetracycline and resistance to ampicillin, sensitivity to tetracycline and resistance to sulfa-trimethoprime[52].

Oxytetracycline and sulphadiazine-trimethoprim combination decreased the mortality rate that indicated its successfulness in termination of bacterial infection in the affected farm which can also reflect the synergistic effect of trimethoprim to both oxytetracycline and sulphadiazine. Bondad-Reantaso *et al.*[5] recorded that oxytetracycline is the most frequently used antibiotic in aquaculture which reflects its value as a treatment. Potentiated sulphonamides are among the commonly used antibacterials in fish farming[53-55].

Ammonia level in the bond water of infected farms was ranged between 0.33-0.72 with a mean value 0.52 ± 0.05 mg L^{-1} which is much higher than acceptable normal value (0.06 mg L^{-1}). Un-ionised ammonia (NH3) is toxic

to fish[10], increased ammonia over the acceptable limit act as a predisposing factor for outbreaks, ammonia act as stress factor lead to impairment of disease response[56]. High ammonia level decreased the fish immunity and subsequently increase disease susceptibility[12]. In current study increased ammonia level may be due to decreased bond water change. Partial daily change (about 30%) of bond water help in decreasing ammonia level to 0.08 mg L^{-1} which is much acceptable than previous value. Current work finding suggested that increased ammonia level is play an important role in occurrence and progression of this disease as high ammonia level decrease the fish immunity turned it more susceptible to bacterial infection. Dissolved oxygen level was ranged between (4.85-6.8) with mean value (5.45±0.32) mg L^{-1} which considered acceptable for tilapia culture (4-5) mg L^{-1}. The pH value was ranged between (7.02-8.13), the mean pH value was (7.72±0.18) which considered in the optimum rage for tilapia culture as described by El-Sayed[10].

CONCLUSION

Advanced diagnostic techniques as VITEK 2 system was valuable for rapid identification of *Enterobacter cloacae*, *Vibrio cholera* and *Plesiomonas shigelloides* responsible for occurrence of the outbreak hits tilapia farms at Kafrelsheikh. These bacteria considered opportunistic pathogens responsible for inducing serious diseases specially under stressful condition as high ammonia level. Medicated feed contains oxytetracycline and sulphadiazine-trimethoprim combination at a dose of 50 and 30 mg kg^{-1} body weight, respectively for ten consecutive days was effective in termination of this disease condition together with improving farm water quality.

SIGNIFICANCE STATEMENT

This article identified twenty-eight *Enterobacter cloacae*, 15 *Vibrio cholera* and 7 *Plesiomonas shigelloides* isolate as the actual causes of the outbreak affecting tilapia farms and considered the high ammonia level as a predisposing cause, it also proves the efficacy of oral antibiotic administration using oxytetracycline and sulphadiazine-trimethoprim combination at a dose of 50 and 30 mg kg^{-1} b.wt., respectively for ten consecutive days in termination of infection delete together with improving farm water quality.

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