



Mansoura University
Faculty of Veterinary Medicine
Department of Internal Medicine,
Infectious and Fish Diseases.

**Advanced Study on Motile *Aeromonas* Septicemia
(MAS) in Cultured Nile tilapia (*Oreochromis niloticus*)**

By

Mona Abd Elkhalek Abd Elhalem Salem

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Master of Veterinary Medical Sciences (Fish Diseases and Management).

Under Supervision of

Prof. Dr. Viola Hassan Zaki

Professor of Fish Diseases and Management and Head Department of Internal Medicine, Infectious and Fish Diseases. Faculty of Veterinary Medicine, Mansoura University.

Prof. Dr. Eman Zahran Abd-Elhamid

Professor of Fish Diseases and Management - Faculty of Veterinary Medicine, Mansoura University.

Dr. Rawia Saad Adawi

Senior Researcher of Fish Disease Animal Health Research Institute
Agricultural Research Center.

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6. Summary

The present work objectives were to survey the bacterial infections affecting Nile tilapia fish farms at different localities; determining their prevalence, molecular characterization and virulence genes detection besides the antimicrobial resistance pattern. Further, we adopted a new ecofriendly method to control the most common bacterial infection based on our results and emphasized the role of this tool in the fish health as well.

This study was done through two experiment:

Experiment 1: Prevalence, molecular characterization, virulotyping, and antibiotic resistance of motile aeromonads isolated from Nile tilapia farms in Egypt

A total number of 280 alive and freshly dead naturally infected Nile tilapia exhibited signs of hemorrhagic septicemia were collected from private fish farms in Kafr El-Sheikh, Manzala and Gamsa during January 2017 - 2018. Fish weight was ranged from 100-150 g and length was ranged from 17-20 cm. The freshly dead fish were kept on ice in a storage ice box till the onset of laboratory examination (dissection, clinical, postmortem, bacteriological and histopathological examination).

Bacterial isolates was identified by conventional biochemical method, API-20E method and molecular identification using 16s rRNA. Then all isolates were examined using a species-specifying primer to determine *A. hydrophila*. Virulence gene was then verified in all isolates confirmed *A. hydrophila*; by PCR using virulence specific primers. Antimicrobials resistance pattern was recorded in all identified *A. hydrophila* strains by the disc diffusion method. Finally, histopathological examination of liver and spleen of naturally diseased Nile tilapia were examined.

Our findings revealed the followings:

1. Clinical signs of naturally diseased Nile Tilapia were manifested as bilateral exophthalmia associated with hemorrhage in gill cover, sever ulceration on body surface, hemorrhage of fin, abdominal distension, and significant mass mortalities. The postmortem examination of naturally diseased Nile tilapia showed hemorrhage on liver surface and hemorrhagic spleen. Kidney and liver were congested and slightly enlarged, distended gall bladder, hemorrhagic kidney and liver with serous fluid in abdominal cavity.
2. *A. hydrophila* was identified depending on different tests applied to demonstrate the biochemical characters by using the API 20 E system and positively amplified at 500 bp by PCR amplification of 16S rDNA and also to the sub spp level by a. *hydrophila* specific primers. Virulence genes detection revealed that out of these isolates, five *Aeromonas hydrophila* (83.3 %) harbored aerolysin gene. Meanwhile, hemolysin and lipase genes positive isolates were lower reaching 16.7 % for both genes.
3. The prevalence of bacterial infection among naturally diseased Nile tilapia were 79.17, 70 and 58.33 in Kafr El-Sheikh, El- Manzala, and Gamsa fish farms; respectively. While, The prevalence of *Aeromonas* infection were of 34.76, 26 and 23.18 in Kafr El-Sheikh, El- Manzala, and Gamasa fish farm; respectively.
4. Aeromonads were the most common prevalent bacterial isolates 128 out of 429 with percent 29.84%, among them; *A. hydrophila* was the predominant species 84 isolates (65.63%) and followed by *A. caviae* 24 isolates and *A. sobria* 20 isolates with 18.75% and 15.63%, respectively.

5. The prevalence of *A. hydrophila* among naturally diseased Nile tilapia was 65.75, 61.53 and 75 in Kafr El-Sheikh, El- Manzala and Gamasa fish farm; respectively. Prevalence of *A. hydrophila* to total No. of *Aeromonas* isolates was 37.5%, 18.75 and 9.37 in Kafr El-Sheikh, El- Manzala and Gamasa fish farm; respectively.
6. In Kafr El-Sheikh, a total of 48 *A. hydrophila* isolates were isolated from different fish farms with prevalence of infection in relation to No. of fish in area as follow 62.5, 45 and 12.5% in Elhamol, Baltim and Torombat seven, respectively. A total of 15 *A. caviae* isolates were isolated from different fish farms as follow 20, 12.5 and 5% in Elhamol, Baltim, and Torombat seven, respectively. Whereas a total of 10 *A. sobria* isolates were isolated from different fish farms as follow, 15%, 7.5% and 2.5% in Elhamol, Baltim and Torombat seven, respectively.
7. The highest prevalence of *A. hydrophila* and *A. caviae* infection was in kidney with 41.83 and 13.63% respectively. The highest prevalence of *A. sobria* was in liver with percent 12.63%.
8. The highest prevalence of *Aeromonas* spp infection was recorded during summer, followed by spring, then autumn and finally, winter with percent 66.36, 53.85, 23.40 and 20.84, respectively.
9. Antimicrobial resistance pattern revealed that *A. hydrophila* showed the highest resistance was recorded against ampicillin, amoxicillin followed by clindamycin and erythromycin. The lowest resistance was against gentamycin. However, *A. hydrophila* strains were intermediate to neomycin and nalidixic acid but highly sensitive to ciprofloxacin, trimethoprim, chloramphenicol, amikacin and tetracyclin. Additionally, Our results revealed that the MAR index

values of six identified *A. hydrophila* was ranged from 0.16-0.42. The identified isolates showed multiple resistant patterns. Four strains were commonly resistant to 5 antibiotics with 66.6% multi-resistance patterns, while one strain was multiple resistances against 2 antibiotics with 16.6% of total strains, Also, one strain showed multiple resistances against 4 antibiotic with 16.6% of total strains.

10. Histopathological examination of naturally diseased Nile tilapia revealed that hepatic tissue showing thickened capsule with congested blood vessels, multifocal areas of necrosis, cytoplasmic vacuolation and fat deposition around hepatopancreas. Also, leukocytic cells infiltration around bile ducts and hepatopancreas. Multifocal hepatopancreatic necrosis with pyknotic nuclei. Meanwhile, splenic tissue showing congestion with edema in red pulp, depletion of lymphocytes from white pulp, hemosiderosis in red pulp fibrinoid necrosis of vessel wall.

Experiment 2: Effect of dietary microalgae supplementation on enhancement of fish health and resistance against *Aeromonas* spp challenge

A total number of ninety apparently healthy Nile tilapia weighed 50g were obtained from a private fish farm at Kafr El-Shiekh governorate, Egypt, and transported alive to the laboratory of fish disease and management, Mansoura Veterinary laboratory of the Animal Health Research Institute of the Agricultural Research Center. Fish were fed basal diet at 3% of their body weight at 9:00 hr and 16:00 hr. After the two weeks acclimation period, Fish (N= 15/ tank, 30/ groups) were allocated into three groups in duplicate as control (basal diet), N5 (basal diet supplemented with 5% of *N. oculata*), and N10 (basal diet supplemented with 10% of *N.*

oculata). Fish fed at 3% of their body weight twice daily for 8 weeks. All fish were weighed at the start and the end of the experimental trial. Fish were deprived of food 24 hr before weighing. Five fish from each aquarium (10 fish /group) were sampled at the completion of the trial after being anaesthetized using MS-222; blood and serum samples were collected, liver and spleen were collected and weighed to determine the HSI and SSI. Besides, liver was collected for oxidant and antioxidant analysis. The entire intestine was also dissected divided into 2 parts; one part was used for microbiological analysis, the 2nd part was used for light and transmission electron (TEM) microscopic examination

Seven days after sampling, all fish in all groups were challenged with *A. hydrophila* intraperitoneally with 0.1 mL of bacterial suspension containing 1.4×10^8 CFU/mL and mortalities were monitored over 2 weeks. Cumulative mortalities was calculated, inoculum from liver and kidney of all dead fish was used to re-isolate the bacteria.

Our findings revealed the following points:

1. Growth performance of Nile tilapia after 8 weeks of feeding revealed that group supplemented with N10 was higher in weight, length and K factor than the other groups; meanwhile, N5 supplemented group was higher in HSI and SSI than other groups.
2. Total leukocytic count (TLC) and lymphocytic counts were in similar trend, they were significantly increased in N5 and N10 groups compared to control. Particularly, they were highly increased in N10 compared to N5 group. Also, Neutrophil was significantly decreased in N5 and N10 groups compared to control group.

3. Serum biochemical parameters of Nile tilapia revealed that there were no adverse effect of dietary microalgae on these parameters including (TP, albumin, globulin ALT, AST, LDH, and ALP)
4. Immunoglobulin (IgM) was significantly increased in N5 and N10 groups compared to control. Interestingly, IgM was highly increased in N10 compared to N5 group. In same trend, serum lysozyme activity was significantly increased in N10 group compared to other groups.
5. Digestive enzymes activities of lipase and amylase activities exhibited a significant increase in N10 group compared to N5 group, while no statistical changes were found between control and both groups.
6. Total bacterial count of the intestinal homogenate was significantly decreased in N10 group compared to N5 and control groups.
7. Oxidative damage marker represented by MDA level was nominally decreased in N10 group compared to N5 group. Similarly, antioxidant enzymes activities in liver homogenate revealed that CAT level was nominally decreased in N10 group compared to others, however, no statistical changes were evident in both MDA and CAT levels. GSH level revealed no significant changes between groups.
8. Light micrograph of Nile tilapia fed N10 supplemented diet revealed immunomodulation of intestinal cell activities compared to other groups.
9. T.E. micrograph of the intestinal villi of N10 group showing proliferated enterocytes formed by more than one layer having large elongated vesicular nucleus and cytoplasm with presence of few goblet cells, mucus globule and numerous intraepithelial lymphocytes with presence of cell in state of apoptosis compared to other groups.

10. Challenging fish with *A. hydrophila* after the feeding period, revealed that same clinical signs and postmortem findings as what have been seen from naturally diseased Nile tilapia. The cumulative mortalities were decreased in both N5 and N10 groups compared to the control, however, in N10 groups cumulative mortalities were much lower than that of N5 group. *A. hydrophila* was re-isolated from the internal organs of moribund and freshly dead fish. Re-isolated *A. hydrophila* bacteria was identified by phenotypic and biochemical method.