E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(3): 197-202

www.fisheriesjournal.com
Received: 25-03-2019
Accepted: 27-04-2019

Mai AM El-Son
Internal Medicine, Infectious and Fish Diseases Department, Faculty of Veterinary Medicine, Mansoura University, Egypt

Nevien KM Abdelkhalek
Internal Medicine, Infectious and Fish Diseases Department, Faculty of Veterinary Medicine, Mansoura University, Egypt

Ahmed MM El-Ashram
Fish Diseases Department, Faculty of Fisheries, Suez Canal University, Egypt

Viola H Zaki
Internal Medicine, Infectious and Fish Diseases Department, Faculty of Veterinary Medicine, Mansoura University, Egypt

Correspondence
Mai AM El-Son
Internal Medicine, Infectious and Fish Diseases Department, Faculty of Veterinary Medicine, Mansoura University, Egypt

Phenotypic and biochemical detection of *Aeromonas hydrophila* isolated from cultured *Oreochromis niloticus* during disease outbreaks

Mai AM El-Son, Nevien KM Abdelkhalek, Ahmed MM El-Ashram and Viola H Zaki

Abstract
This study was focused on detection of the main causes of mass mortalities in Al-manzala fish farms in Dakahlia governorate during disease outbreaks in the summer season of 2018. Four hundred *Oreochromis niloticus* (Nile tilapia) were collected randomly from semi-intensive earthen ponds. The clinical signs and postmortem lesions were recorded. Phenotypic characterization, biochemical detection and sensitivity testing of the obtained strains to various antibiotics were applied. The results revealed that 175 *Aeromonas* species were isolated from the examined fish, whereas *A. hydrophila* was the most prevalent strain (57.1 %). *A. hydrophila* was isolated and identified by the API 20 E system. The experimental infection using the isolated *A. hydrophila* to detect the virulence of the strain recorded 60 % of mortality rate. Regarding the antibiotic sensitivity test, *A. hydrophila* isolate was sensitive to Aztreonam, and resistant to Amoxicillin, Ampicillin, Streptomycin, Cefotaxime, and Erythromycin. It can be concluded that Aztreonam can control *A. hydrophila* infection in tilapia farms.

Keywords: A. hydrophila, phenotypic and biochemical detection, antibiotic resistance

1. Introduction
Tilapia is a fast growing, well-adapted fish species that has been commercially farmed all over 100 countries in the tropical and subtropical regions [1]. Like other aquaculture fish species, under stress conditions of intensive culture, tilapia is also susceptible to different bacterial infections such as hemorrhagic septicemia caused by Aeromonas spp. [2]. *Aeromonas* species are facultatively anaerobic, motile gram-negative bacteria [3] that normal inhabitant in aquatic environments and in gastrointestinal tracts of healthy fish [4] which become pathogenic under different stressors. *A. hydrophila*, *A. caviae*, *A. sobria* were previously known as a stress-related fish pathogen causing Motile Aeromonas septicemia (MAS) either acute or chronic with vulnerable dermal ulcer bacteria hemorrhagic septicemia, tail and fin rot, bacterial gill rot and dropsy [5, 6].
A. hydrophila is a predominant bacteria in warm water aquaculture in Egypt causing high mortalities even without symptoms in per-acute phase due to disease known as Motile Aeromonas Septicemia (MAS) but in acute phase, the most recorded lesions were skin and fin ulcers appear on the external surface of fish with ascites in the abdomen and exophthalmia with significant economic losses in aquaculture industry due to low survival rate of the cured fish [7, 8]. The disease caused by *Aeromonas* spp infection is a result of a complex interaction between host, environment, and bacterium. There are numerous virulence factors share between members of A. hydrophila responsible for the recorded symptoms during the disease outbreak [9, 10], such as Aerolysin (aerA), Haemolysin (hly), Cytotoxic enterotoxin (act), Cytotoxic enterotoxins- heat-stable (ast), enterotoxins- heat-labile (alt) that are related to its pathogenicity [11, 12].
Extraordinary antibiotic resistance is recorded in bacterial infections caused by members of the genus Aeromonas [13]. Worldwide reports of the members of Aeromonas spp. suggests that Aeromonads are readily developing single or multiple antimicrobial resistance phenotypes [14]. Aeromonas spp are known to be resistant to β-lactams, tetracyclines, quinolones, second and third-generation cephalosporins [15-18]. The present study aimed to identify the causative agents responsible for high tilapia mortalities in Al-manzala farms with reference to test the most effective antibiotic that can be used against the obtained strains.

Materials and Methods

1. Fish samples

Four hundred (200 fish/farm) live and freshly dead *O. niloticus* were collected from two private farms suffered from mass mortalities in Al-manzala in Dakahlia governorate. Fish weight ranged between 150-250 g that was packed alive in a labeled plastic bag and freshly dead fish in ice box then transported to Fish Diseases and Management laboratory, Faculty of Veterinary Medicine, Mansoura University, Egypt. The clinical and post-mortem examination was performed [19].

2. Bacteriological examination

For bacteriological examination of the collected samples, the fish abdominal cavity was opened under the complete sterile condition and loopful from liver, spleen, kidney, and skin ulcers were obtained and inoculated on tryptic soya broth (TSB) (Oxoid), incubated at 25 °C for 18-24 hrs, then streaked on different laboratory media, as nutrient agar, tryptic soya agar (TSA), Rimler-Shotts media (R-S media) (Oxoid). The inoculated plates were incubated at 25 °C for 18-24 hrs [20-22].

3. Identification of the obtained bacterial isolates

The morphological characteristics of the bacterial isolates were examined under a microscope after by stained with Gram stain [23]. Detection of motility on semisolid media was done [21]. Other biochemical criteria and final identification of each isolate were achieved by using an analytical profile index system API 20 E system (Bio Mérieux) according to manufacture guide [21]. Regarding the esculin hydrolysis test, bile esculin agar medium (Difco™) was used to identify the *Aeromonas hydrophila*. This was done by inoculation of obtained isolates on sterile bile esculin agar slants then the reacion color was recorded after incubation at 24 °C for 24 hrs.

4. Histopathological examination

To detect the pathological changes in the infected tissue during bacterial infection, tissue specimens from gills, liver, spleen, and kidney of naturally infected fish were collected and fixed in 10% neutral buffered formalin for 24 hrs. 5 µm thick sections were cut and stained with hematoxylin and eosin (H and E) and then examined by light microscopy [24].

5. Experimental infection for studying the pathogenicity of *A. hydrophila* to *O. niloticus*

Another sixty apparently healthy *O. niloticus* with an average body weight of 100 ± 10 g were obtained from Kafr Elsheikh fish farm and transported alive to the laboratory of Fish Diseases and Management, Faculty of Veterinary Medicine, Mansoura University for experimental challenge [25]. Fish were kept for two weeks for adaptation after divided into two groups (30 fish/group), three replicates in six glass aquaria (80 X 40 X 30 Cm) each. The first group was injected I/P with 1.7x 10^6 CFU/mL with an *A. hydrophila* strain (18 hrs culture), and the second group was I/P injected by sterile normal saline as a sham control. During the experiment, fish were adapted on the feeding of fish basal diet twice daily at 3% of their body weight. Water was changed every 5 days to maintain good water quality. The aquaria were supplied with enough dechlorinated tap water; aeration was carried by the electric aerator. The temperature and pH were adjusted at 25 ± 2 ˚C and 7.4 respectively.

6. Antibiogram testing

The antibiotic sensitivity test was carried out using the disc diffusion method on Muller-Hinton Agar (Oxoid) and the results were interpreted in accordance with the Clinical and Laboratory Standards Institute guidelines [26]. Six antibiotic discs namely Amoxicillin (AX, 25 ug), Ampicillin (AM, 10 ug), Streptomycin (S, 10 ug), Aztreonam (ATM30 ug), Cefotaxime (CTX30) and Erythromycin (E, 15ug) (Oxoid). After 24 hrs incubation, the zone of inhibition was measured and compared according to the manufacturer's instruction.

Results

1. Clinical and post-mortem findings

Fish from both farms showed off food, sluggish movement, dullness, and swimming near the water surface with the absence of different reflexes. Postmortem examination of diseased fish exhibited superficial and deep ulcers (Plate; 1B), hemorrhagic eye (Plate; 1D) and hemorrhages spreading ventrally and on pectoral fins (Plate; 1F). The post-mortem examination of diseased fish exposed septicemia represented by kidney congestion (Plate;1A), hemorrhagic spots in liver (Plate; 1C), enlarged spleen and gathering of bloody exudates in abdominal cavity (Plate; 1C) and intestine is devoid of food and filled with bloody mucus-like material (Plate; 1D).

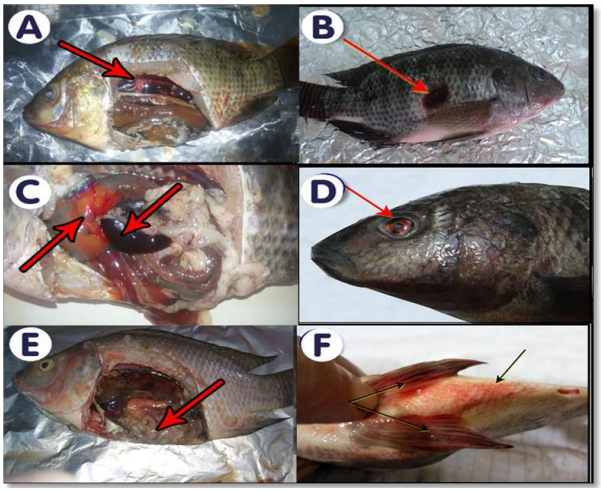

Plate 1: Naturally infected tilapia show (A) kidney congestion (B) deep external ulcer C) enlarged spleen and hemorrhagic batches on liver D) hemorrhagic eye E) intestine is devoid of food and filled with bloody mucus-like material f) sever hemorrhage on ventral aspect and on pectoral fins

2. Phenotyping and biochemical identification of *A. hydrophila*: -

One hundred *A. hydrophila* isolates characterized by gram-negative short rods, Oxidase positive, resistant to Vibrio-static reagent (0/129) (150 µg ml-1), positive esculin hydrolysis test (Dark brown compound esculetin) and small, circular, translucent, pinpoint colonies on TSA and large flattened yellow colonies on RS media. The bacteria could produce acid from arabinose. Consequently, the isolates showed growth at 37 °C with the optimum at 24 °C but no growth found at 4°C and 40 °C. Additionally, they could grow in 0-1% NaCl, however, no growth was noted in 2-4% NaCl media.

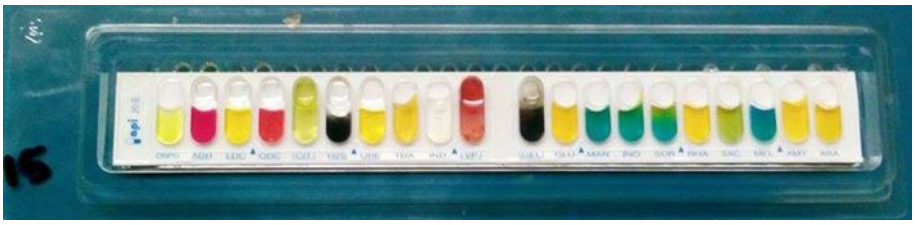

Fig 1: Representative sample of API 20E profile system for A. hydrophila

Table 1: Biochemical reactions of the identified *A. hydrophila* isolates by API 20E kits

Item	Isolated *A. hydrophila*
Sorbitol fermentation "SOR"	-ve
Rhamnose fermentation RHA	+ve
Sucrose fermentation SAC	-ve
Melibiose fermentation MEL	-ve
Amygdalin fermentation AMY	+ve
Arabinose fermentation ARA	+ve
Cytochrome oxidase OX	+ve
o-Nitro phenyl Galactoside ONPG	+ve
Arginine dihydrolase ADH	+ve
Lysine decarboxylase LDC	-ve
Ornithine decarboxylase ODC	+ve
Citrate utilization CIT	-ve
H_2S production H_2S	+ve
Urease production URE	-ve
Tryptophane deaminase TDA	-ve
Indole Production IND	-ve
Voges-Proskauer Test "VP"	+ve
Gelatine hydrolysis "GEL"	+ve
Glucose fermentation "GLU"	+ve
Mannitol fermentation "MAn"	-ve
Inositol fermentation "INO"	-ve

3. Histopathological results of naturally infected *O. niloticus*

The histopathological lesions were noticed mainly in kidney, liver, and spleen. Spleen showed hyperplasia of melanomacrophage center (Plate; 2A). Regarding the liver, there was vacuolation of hepatocytes and lymphocytic infiltrates into the hepatic tissue (Plate; 2B). Proliferative glomerulonephritis, degeneration of the renal tubules and lymphocytic infiltration in interstitial tissue of kidney (Plate; 2C).

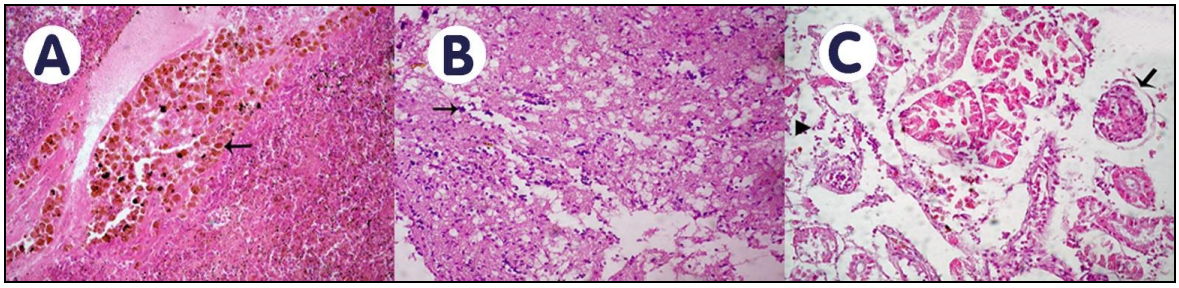

Plate 2: A: Spleen of naturally infected *O. niloticus* show hyperplasia of melanomacrophage center (arrow). (HE, 400x)**, B** Liver of naturally infected *O. niloticus* showing vacuolation of hepatocytes and lymphocytic infiltrates (arrow) into the hepatic tissue. (HE, 400x), **C** Kidney of naturally infected *O. niloticus* showing proliferative glomerulonephritis (arrow), degeneration of the renal tubules and lymphocytic infiltration in interstitial tissue (arrowhead). (HE, 400x)

4. Results of challenge test

The obtained isolate of *A. hydrophila* was highly pathogenic to the examined fish caused mortality of 60% (Table, 2) after I/P injection with (1.7 x 10^6 CFU/ml) with no mortality in control groups. The clinical signs and postmortem changes were like those described during the natural infection. 18 hours following the infection, all fish were observed to be motionless. During the experiment there were many signs

appear on the fish which increase in their severity gradually as off food, darkening in skin and mild hyperemia of the fin bases, focal hemorrhagic batches of the skin over the pectoral fins and unstable swimming on the bottom of the aquarium, in addition to macroscopic lesions in internal organs as congestion in gas bladder, anterior and posterior kidneys, liver, spleen, gall bladder, heart, brain and intestine. *A. hydrophila* was re-isolated from the internal organs of moribund and freshly dead fish. Phenotypically and biochemical confirmations for re-isolated bacteria were performed.

Table 2: The mortality rate of *O. niloticus* challenged with *A. hydrophila*

Fish group	No of fish	1st	2nd	3rd	4th	5th	6th	7th	Mortality rate %	Survivability %
Aeromonas injected group	30	0	5	8	0	3	2	0	60%	40%
Control group	30	0	0	0	0	0	0	0	0%	100%

5. Susceptibility of *A. hydrophila* isolates to different antibiotics

A. hydrophilia isolates were completely resistant to Ampicillin (AM, 10 µg), Amoxicillin (AX, 25 µg), Streptomycin (S, 10 µg), Cefotaxime (CTX30µg), Erythromycin (E, 15 µg) and sensitive to Aztreonam (ATM30 µg).

Discussion

In Egypt, *O. niloticus* is one of the most cultured freshwater fish and an important species in the commercial fisheries [27, 28] Biochemical reactions are very important for biotyping or speciating the Aeromonas isolates. Previous authors have successfully used conventional biochemical tests to separate at least the major species and able to correctly identify 97% of their isolates. The identification and characterization of isolates require many test media. To reduce the work burden, various rapid test systems have commercially been developed to aid microbiologists in identifying their isolates. The API system is one of these and has a database of biochemical reactions for Aeromonas that can be called upon for identifying the isolates [29]. Fish diseases caused by Aeromonas species is considered one of the main bacterial problems facing aquaculture growth and lead to mass mortalities and reduce production rate [30]. The present study revealed that the prevalence of motile Aeromonas septicemia was 57.1 % in the collected naturally infected samples which is relatively similar to [31] who reported that the prevalence of the *A. hydrophila* was 47.3% among the diseased cultured tilapias. In the present work, we spotlight on detection the main causes of mass mortalities in Al-manzala fish farms in Dakahlia governorate during disease outbreaks in the summer season of 2018 by focusing on the clinical picture, PM lesions of *A. hydrophila* as the most predominant bacterial pathogen affecting the examined farms and API20E.

Concerning the clinical signs and postmortem lesions of the diseased fish in the present study; the clinical picture and gross lesions include dermal ulceration, hemorrhagic septicemia, red sore disease, exophthalmia, reddening of the skin, ascites, abraded gills, fins, and tail. Internally, petechial hemorrhages sometimes occur throughout the peritoneum and musculature. The lower intestine and vent were protruded from the body, which is often swollen and inflamed. Additionally, the intestine is empty and filled with a yellow mucus-like material. These results the same reported by [2, 32, 33]**.**The main histopathological lesions that recorded in such fish present in kidney, liver, and spleen in which there were showed hyperplasia of the melanomacrophage center of the spleen, vacuolation of hepatocytes and lymphocytic infiltrates into the hepatic tissue and proliferative glomerulonephritis, degeneration of the renal tubules and lymphocytic infiltration in interstitial tissue [34, 35].

Identification of *A. hydrophila* depends on morphological characters of obtained colonies which characterized by gram-negative, short bacilli, motile and yellow convex on R-S media with diameter ranged from 2-3 mm [36]**.** It was found that the API 20E system could be used to identify the *Aeromonas* strains under certain circumstances [6, 37]. In the current study, API 20E kits were used to identify *A. hydrophila* along with several conventional analyses. According to the results from the API 20E system, it has been shown the evidence of Aeromonad group (Table 1). In addition, the gram-negative, motile, oxidative, acid forming (from arabinose), fermentative, and O/129 resistance potentiality of the causal organism also conclusively proved the Aeromonad bacterial infection. Simultaneously, a positive result from the esculin hydrolysis test was indicated the *A. hydrophila* infections that agreed with the finding of an earlier study [38].

Antimicrobial resistance is mostly attributed to the misapplication of antimicrobial drugs as treatments, prophylactics and as growth promoters [39, 40]**.** Usually, *A. hydrophila* is considered sensitive to antimicrobials, but during the last few years, the antimicrobial resistance has developed in many bacterial genera due to the unwarranted use of antimicrobials in agriculture and aquaculture structures [41, 42]**.** In our current study isolated *A. hydrophila* also showed variable resistance to the Ampicillin (AM, 10 µg), Amoxicillin (AX, 25 µg), Streptomycin (S, 10 µg), Cefotaxime (CTX30 µg), Erythromycin (E, 15 µg) and sensitive to Aztreonam (ATM30 µg) similar to results obtained by [15, 43].

Regarding to experimental infection results, the examined isolate was highly pathogenic to fish in a dose of (1.7 x 10^6cfu/ml) and give the typical clinical signs and postmortem lesions related to *A. hydrophila* infection according to [44, 45] who confirmed the pathogenicity of the isolated species by hemorrhage all over the body surface and ulcers with muscular necrosis in addition to macroscopic lesions in internal organs as congestion in gas bladder, anterior and posterior kidneys, liver, spleen, gall bladder, heart, brain and intestine and the mortality in the experimental infection was 70% which related to expression of virulence factors as proteolytic enzymes, haemolysin, enterotoxins, cytotoxin, dermonecrotic factors, gelatinase, and elastase in addition to toxic metabolites [7, 36], which consider good point for research to identify the different virulence factors of *A. hydrophila* that cause high mortality rates in fish farms.

Conclusions

A. hydrophila is the predominant pathogenic bacterium in examined tilapia fish farms, which cause mass mortalities in these farms during the summer season of 2018. The initial evidence of the isolation, identification and biochemical

characterization of *A. hydrophila* by using API20E were demonstrated. The result shows that obtained strain is virulent and resistant to the most used antibiotics except Aztreonam (ATM30 µg). However; further studies are needed to demonstrate the molecular and serological characterization of the organism to help in accurate identification of the bacteria. In addition, identification of virulence factors and antibiotics resistance genes of *A. hydrophila* from *O. niloticus* needs to be studied to help in control strategies for such economically important bacteria.

References

1. El-Sayed A-FM. Tilapia culture. CABI. 2006.
2. Eissa I *et al*. Aeromonas veronii biovar sobria a Causative Agent of Mass Mortalities in Cultured Nile Tilapia in El-Sharkia governorate Egypt. 2015; 12:90-97.
3. Hazen T *et al*. Prevalence and distribution of *Aeromonas hydrophila* in the United States. 1978. 36(5):731-738.
4. Laith A, Najiah M. *Aeromonas hydrophila*: antimicrobial susceptibility and histopathology of isolates from diseased catfish, Clarias gariepinus (Burchell). Journal of Aquaculture Research and Development. 2013; 5:215.
5. Cai S-H *et al*. Characterization of pathogenic Aeromonas veronii bv. veronii associated with ulcerative syndrome from Chinese longsnout catfish (*Leiocassis* longirostris Günther). 2012; 43(1):382-388.
6. Paniagua C *et al*., Pathogenicity factors and virulence for rainbow trout (Salmo gairdneri) of motile Aeromonas spp. isolated from a river. Journal of Clinical Microbiology. 1990; 28(2):350-355.
7. Pachanawan A, Phumkhachorn P, Rattanachaikunsopon P. Potential of Psidium guajava supplemented fish diets in controlling *Aeromonas hydrophila* infection in tilapia (*Oreochromis niloticus*). Journal of bioscience and bioengineering. 2008; 106(5):419-424.
8. Wassif I. Biochemical and Molecular Characterization of Aeromonas Species Isolated from Fish. 2018; 57:38.
9. Subashkumar R *et al*. RAPD and ERIC–PCR typing of virulent *Aeromonas hydrophila* isolated from children with acute diarrhea, 2014, 12.
10. Rasmussen-Ivey CR *et al*. Virulence factors of *Aeromonas hydrophila*: in the wake of reclassification. 2016; 7:1337.
11. Chacón M *et al*. Distribution of virulence genes in clinical and environmental isolates of Aeromonas spp. 2003; 84(4):269-278.
12. Hu M *et al*. Identity and virulence properties of Aeromonas isolates from diseased fish, healthy controls and water environment in China. 2012; 55(3):224-233.
13. Shak JR *et al*. Aminoglycoside-resistant *Aeromonas hydrophila* as part of a polymicrobial infection following a traumatic fall into freshwater. Journal of clinical microbiology. 2011; 49(3):1169-1170.
14. Janda JM, Abbott SLJCMR. The genus Aeromonas: taxonomy, pathogenicity, and infection. 2010; 23(1):35-73.
15. Saavedra MJ *et al*. Resistance to β-lactam antibiotics in *Aeromonas hydrophila* isolated from rainbow trout (Onchorhynchus mykiss). International microbiology. 2004; 7(3):207-211.
16. Jacobs L, Chenia HYJIJOFM. Characterization of integrons and tetracycline resistance determinants in Aeromonas spp. isolated from South African aquaculture systems. 2007; 114(3):295-306.
17. Jun JW *et al*. Isolation and molecular detection of Plesiomonas shigelloides containing tet A gene from Asian arowana (*Scleropages formosus*) in a Korean aquarium. 2011; 5(28):5019-5021.
18. Igbinosa IH, Okoh AI. Antibiotic susceptibility profile of Aeromonas species isolated from wastewater treatment plant. The Scientific World Journal, 2012.
19. Noga EJ. Fish disease: diagnosis and treatment. John Wiley & Sons, 2011.
20. Zorrilla M *et al*. Bacteria recovered from diseased cultured gilthead sea bream, *parus aurata* L. southwestern Spain. Aquac. 2003; 218:11-20.
21. Buller NB. Bacteria from Fish and Other Aquatic Animals: A Practical Identification Manual. Cambridge: CABI Publishing, 2004.
22. El-Gendy MY. Epizootiologyical studies on some bacterial infections in marine fishes. Cairo Univ, 2007.
23. Bergmans L *et al*. Microscopic observation of bacteria: review highlighting the use of environmental SEM. International Endodontic Journal. 2005; 38(11):775-788.
24. Roberts RJ. Fish pathology. John Wiley & Sons, 2012.
25. Schäperclaus W, Kulow H, Schreckenbach K. Fish Disease–AA Balkema. Rotterdam, the Netherlands, 1992.
26. CLSI. Performance Standards for Antimicrobial Susceptibility Testing of Anaerobic Bacteria: Informational Supplement. Clinical and Laboratory Standards Institute (CLSI), 2009.
27. Abdel-Hakim N, Moustafa SJEJOAB. Fisheries, Performance of Nile tilapia (*Oreochromis niloticus*) raised in cages as affected with stocking density and dietary protein level. 2000; 4:95.
28. El-Sayed A-FM. Effects of stocking density and feeding levels on growth and feed efficiency of Nile tilapia (*Oreochromis niloticus* L.) fry. 2002; 33(8):621-626.
29. Awan MB *et al*. Biochemical characterization of the Aeromonas species isolated from food and environment. Pakistan. J Physiol. 2005; 1:1-2.
30. Ahmed M, Shoreit AAM. Bacterial haemorrhagic septicaemia in *Oreochromis niloticus* at Aswan fish hatcheries. Assiut. Vet. Med. J. 2001; 45(89):200-206.
31. El-Ashram AMM. On *Aeromonas Hydrophila* Infection among Cultured Tilapias: A Biological, Histopathological and Management Study. Egyptian Journal of Aquatic Biology and Fisheries. 2002; 6(3):181-202.
32. Stoskopf MK. Fish medicine, 1993.
33. Afifi SH, Al-Thobiati S, Hazaa MS. Bacteriological and histopathological studies on *Aeromonas hydrophila* infection of Nile tilapia (*Oreochromis niloticus*) from fish farms in Saudi Arabia. Assiut Veterinary Medical Journal. 2000; 42(84):195-205.
34. Harikrishnan R, Balasundaram C. Modern trends in *Aeromonas hydrophila* disease management with fish. Reviews in Fisheries Science. 2005; 13(4):281-320.
35. Suprarto H *et al*. The isolation of *Aeromonas hydrophila* and Escherichia coli from Lou Han Cichlasoma synspilum and studies of their histopathology changes. Journal of Biological Researches, 2014, 10(2).
36. Austin B, Austin DA. Bacterial Fish Pathogens: Disease of Farmed and Wild Fish. Sixth ed. Springer, 2012.
37. Toranzo AE. *et al*. Plasmid coding for transferable drug resistance in bacteria isolated from cultured rainbow trout. Appl. Environ. Microbiol. 1984; 48(4):872-877.
38. Erdem B *et al*. Biochemical identification and numerical

taxonomy of Aeromonas spp. isolated from food samples in Turkey. Turkish Journal of Biology. 2011; 35(4):463-472.
39. Frye JG, Jackson CRJFIM. Genetic mechanisms of antimicrobial resistance identified in Salmonella enterica, Escherichia coli, and Enterococcus spp. isolated from US food animals. 2013; 4:135.
40. Adenipekun EO *et al*. Prevalence and multidrug resistance of Escherichia coli from community-acquired infections in Lagos, Nigeria. 2016; 10(09):920-931.
41. Cabello FCJEM. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. 2006; 8(7):1137-1144.
42. Chen Y *et al*. Identification and Antibiotic susceptibility of Pathogenic Bacteriium *Aeromonas hydrophila* from diseased Scortum barcoo. Fisheries Science, 2015, 7(1).
43. Duma ST. Characterization of Factors Involved in and Affecting Biofilm Formation by Aeromonas Spp. Isolates. University of KwaZulu-Natal, Westville, 2012.
44. Yardimci B, Aydin Y. Pathological findings of experimental *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*). 2011; 58:47-54.
45. Austin B, Austin DA. Aeromonadaceae representatives (motile aeromonads), in Bacterial Fish Pathogens. Springer, 2016, 161-214.