Prevalence of some bacterial agents affecting the gills of some cultured fishes in Egypt

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Abstract

A number of 3010 apparently healthy and naturally infected fishes of different species (tilapia sp., catfish, carp sp., mullets and byad were collected from different fish farms in Egypt. These fishes were subjected to full clinical, bacterial and histopathological examinations. The results revealed that the most common clinical signs were mainly respiratory manifestations, where the infested fishes showed slow and surface swimming usually near to Oxygen pumps, blackness of the skin as well as mass mortalities. With regards to post-mortem lesions, the gills were either congested in acute cases or anaemic and pale with emaciation and excessive mucus secretion in chronic cases. Excessive blood oozing was noticed in gills of *O. niloticus* with acute bacterial co-infections. The isolated bacteria were *Aeromonas hydrophila, A. caviae, Pseudomonas putida, P. fluorescens* and *Vibrio anguillarum.* The means of Nitro Blue Tetrazolium (NBT) estimates, which in turn represent the activity of phagocytosis, reached their maximum levels 1 hour after I/P injection of *O. niloticus* with 3 bacterial isolates *(A. caviae, P. putida* and *V. anguillarun)*. There were a significant difference between NBT estimates in the 3 bacterial isolates and the control group, which showed lower NBT estimates in all time intervals.

Introduction

Fish diseases caused by aeromonads and pseudomonads considered to be the major bacterial problems facing the aquaculture development causing mass mortalities, reduced production and low quality of aquatic organisms. Both Aeromonas spp. (*A. hydrophila, A. sobria and A. caviae*) and Pseudomonas spp. (*P. fluorescens, P. putida and P. aeruginosa*) were incorporated in severe outbreaks among *O. niloticus* in fish hatcheries (Ahmed and Shoreit, 2001). *A. hydrophila* alone was isolated from gills of the naturally infected male monosex *O. niloticus* suffering from Motile Aeromonas septicemia in floating cages (Gamal et al., 2002).

The present study was carried out to address the following objectives:

1- Determination of the prevalence of bacterial gill affections in cultured fish species in different fish farms.
2- Detecting the gill affections in different fish species, different growth Stages and different seasons.

Materials and methods

Fish

A number of 3010 apparently healthy and naturally infected fishes of different species; 941 frys & fingerlings of *Oreochromis niloticus*, 1686 adult tilapia spp. (*O. niloticus, O. aureus, Sarothredon galilaeus* and *Tilapia zillii*), 115 catfish *(Clarias gariepinus),* 133 carp spp including common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), 108 mullet spp. (*Mugil cephalus* and *M. capito*) and 27 bayad sp. *(Bagrus byad)* were collected from different localities in Egypt. These fishes were subjected to full clinical examination.

Bacteriological examination

The bacteriological examination was carried out using the API and also using the traditional method according to the scheme of Plumb (1994).

Histopathological examination was carried out according to Robert (1989).

The immunological evaluation was conducted by measuring NBT estimates in the serum of *O. niloticus*, I/P injected with *A. caviae, P. putida* and *V. anguillarum:*
A total of 120 *O. niloticus* were collected from Abbassa fish farm and kept in glass aquaria for 2 weeks prior to injection for acclimatization. The fish were equally divided into 4 groups (1, 2, 3 and 4). The fish in each group were anaesthetized using MS 222 (100 mg/l) combined with sodium bicarbonate (200 mg/l). Saline solution 1.0 ml containing ($2x10^6$) cells of the bacterial isolates, *A. caviae, P. putida and V. anguillarum* was injected I/P into the fish in groups 1, 2 and 3 respectively. The group 4 was injected with 1.0 ml of sterile saline solution per fish and kept as control. The experimented fishes were kept in aquaria of 60 liters water capacity, supplied with air stones and observed for 4 weeks post inoculation. The temperature, dissolved Oxygen, Ammonia level and the pH of the water were 30±2 ºC, 6±1 mg/l, 3 ppm and 8.5 respectively. Blood samples were taken from the caudal veins of 3 fish in order to be as 3 replicates per each treatment per each time.

Times of blood samples 10 blood samples were taken after 1 hour, 6 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks and finally after 4 weeks respectively. Immunological test including blood nitroblue tetrazolium (NBT) test was carried out according to Abu El-Magd (1996).

Antibiotic sensitivity test was conducted

Results and discussion

Clinical signs

Heavy mortalities were noticed in *O. niloticus* with acute bacterial infections with *A. hydrophila, A. caviae, P. putida, P. fluorescens* and *Vibrio anguillarum*, where the affected fish showed blackness of skin, scale loss, tail & fin rot, excessive and wide spread cases of corneal opacity (Fig. 1 plate I). The gills were hyperemic and in some cases of severe bacterial outbreaks, gills were not only found severely congested (Fig. 2 plate I) but also excessive blood oozing was noticed in gills of *O. niloticus* with acute mixed infection with *V. anguillarum* and *A. caviae* (Fig. 3 plate I). Congested liver and internal organs were also noticed in the affected fishes (Fig. 4 plate I)**.** Similar clinical signs and postmortem lesions were described by Ahmed and Shoreit (2001) and Gamal et al. (2002).

Prevalence of the isolated bacteria

The isolated bacteria were *Aeromonas hydrophila, A. caviae, Pseudomonas putida, P. fluorescens* and *Vibrio anguillarum.* Similar results and isolates were recorded by Ahmed and Shoreit (2001) and Laila El-Seedy et al. (2004).
Concerning *A. hydrophila*, it was isolated from gills of *O.niloticus* and *C. carpio* of Abbassa fish farms with a prevalence of 7.5 % & 0.8 % respectively. *A. hydrophila* was isolated also from gills and spleen of *O. niloticus* in Damietta cages (with a prevalence of 3.1 %) mixed with *A. caviae* isolated from liver and *P. putida* isolated

from the eye and internal organs (liver, kidney and spleen). Similar results and prevalences were recorded by Ahmed and Shoreit (2001); Taylor (2003) and Laila El-Seedy et al. (2004). Higher prevalence was recorded by Gamal et al. (2002).

Regarding *A. caviae*, it was isolated from internal organs (liver and kidney) of *O. niloticus* of Wady Al-Rayan fish farms in Fayium with a prevalence of 1.5 %. While its prevalence between *M. cephalus* of the same locality was 6.5 % where mullets were more severely affected and showed higher mortality than *O. niloticus*. On the other hand *A. caviae* was also isolated from liver of *O. niloticus* of Damietta cages with a prevalence of 3.1 %. Similar results were recorded by Ahmed and Shoreit (2001).

With respect to *P. putida*, it was isolated from gills and liver of *O. niloticus'* fingerlings of Abbassa with a prevalence of 5.4 %. Similar results and prevalence were reported by Eissa et al. (1996) who isolated *P. putida* from cultured tilapia spp. in Abbassa fish farms with a prevalence of 6.8 %. While in case of *O. niloticus* from Damietta cages, *P. putida* was incorporated in all eye lesions of corneal opacity and was also isolated from all internal organs (liver, kidney & spleen) with a prevalence of 2.1 %. Similar results were recorded by Robert (1989); Ahmed and Shoreit (2001).

P. fluorescens was isolated from liver of adult *O. niloticus* of Abbassa (with a prevalence of 7.5 %) as mixed infection with *A. hydrophila* in gills and livers. Similar results and prevalence were reported by Eissa et al. (1996); Ahmed and Shoreit (2001) and Laila El-Seedy et al. (2004).

Regarding, *V. anguillarum*, it was isolated from gills of *O. niloticus* & *M. cephalus* of Wady Al-Rayan fish farms with a prevalence of 1.5 % & 6.5 % respectively. In this study, *V. anguillarum* was isolated from Fayium only and this may be attributed to the relatively higher water salinity (1-3 ppt) of Wady Al-Rayan fish ponds compared with water salinity of Abbassa and Damietta branch of the River Nile. Besides, the fish ponds of Wady Al-Rayan are close to Qarun Lake with its very high water salinity (more than 40 ppt), where *Vibrio sp*. are more prevalent and common in this marine lake with the high possibility of contamination and transfer of Vibrios to other parts of Fayium governorate via different vectors such as human, mammals or aquatic birds.

All bacterial infections were found as mixed infections. Mixed bacterial infections with Aeromonas & Pseudomonas spp. was also recorded by Ahmed and Shoreit (2001).

Concerning the seasonality of different isolated bacteria from Abbassa fish farms, *A. hydrophila* and *P. fluorescens* were more prevalent in winter season while *P. putida* was isolated from fingerlings of *O. niloticus* in summer season. On the other hand, in Damietta floating cages, *A. hydrophila* and *P. putida* were isolated in both winter and summer seasons, while *A. caviae* was isolated during winter season only. However, *A. caviae* and *V. anguillarum* were isolated during summer season from Wady Al-Rayan fish farms in Fayium but no samples were examined during winter season. Thus, it could be concluded that both Aeromonad and Pseudomonad septicemias were more prevalent during winter season. This could be attributed to the suppressed immunity of the cultured fishes caused by cold weather and low water temperature, which most warm water fishes couldn't tolerate especially tilapia spp. rendering the fishes more vulnerable for different disease agents.

Bacterial examination of *O. niloticus* experimentally injected I/P with *A. caviae, P. putida and V. anguillarum* revealed that the 3 bacterial isolates were re-isolated from gills, liver, kidney, muscles and brain. However, the re-isolation and the presence of the 3 bacterial isolates in muscles & brain were irregular (sometimes –ve and sometimes +ve). On the contrary, the 3 bacterial isolates were present and re-isolated regularly from gills, livers and kidneys of the examined fishes 4 weeks after injection. The results were supported by Robert (1989); Ahmed and Shoreit (2001).

NBT estimates, which in turn represent the activity of phagocytosis reached their maximum levels 1 hour after injection with the 3 bacterial isolates and lasted for 6 hours after injection after that the NBT estimates began to decrease gradually after 6 hours and pursued lowering until the 4^{th} week from injection. Thus, those NBT

estimates 1 hour after injection were significantly higher than other time intervals during which the NBT estimates gradually decreased with no significant differences between them until the 4th week. The results also showed that there were a significant difference between NBT estimates in the 3 bacterial isolates and the control group, which showed lower NBT estimates in all time intervals. However, there was no significant difference between NBT estimates in case of *V. anguillarum* (although they still higher) and the control group in all time intervals. These results agreed with those recorded by Abu El-Magd (1996).

Fig. 1 *O. niloticus* showing blackness, corneal opacity, scale loss and tail rot, where *A. hydrophila, A. caviae and P. putida* were isolated.

Fig. 2 *O. niloticus* showing severe congestion of the gills and blackness of the skin, where *A. hydrophila, A. caviae and P. putida* were isolated.

Fig. 3 *O. niloticus* showing excessive blood oozing and congestion of gills, from which *Aeromonas caviae and Vibrio anguillarum* were isolated.

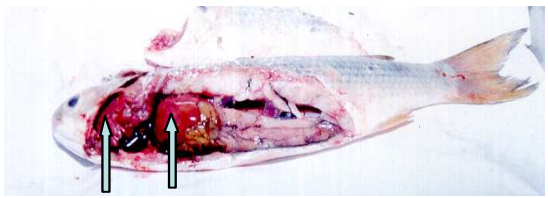

Fig. 4 *Mugil cephalus* showing congestion of gills and liver, from which mixed infection with *A. caviae and V. anguillarum* was recorded.

Plate I

Antimicrobial sensitivity

All bacterial isolates were resistant to Penicillin and Erythromycin. However, they were sensitive to Ciprofloxacin (except *V. anguillarum*), Nalidixic acid, Garamycin, Streptomycin and Kanamycin. Similar results were recorded by Gamal et al. (2002). On the other hand, the results disagreed with those reported by Taylor (2003).

Histopathological alterations

The histopathological alterations revealed that *O. niloticus* with mixed infection of *A. caviae and V. anguillarum* revealed primary lamellar oedema and separation of epithelium at the base of secondary lamellae (Fig. 5 plate II). Liver of the same fish showed congestion of the portal vein (Fig. 6 plate II). On the other hand, gills of *O. niloticus* experimentally infected with *P. putida* displayed telangiectasis or anurysm of blood vessels in the secondary gill lamellae (Fig. 7 plate II). Besides, kidney of *O. niloticus* experimentally infected with *A. caviae* showed condensation of glomeruli with edema in Bowman's capsule with focal coagulative necrosis (Fig. 8 plate II). The results agreed with those recorded by Ahmed & Shoreit (2001) and Taylor (2003).

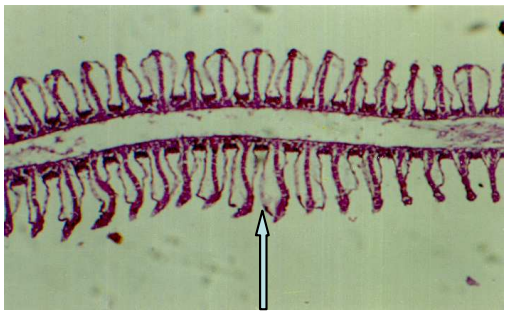

Fig. 5 Gill lamellae of *O. niloticus* showing oedema, where *A. caviae* and *V. anguillarum* were isolated. (x 200) (H&E)

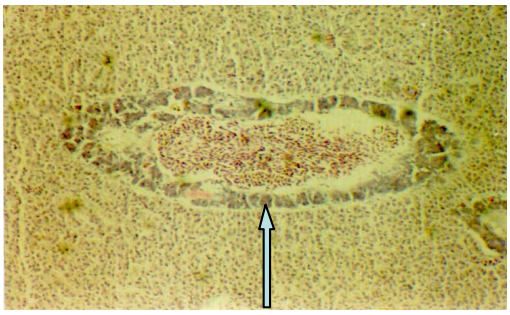

Fig. 6 Liver of *O. niloticus* showing congested portal vein, where *A. caviae* and *V. anguillarum co-infection* was recorded. (x 100) (H&E)

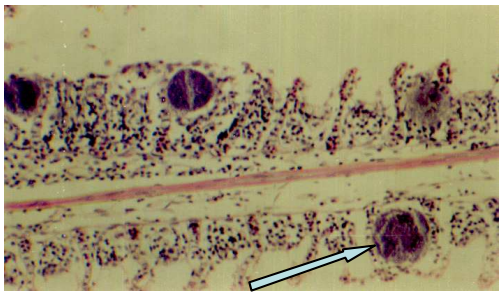

Fig. 7 Gills of *O. niloticus* experimentally infected with *P. putida* showing anurysm of blood vessels. (x 40) (H&E)

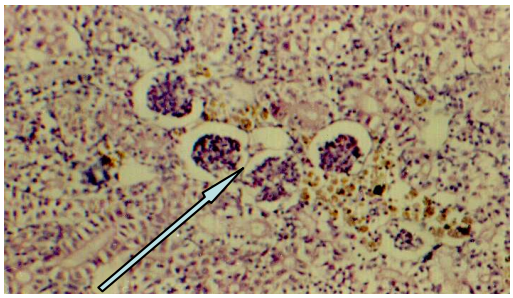

Fig. 8 Kidney of *O. niloticus* experimentally infected with *A. caviae* showing condensation of glomeruli with oedema in Bowman's capsule and coagulative necrosis. (x 200) (H&E)

Plate II

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