ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# A contribution on *Pseudomonas aeruginosa* infection in African Catfish (*Clarias gariepinus*)

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**ABSTRACT**

In this study, samples from cultured Common carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*) and African catfish(*Clarias gariepinus*) fishes were collected from Kafr el-Sheikh, Menofya, Behira and Sharkia Governorates in Egypt for detection of *Pseudomonas aeruginosa* infection. Isolation and identification of *Pseudomonas aeruginosa* was done by traditional methods then confirmed using regular PCR technique. *Pseudomonas aeruginosa* gave 956 bp product size specific for 16S rDNA. The experimental inoculation of *Clarias gariepinus* with *Pseudomonas aeruginosa* was fully demonstrated. The most common clinical signs were external haemorrhage and ulcer with mortality rate 40%. Histopathological changes revealed degeneration and necrosis in all internal organs associated with hyperplesia in the wall of the blood vessels. Chronic inflammatory cell infiltration and melanomacrophage cells were detected in all fish tissues. The effect on some oxidative stress and immunological parameters of experimentally inoculated *Clarias gariepinus* with *Pseudomonas aeruginosa* were studied. Results revealed that there were significant increase in lipid peroxidation product (malondialdehyde) , hypoprotineamia, hypoalbuniaemia and hypoglobulinaemia. In-vitro sensitivity test of isolated *Pseudomonas aeruginosa* isolates to different chemotherapeutic agents was conducted. In conclusion, this study showed that *P.aeruginosa* infection is common in some common cultured freshwater fishes in Egypt. It can be diagnosed easily using PCR technique.The experimental infection of Clarias gariepinus with *P.aeruginosa* was associated with mortality rate 40 % , severe histopathological changes in all organs of infected fishes, immunosuppression and oxidative damage of tissues which reflect the significant economic importance of Pseudomonas aeruginosa infection in cultured fishes. Colistin sulphate, Danofloxacin, Nalidixic acid, Oxolonic acid and Oxytetracyclinen are recommended for control of Pseudomonas aeruginosa infection in cultured fishes.

**Keywords:** *Pseudomonas aeruginosa , histopathological changes*, sensitivity test, oxidative stress , PCR, Clinical signs.

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ISSN: 0975-8585

## INTRODUCTION

Fish diseases are one of the major problems in fish farm industry. Disease causes economic losses because of fish mortality ,treatment expenses, postponement or loss of the opportunity to sell the fish and contraction of zoonotic diseases by the handler and final consumer of the affected fish**.** *Pseudomonas aeruginosa* is a gram-negative rod shape bacterium belonging to the family Pseudomonadaceae. This species is highly adaptable opportunistic pathogen, capable of surviving in a variety of environment, including aquaculture environment **(Abdullahi R. *et al*., 2013)**. The most prevalent diseases affecting fish farms in Egypt were Motile *Aeromonads, Pseudomonas* species, *Streptococcus* Spp*. and Staphylococcus* Spp*.; Vibrio* Spp. and *Flexibacter* Spp. (**Khalil et al., 2001**).

*Pseudomonas* spp. are widely spread in natural sources of water and associated with septicaemia in aquatic animals (**Roberts, 2001**). These bacteria are considered opportunistic pathogens, causing disease when the host is subjected to stress. A number of aquatic animals including fish, frogs and soft-shelled turtles are reported to be susceptible to *Pseudomonas* spp. with moderate to high losses (**Somsiri and Soontornvit, 2002**). The etiological agents commonly found are *P. diminuta, P. fluorescens, P. putida* and *P. aeruginosa* with different degrees of virulence**.** The characteristic symptom of the disease produced by Pseudomonas bacteria is a remarkable septicaemic hemorrhage in the skin of the mouth region, opercula and ventral side of the body (**Wakabayashi and Egusa, 1972**).

During 2004, *Pseudomonas* spp. was isolated from Nile tilapia and African catfish (*Clarias gariepinus),* silver carp (*Hypophthalmichthys molitrix*) and grey mullet (*Mugil cephalus*) that were being reared in seventeen commercial fish farms in Kafr EI-Sheikh Governorate (**Mesalhy, 2013**). Bacteriological examinations of private fish farms in Kafr EI-Sheikh Governorate suffered from high mortalities, ranging from 17.6 to 22.9%. revealed that 38 fish (36.9%) were infected with *Pseudomonas fluorescens*, 30 (29.1%) with *Pseudomonas aureginosa*, 19 (18.5%) with *Pseudomonas anguilliseptica* and 16 (15.5%) with *Pseudomonas pseudoalkaligene* **(Masbouba,2004).** *Pseudomonas* infection has been incriminated as the most common bacterial infection among fish and appear to be stress related disease of freshwater fish especially under culture conditions ( **Kitao*, et al* 1993** ) . *Pseudomonas aerugenosa* was isolated from skin, gills and stomach content of cultured *Clarias gariepinus* fingerlings in Nigeria(**Oni *et al*.,2013**).

Conventional microbiological methods needed to identify bacteria from fish are often limited by the length of time required to complete the assays. In recent years, PCR have overcome problems associated with culture- based techniques, enabling the detection of bacteria directly in clinical samples without the need for previous culturing **(Gonzalez et al., 2004).**

So, this study was designed to make a survey of *Pseudomonas aeruginosa* infection in some private fish farms in Kafr- Elsheikh Governorate, Egypt during the Summer of 2013 followed by full identification and characterization scheme of the retrieved isolates. Also, experimental infection of ***Clarias gariepinus*** with the confirmed isolate of *P.aeruginosa* to study some clinical, biochemical and histopathological changes associated with *P.aeruginosa* infection.

## MATERIAL AND METHODS

### Fishes

A total number of 260 cultured fishes (160 *Oreochromis niloticus,* 60 *Cyprinus carpio* and 40 *Clarias gariepinus*) were collected from different fish farms at different localities (Table,1) during Summer, 2013. Fishes were transported alive from fish farms to the Fish Disease Department at Animal Health Research Institute. Fish were kept in glass aquaria, supplied with aerated chlorine free tap water.

### Clinical and postmortem examination of naturally infected fishes

Fish were examined clinically for any abnormal lesions according to **Noga (1996)** and **Austin and Austin (2007).**

**Isolation and identification of *Pseudomonas aeroginosa* From fishes**

Samples from internal organs of examined fishes were streaked onto nutrient agar, trypticase soy agar, Rimler- Shotts medium (RS) and Pseudomonas agar medium plates then incubated at 28ºC for 24 - 48 hr. The growing colonies were picked up in pure form and reinoculated into trypticase soy agar for further identification. Identification of all isolates was done by cultural, morphological and biochemical characters according to **Quinn *et al.,* (2002), Austin and Austin, (2007)** and through using API-20E **(Biomérieux)** for gram-negative fish pathogen**.**

**Molecular identification of *Pseudomonas aeroginosa***

**Bacterial DNA Extraction**

DNA Extraction was done by boiling method **(Bansal, 1996).** The bacterial pellets were washed once with 300 µl phosphate buffered saline (PBS), pH 7.4, centrifuged at 12.000 RPM, and resuspended again in PBS, incubated in hot plate at $105^{O}$C for 10 min. Then rapidly cooled at freeze for another 10 min. The clear supernatants obtained after a 5 min centrifugation at 12000g is the DNA used for PCR reaction.

**Molecular identification of the bacterial DNA using Polymerase Chain Reaction Technique (PCR)**

PCR was done according to **Spilker et al. (2004)** using PA-SS-F GGGGGATCTTCGGACCTCA, Location 189–206 and PA-SS-R TCCTTAGAGTGCCCACCCG located 1124–1144 to amplify 956 bp (Position and size relative to 16S rDNA sequence of *P. aeruginosa* AT2 (AB091760). The used thermal profile was initial denaturization for 2 min at 95°C, 25 cycles of 20 s at 94°C, 20 s at 58°C, and 40 s at 72°C. A final extension of 10 min at 72°C. the PCR product was visualized and photographed on BioRad Gel Documentation system using 1.5% agarose in TBE with Ethidium Bromide.

**Experimental infection of African Catfish (*Clarias gariepinus*) with *Pseudomonas aeroginosa***

A total number of 60 apparently healthy *Clarias gariepinus* were divided into two groups (three replicates for each group). The first group injected intra-peritoneal with 0.2 ml of trypticase soya broth and acts as a control group while the second group injected intra peritoneal with 0.2 ml of trypticase soya broth containing $3x10^{7}$ CFU /ml and acts as experimentally infected group ( **Eissa *et al*., 2010).** Fish were observed for seven days where clinical signs and mortality were recorded.

**Blood analysis**

Blood samples taken from the caudal vessels of experimentally infected ***Clarias gariepinus*** at the seventh day post infection were allowed to clot at room temperature and centrifuged at 3000 r.p.m for 15 min. to separate serum. Serum samples were used to determine malondialdahyde (**Albero *et al*., 1986**)and the nitrite level in the collected serum samples was calculated according to the method described by **Green et al. (1982).** Total serum protein was measured according to the method described by **Richardson (1977)** and Polyacrylamide gel electrophoresis was made according to **Hamesh and Rickwood(1990).**

**Histopathological studies**

Tissue specimens from skin, gills, liver, kidneys, spleen, ovary , testis and brain were taken from both control and experimentally infected ***Clarias gariepinus*** at the seventh day post infection The samples were fixed in 10% formal saline, processed by conventional method, sectioned at 4 µm and stained with Haematoxylin and Eosin (**Roberts, 2001).**

**In-vitro sensitivity test of *Pseudomonas aeruginosa***

Antibiotic susceptibility testing was performed by a disk diffusion method and interpreted in accordance with criteria of the National Committee for Clinical Laboratory Standards **(NCCLS .,1994) and Quinn *et al.,* (2002).**

**Statistical Analysis**

The data obtained in this study were statistically analyzed according to the method described by **Petrie and Watson (1999).**

## RESULTS & DISCUSSION

Clinical and post-mortem examination of both naturally and experimentally infected fishes: Naturally infected fishes showed hemorrhages all over the fish body especially at the base of fins, tail and fins rot, detachment of scales and skin ulceration and abdominal distention. Internally these fishes showed abdominal dropsy with reddish ascitic exudates, liver paleness and enlargement in some fishes and congested with necrotic patches in other fishes, spleen was congested and enlarged and hemorrhagic enteritis in some fishes (Photos 1, 2, 3 & 4). Experimentally infected *Clarias gariepinus* showed external haemorrhage, tail and fin rot and skin ulceration and mortality rate 40 %. The incidence of Pseudomonas aeruginosa infection in the examined cultured fishes were 34.4 %, 30 % and 27.5 % in Oreochromis niloticus, Cyprinus carpio and Clarias gariepinus respectively with an average 32.3 % in all examined cultured fishes. Similar lesions were recorded by **Okaeme (1989)** who recorded that Gram-negative bacteria as *Pseudomonas* sp., *P. fluorescens, P. putrefaciens and P. aeruginosa* were among the bacterial infection causing mortalities in the culture of the tilapias, *Hererobranchus bidorsalis* and *Clarias lazera*. He recorded ulceration and necrotic lesions of skin, fin and gill rot, pop-eye, abdominal dropsy, and haemorrhagic septicaemia beside fish mortality in infected fishes. Also, **Eissa et al.(2010)** isolated different strains of Pseudomonas species namely *P .putida, P.aeruginosa*, *P. fluorescens and P. anguilliseptica* from Oreochromis niloticus in Qaroun and Wadi-El-Rayan Lakes, Egypt. They recorded that infected fishes showed irregular hemorrhages on body surface, especially at the ventral part of abdomen. , eyes cloudiness, scales detachment and congested gills were observed. Internally, there were sanguineous fluids in the abdominal cavity of some fish. Also, these results are in agreement with those of **Maha A. EL-Hady and** and **Samy (2011)** who isolated *Ps. fluorescence, Ps. putida, Ps. aeruginosa and Ps. anguilliseptica* from cultured *Oreochromis niloticus* and *Cyprinus carpio* from different fish farms at different localities in Egypt. These lesions could be attributed to the different types of toxins produced by *Pseudomonas aeruginosa* **( Olgerts & Bengt, 1982).**

Isolation and identification of *Pseudomonas aeruginosa* From fishes: *Pseudomonas aeruginosa* were gram negative short bacilli, positive for oxidase, catalase, and citrate and negative for indol, urease test and $H_2S$ production (Table, 2). API 20 E was used to confirm the results of biochemical tests. Pseudomonas *aeruginosa* isolated from *Oreochromis niloticus* with a percent of 34.4, while isolated from *Cyprinus carpio* with % of 30 and isolated from *Clarias gariepinus* with % of 27.5 (Table, 3). In this respect **Akinyemi (2012)** isolated *P.aeruginosa* from the gill, buccal cavity and skin of *Clarias gariepinus* juvenile. He found that the isolated *Pseudomonas aeruginosa* was a gram negative. The biochemical test indicated that *Pseudomonas aeruginosa* reacted negative to gram reaction, positive to citrate utilization test, positive to catalyze test, negative to coagulase test, positive to motility test, negative to indole test and produced acid in sugar fermentation test using glucose, lactose and sucrose sugar. Also, these results are in agreement with those of **Hossain et al.(2006)** and **Musa et al.(2009).**

**Molecular identification of *Pseudomonas aeruginosa***

photo (5) showed the electrophoresis of *P. aeroginosa* PCR product with the specific band at 956 bp. This result is simulating that of **Spiker et al.(2004)** who designed 16S rDNA-based PCR assays that provide rapid, simple, and reliable identification of *P.aeruginosa* and its differentiation from other phylogenetically closely related *Pseudomonas* species. Both assays have 100% sensitivity and specificity for their intended targets.They have also demonstrated the utility of these PCR assays in accurately identifying *P. aeruginosa* among isolates not correctly identified by phenotypic analyses.

Blood Analysis

The effect of experimental infection with *Pseudomonas aeruginosa* in cultured *Clarias gariepinus* on the electrophoretic pattern of serum protein, total protein and albumin were demonstrated in photo, 6 and table, 4**.** The electrophoretic pattern of serum protein pointed out the presence of 8 fractions; *P.aeruginosa* induced a significant decrease in total protein , albumin and globulin. These results are agreed with those of

**Adonova et al.(2014)** who recorded hypoalbuminaemia , mild hyperglobulinaemia, in dogs infected with *Pseudomonas aeruginosa*. They attributed the decrease in albumin level to increased vascular permeability due to bacterial toxins resulting in the passage of albumin to the surrounding interstitial tissue. Also with those of **Ramalingam and Ramarani (2006).** The liver disorders observed in this study can explain the recorded hypoproteinaemia. In this respect, **Saad et al.(2014)** discussed that the decrease in protein and globulin can explain the drastic effect of Pseudomonas infection on immune response of infected fish with subsequently increased the drastic damage effects of bacterial diseases. The causes of decreased serum total protein may be occurred after vascular leaking due to increasing permeability after histamine release **(Ellis, 1981)**, liver damage and anorexia, non-specific proteolysis**.**

Concerning the effect of *Pseudomonas aeruginosa* infection on lipid peroxidation and nitric oxide production . Results in table, 5 showed an increase in the level of both nitric oxide (NO) and lipid peroxidation product. Estimation of lipid peroxidation is complicated due the large number of potential peroxidation products and by the reactivity of these metabolites, thus the most common technique for measuring lipid peroxide involves the use of TBARS.These results are agreed with those of **Suntres et al.(2002)** who found that the challenge of rats with *P. aeruginosa* resulted in increases in lipid peroxidation and decreases in glutathione content, which were associated with the indices of lung injury and neutrophil infiltration. Such a challenge also resulted in weakening the antioxidant defense system, as evidenced by decreases in superoxide dismutase, catalase and glutathione peroxidase activities. Also, with those of **Adeyemi (2014)** who concluded in a study on the effect of experimental bacterial infection on oxidative stress in African catfish that Fish inoculated with bacteria (either E. coli or V. fischeri) had a significant higher levels of tissue LPO. The results of this study indicate that bacterial inoculation could result in oxidative stress in fish.The rise of TBARS may be considered as a sign of the oxidation of cellular compounds **( Salvayre & Salvayre,1992).** The recoded increase in nitric oxide production is in agreement with those of **Ashraf et al.(2004)** and Evora et al.(1998). These results could be attributed to that *pseudomonas* endotoxin induces the synthesis of nitric oxide from L- arginine by the vascular endothelium **(Evora et al. 1998).**

The histopathological changes: Pseudomonas sp. could be considered as an opportunistic pathogen, which can survive on the fish surface or in water or in the gut and may cause disease when unfavorable conditions developed **(Kumaran *et al.,* 2010)**.In our results, the African Catfish, *Clarias gariepinus* were inoculated with *P.aeruginosa* for 7 days. Results showed marked histopathological alterations in all fish organs. These findings were paralleled with those described by **Devakumar*et al.* (2013)** who mentioned that in the crabs are in stress due to *P.aeruginosa* infection and occurred at tissue damage in all the tissues (brain, ovary and gills).Skin showed vacuolar degeneration and necrosis in the epidermal cells with mononuclear inflammatory cells infiltration in between the epidermal cells (Fig.1).Activation of the goblet (mucus) cells were detected. There was also accumulation of edematous fluid sub-epidermal leading to splitting of the sub-epidermal connective tissue (Fig. 2). zIn our opinion, based on the above information, the proteolytic enzymes produced by *P. aeruginosa* are responsible for the hemorrhagic and necrotic changes in the skin**(Pinghui,1974).** The gills changes revealed hypertrophy and hyperplasia of the epithelial cells lining the secondary lamellae resulting in lamellar fusion (Fig.3).Epithelial lifting, epithelial necrosis, edema and desquamation of lamellar epithelium were also observed. The cartilaginous rod at the core of primary lamellae was seen to be disrupted in numerous areas aneurism as well as curling of secondary lamellae were also detected(Fig. 4). The results of the gill histology also clearly suggest the effect of *P.aeruginosa*. In aquatic organisms, the gills represent a vital organ, since they play an important role in the transport of respiratory gases and regulate the osmotic and ionic balance. Toxic substances may cause damage to gill tissues, thereby reducing the oxygen consumption and disrupting the osmoregulatory function of aquatic organisms **(Ghate and Mulherkar1979).** Similar lesions have been reported to occur in *P.aeruginosa* infected crabs gills where haemocytes(HE) are accumulated in the haemocoelic space. Necrosis (NCR) is seen in most of the gill rachis. Epithelial lifting (EL) and Disrupted Pillar cells (DPC) were also observed.**(Devakumar*et al.,* 2013).** The liver showed degenerative changes in the hepatocytes in the form of cytoplasmic vacuolization, lateralization and condensation of the nuclei. Mononuclear infiltration in between the hepatic parenchyma were also observed (Fig. 5).Shrinkage of hepatocytes with increased sinusoidal blood spaces and hepatic blood vessels associated with hyperplasia in the wall of blood vessels with congestion was marked (Fig.6). Most of these findings are similar to a great extent to **Amosu (2012)** who pointed out that the observed pathological lesions of liver in African Catfish, *Clarias gariepinus* which were inoculated with different concentrations ($10^{-5}$, $10^{-7}$ and$10^{-9}$Colony Forming Unit/ml) of *P.aeruginosa* over 14days period. The observed pathological lesions of liver include widespread hepatic degeneration i.e. vacuolar degeneration of liver cells, focal area of cellular

infiltration consisting mainly of heterophils and area of necrosis or disorganization of the hepatic cells and the fish inoculated with $10^{-5}$, $10^{-7}$ and $10^{-9}$CFU/ml concentration of *P.aeruginosa* showed the same degree of damages at 7days post infection while at 14days post infection, severe damages were recorded such as area of necrosis (loss of hepatocytes) and disorganization of the hepatic cells. The kidneys revealed degenerative changes in the form of distinct vacuolization and necrotic changes in the tubular epithelium with sloughing off of the epithelial cells. Hypertrophy of epithelial cells of the renal tubules with the consequent reduction in tubular lumens was observed. Contraction of glomeruli as a result of degenerative changes in the endothelial lining the glomerular tuft and disruption of hematopoietic tissues were also observed, widening of the blood vessels with slight congestion accompanied with severe hyperplasia in the wall of all branches of renal blood vessels. Melanomacrophage cells aggregation also appeared in between the interstitial tissues. Interstitial nephritis was manifested in these cases by necrosis of various elements of the reticular haemopoeitic tissue and diffuses infiltration of an individual numbers of mononuclear inflammatory cells (Fig, 8). Similar findings were detected by **Amosu (2012)** who reported that the observed pathological lesions of kidneys in African Catfish,(*Clarias gariepinus*) which were inoculated with different concentrations ($10^{-5}$, $10^{-7}$ and $10^{-9}$Colony Forming Unit/ml) of *Pseudomonas aeruginosa* over 14 days period revealed widespread tubular degeneration with loss of epithelial cells, diffuse cellular infiltration predominantly of heterophils and necrosis of the tubules with sloughing off of the epithelial cells were noticed as pathological lesion of the kidney. At 7days post infection, the renal corpuscles of the kidney were scattered resulting in their disorganization and consequently obstruction to their physiological functions. It appeared that the fish kidney at 14 days post infection showed necrosis of the tubules with sloughing off of the epithelial cells which is absent in 7days post infection. The spleen showed clusters of melanomacrophage centers consisting of polyhedral large cells appeared brownish or dark brown in color. These were increased both in number and extension in the splenic parenchyma, depletion of lymphocytic elements also detected (Fig, 9). These changes may be resulted from the toxins produced by bacteria where *P.aeruginosa* produces a large number of extracellular toxins, which include phytotoxic factor, pigments, hydrocyanic acid, proteolytic enzymes, phospholipase, enterotoxin, exotoxin, and slime. The most important factor in the pathogenicity of *P. aeruginosa* is the elaboration of a group of protein exotoxins. These exotoxins can produce necrosis of liver, pulmonary edema, hemorrhage, and tubular necrosis of kidneys**(Pinghui,1974).** In the intestine the columnar epithelium of the intestinal villi and crypts undergo hypertrophy,where increased secretory activity with vacuolated clear cytoplasm and were infiltrated by inflammatory cells. A mass of necrotic debris, mucous, and desquamated epithelial cells were seen in the lumen (Fig, 10). The ovary showed stromal edema and numerous mature and immature follicles (primary and secondary oocytes) some of them are degenerated and necrotic, hemorrhage and hemosiderin pigments also detected in between. Similar observations were also mentioned by **Devakumar *et al.*,(2013)** who stated that the histopathological studies of infected crab's ovary with *P. aerginosa* showed thin ovarian wall and ruptured oocytes, and some oocytes shows disintegration. The oocytes were poorly developed and very less in number and all the oocytes lost their cytoplasmic contents. The testis showed degeneration and necrosis leading to decrease in the number of spermatogenic cells in the numerous siminiferous tubules associated with hypertrophy and vacuolar degeneration in the germinal epitheliumlining the seminiferous tubules (Fig, 13&14). The brain showed degenerative changes including neuronal shrinkage assuming deep esinophilic staining with neuropilarvacuolation(Fig, 15) and glial cell infiltration. Slight congestion and hyperplasia in the wall of blood vessels also recorded (Fig, 16).

Our results agreed with **Kulasekharan(1994)** who stated that the bacterial infection in the crabs leads to an extensive damage of the brain. Some crabs showed clustered NSCs pushed to one corner and have no neurosecretory materials (NSM). The cytoplasm is unclear. The nucleus and the nucleolus are invisible. The shape and size of the NSCs are indistinguishable and are found scattered on the periphery. Other crabs showed more number of NSCs distributed along the peripheral region. The NSCs are clear with moderate staining intensity. The NSCs are with nucleus and nucleolus **(Devakumar *et al.*,2013).**The same findings were observed by **Amosu (2012)**who stated that the brain of the fish of all treatments at 7days post infection showed the same degree of neuronal degeneration, multifocal areas of gliosis, areas of vacuolation and satellitosis which was found to be more severe at 14 days post infection. All of these damages are of various degrees. The degree of all these recorded anomalies is not really concentration dependent but depends mostly on the infection period.

In-vitro sensitivity test of *Pseudomonas aeruginosa* isolates: As shown in table (6) all *Pseudomonas aeroginosa* isolates were sensitive to Colistin sulphate,danofloxacin, Nalidixic acid, Oxolonic acid, Oxytetracycline. While all the examined isolates were resistant to amoxicillin, cephalothin, erythromycin,

lincomycine, nitrofurantoin and (sulphamethoxazole + trimethoprim). Also *Pseudomonas aeroginosa* isolates were with intermediate sensitivity to Gentamycin. Concerning this point, **Algama et al.(2012)** tested the sensitivity of *P. aeruginosa*. They found that high resistance was observed against Kanamysin, Cephalothin, Sulphatriad, Colistin methane sulphonate, Tobramysin, Nitro Furantoin, Cefpodoxime, Cefoxitin and Aztreonam.Higher sensitivity performed by Tetracyclin, Cephotaxime, Ciprofloxacin, Norfloxacin, Ofloxacin, Moxifloxacin, Gatifloxacin and Levofloxacin. Out of that highest sensitivity recorded with Moxifloxacin. Also, **Eissa et al.(2010)** studied the antibiogram sensitivity of *P.anguilliseptica, P. putida* and *P. aureginosa.* Results revealed that almost all of them were highly sensitive to Avatryl and Amikicin and sensitive to Gentamicin, Erythromycin Novobiocin and Sulfa-trimethoprime. The variation in antibiotic sensitivity test of isolated *Pseudomonas aeroginosa* was described by **Hancock(1998)** who reported that P. aeruginosa is intrinsically resistant to several antibiotics because of the low permeability of its outer-membrane, the constitutive expression of various efflux pumps, and the production of antibiotic-inactivating enzymes (e.g.,cephalosporinases). **Vaisvila et al.(2001)** reported that these variation may be related to the large size and the versatility of its genome, and to its distribution in aquatic habitats, which could constitute a reservoir for bacteria carrying other resistance genes. Finally, the findings of **Dominic et al.(2005)** illustrated that antibiotic sensitivities are dependent on culture conditions and highlight the complexities of choosing appropriate combination therapy for multidrug-resistant *P. aeruginosa* in the cystic fibrosis lung.In conclusion, this study showed that *P.aeruginosa* infection is common in some common cultured freshwater fishes in Egypt. It can be diagnosed easily using PCR technique.The experimental infection of Clarias gariepinus with *P.aeruginosa* was associated with mortality rate 40 % , severe histopathological changes in all organs of infected fishes, immunosuppression and oxidative damage of tissues which reflect the significant economic importance of Pseudomonas aeruginosa infection in cultured fishes. Colistin sulphate, Danofloxacin, Nalidixic acid, Oxolonic acid and Oxytetracyclinen are recommended for control of Pseudomonas aeruginosa infection in cultured fishes.

**Table 1: Types, areas of collection and numbers of examined cultured fishes**

| Fish species | Location ( Province) | No of examined fishes |
|---|---|---|
| *Oreochromis niloticus* | Kafr el-Sheikh, Behira, Menofya, Sharkia | 160 |
| *Cyprinus carpio* | Kafr el-Sheikh, Sharkia, Menofya. | 60 |
| *Clarias gariepinus* | Behira, Kafr el-Sheikh, Sharkia and Menofya Province | 40 |

**Table 2: Biochemical characters of *Pseudomonas aeruginosa* isolated from cultured fishes.**

| Item | *P. aeruginosa* |
|---|---|
| Gram stain | - |
| Pigment | + |
| Motility | + |
| Oxidase | + |
| Catlase | + |
| H2S production | - |
| Urease | - |
| Citrate | + |
| TSI | K/K |
| Indol production | - |
| Vogas Proskour | - |
| Methyl- Red | - |
| Gelatin liquefaction | V** |
| O/F test glucose | O |
| O/F test Lactose | - |
| Glucose | + |
| Sucrose | - |
| Mannitol | - |
| Lactose | - |

* k/k = alkaline/ alkaline **V = variable

ISSN: 0975-8585

**Table 3: Incidence of *Pseudomonas aeruginosa* isolation from examined fishes.**

| Fish species | No of examined fishes | Positive cases for *Pseudomonas aeruginosa* | |
|---|---|---|---|
| | | NO. | % |
| *Oreochromis niloticus* | 160 | 55 | 34.4 |
| *Cyprinus carpio* | 60 | 18 | 30 |
| *Clarias gariepinus* | 40 | 11 | 27.5 |
| Total | 260 | 84 | 32.3 |

**Table 4: Percentage of different serum total protein fractions(gm/dl) of control and experimentally infected *Clarias gariepinus* with *Pseudomonas aeruginosa*.**

| group / protein fractions | Control | Experimentally Infected fish |
|---|---|---|
| **Total protein** | 4.23±025 | 2.93±0.17* |
| **albumin** | 1.54±0.05 | 1.10±0.02* |
| **1** | 0.17±0.01 | 0.09±0.01* |
| **2** | 0.50±0.02 | 0.27±0.08* |
| **3** | 0.20±0.02 | 0.12±0.02* |
| **4** | 0.21±0.02 | 0.17±0.03 |
| **5** | 0.32±0.02 | 0.29±0.06 |
| **6** | 1.29±0.20 | 0.89±0.08 |

**Mean ± Standard error. * , significant at level of Probability at ≤ 0.05**

**Table 5: Malondialdehyde (MDL) and nitric oxide (NO) , in control and experimentally infected group.**

| group / parameter | Control | Experimentally Infected fish |
|---|---|---|
| **MDL (mmol/ml)** | **1.3465 ± 0.08** | **3.952285 ±0.23*** |
| **NO (mmol/ml)** | **27.51270656 ± 0.16** | **32.76598499 ± 0.19*** |

**Mean ± Standard error. * , significant at level of Probability at ≤ 0.05**

**Table 6: In-vitro sensitivity of *Pseudomonas aeruginosa* isolates to different chemotherapeutic agents.**

| Chemotherapeutic agents | Concentration per disc | *P. aeruginosa* |
|---|---|---|
| Amoxycillin | 10 µg | R |
| Cephalothin | 30 µg | R |
| Colistin sulphate | 25 µg | S |
| Danofloxacin | 5 µg | S |
| Erythromycin | 15mcg | R |
| Gentamycin | 10 µg | I |
| Lincomycin | 10 µg | R |
| Nalidixic acid | 30 µg | S |
| Nitrofurantoin | 300 µg | R |
| Oxolonic acid | 2 µg | S |
| oxytetracycline | 30 µg | S |
| Sulphamethoxazole 23.7ug/ Trimethoprim 10.25 ug | 25 µg | R |

S: Sensitive I: Intermediate R: Resistant

ISSN: 0975-8585

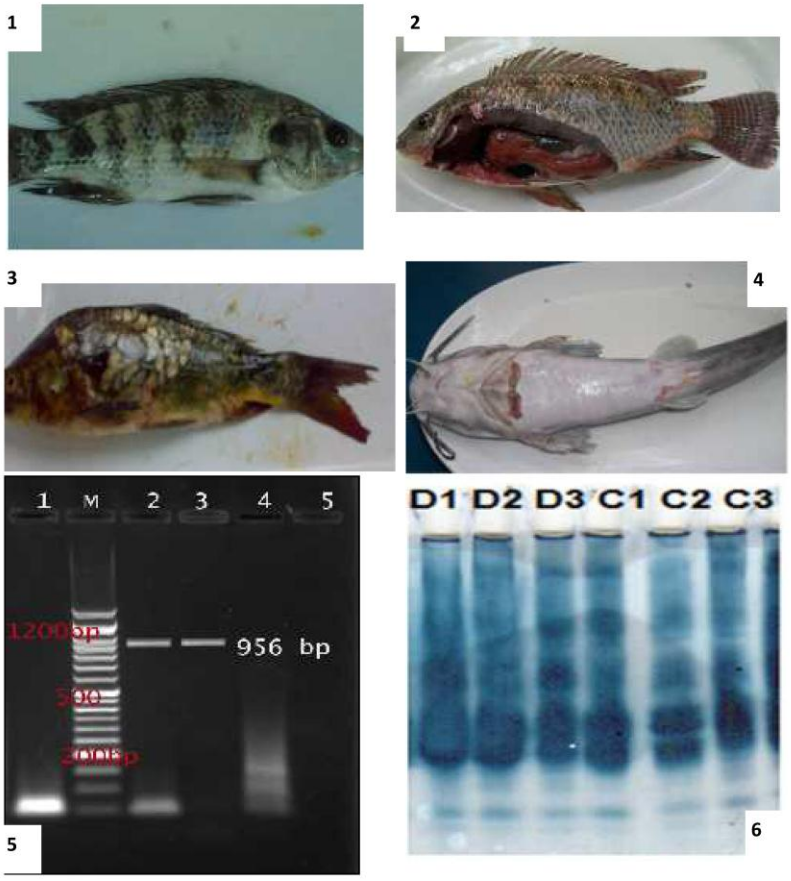


Photo 1: Naturally infected Oreochromis niloticus with Pseudomonas aeruginosa showing ulceration

Photo 2: Naturally infected Oreochromis niloticus with Pseudomonas aeruginosa showing congestion of gills , congested enlarged liver.

Photo 3: Naturally infected Cyprinus carpio with Pseudomonas aeruginosa showing hemorrhages on belly and tail.

Photo 4: Naturally infected Clarias gariepinus with Pseudomonas aeruginosa showing hemorrhages.

Photo 5: showing the electrophoresis of P. aeroginosa PCR product with the specific band at 956 bp

Photo 6: showing the electrophoretic pattern of serum protein fractions separated in experimentally infected group (lanes D1, D2, D3) and control group (lanes C1, C2, C3).

ISSN: 0975-8585

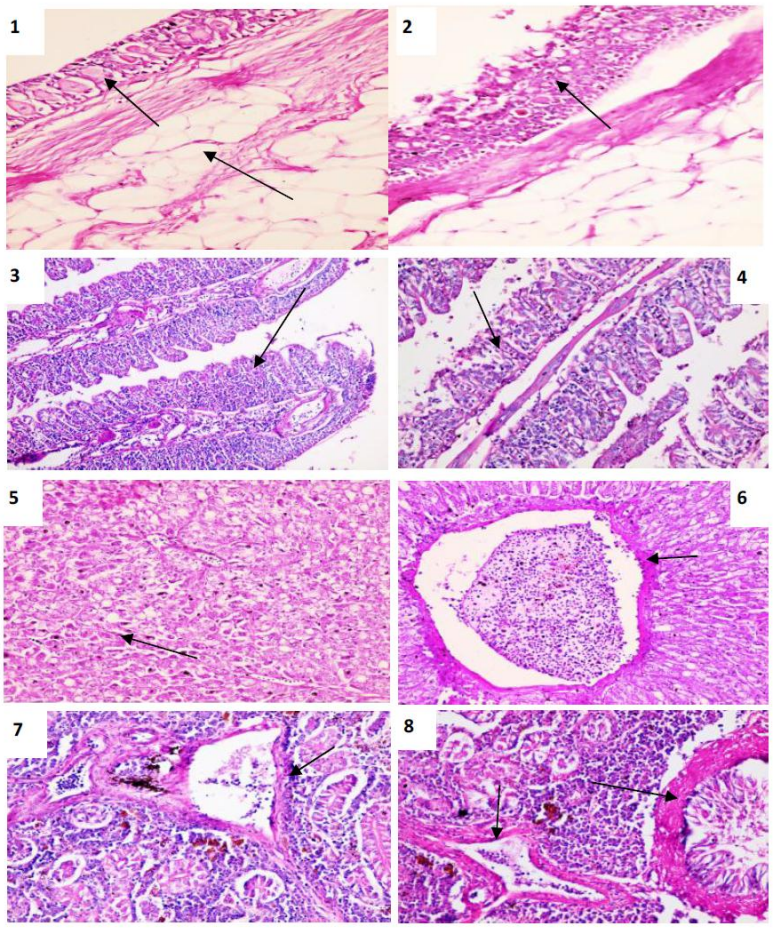


**Fig. 1:** Skin showed vacuolar degeneration and necrosis in the epidermal cells with mononuclear inflammatory cells infiltration in between the epidermal cells **H&E, X400.**

**Fig. 2:** Skin showed Activation of the goblet (mucus) cells and accumulation of edematous fluid sub-epidermal leading to splitting of the sub-epidermal connective tissue **H&E, X400.**

**Fig. 3:** Gills showed hypertrophy and hyperplasia of the epithelial cells lining the secondary lamellae resulting in lamellar fusion **H&E, X200.**

**Fig. 4:** Gills showed Epithelial lifting, epithelial necrosis, edema and desquamation of lamellar epithelium **H&E, X400.**

**Fig. 5:** liver showed vacuolar degeneration in the hepatocytes, condensation of the nuclei and mononuclear infiltration in between the hepatic parenchyma **H&E, X400.**

**Fig. 6:** liver showed shrinkage of hepatocytes, increased sinusoidal blood spaces and hepatic blood vessels, hyperplasia in the wall of blood vessels and congestion **H&E, X400.**

**Fig. 7:** kidneys showed vacuolization and necrotic changes in the tubular epithelium with sloughing off of the epithelial cells, hypertrophy of epithelial cells of the renal tubules with reduction in tubular lumens and contraction of glomeruli **H&E, X400.**

**Fig. 8:** kidneys showed widening of the blood vessels with slight congestion, severe hyperplasia in the wall of renal blood vessels, melanomacrophage cells aggregation and interstitial nephritis **H&E,X400.**

ISSN: 0975-8585

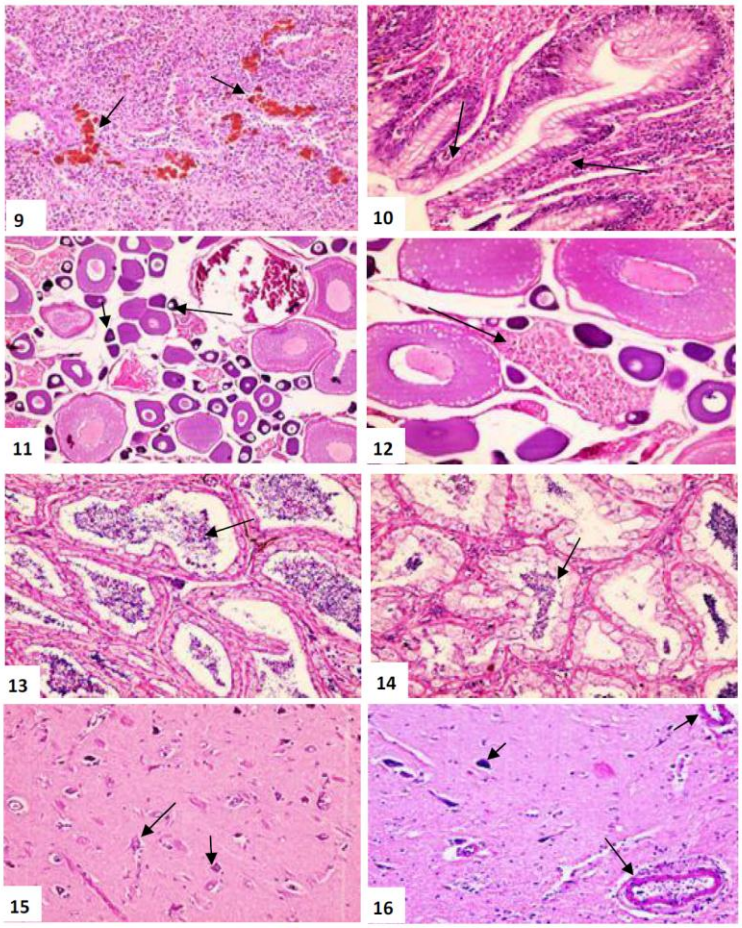


**Fig. 9:** Spleen showed clusters of melanomacrophage centers increased both in number and extension in the splenic parenchyma and depletion of lymphocytic elements **H&E, X200.**

**Fig. 10:** Intestine showed hypertrophy in the columnar epithelium of the intestinal villi and crypts, increased secretory activity with vacuolated clear cytoplasm and infiltration of inflammatory cells **H&E, X400.**

**Fig. 11:** Ovary showed stromal edema and numerous mature and immature follicles (primary and secandry oocytes) some of them are degenerated and necrotic **H&E, X200.**

**Fig. 12:** Ovary showed hemorrhage and hemosiderin pigments in the stroma **H&E, X400.**

**Fig. 13:** Testis showed degeneration, necrosis and decrease in the number of spermatogenic cells **H&E, X400.**

**Fig. 14:** Testis showed hypertrophy and vacuolar degeneration in the germinal epithelium lining the seminiferous tubules **H&E, X400.**

**Fig. 15:** Brain showed degenerative changes, neuronal shrinkage assuming deep esinophilic staining and neuropilar vacuolation **H&E, X400.**

**Fig. 16:** Brain showed degenerative changes, glial cell infiltration. Slight congestion and hyperplasia in the wall of blood vessels **H&E,X400.**

ISSN: 0975-8585

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