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Studies on Pseudomonas Septicemia among Cultured *Oreochromus niloticus*

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ABSTRACT

Pseudomonas septicemia is one of the most pathogenic bacteria affecting fish farm in Egypt, especially *Oreochromis niloticus.* In this study, *Pseudomonas aerogenosa* was isolated during an outbreaks among cultured *Oreochromis niloticus*. The isolated strain from examined fish were indol –ve , nitrate +ve , glucose +ve, lactose –ve and sensitive to Amikacin and Gentamicin .The most clinical signs in both natural and experimental infected fish were darkening of body, loss of scales, tail rot and congestion of all internal organs.

Keywords:

INTRODUCTION

Fish diseases due to bacterial infections are the major problems in aquaculture as it found naturally in the fish environment and under certain stress condition causes severe economic losses to fish farms (Post, 1987 and Olsson *et al.,* 1998).

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) .

The green fluorescent species, *P. fluorescens* and *P. putida* are the genus of fresh and brackish water fish. *P. fluorescens* participated in causing freshwater Eel disease (Red pest, Red fish disease), Red spot disease of pike (Red sore disease) and Red spot disease of *Cyprinids, Percids and Coregonids* (Schaperclaus *et al.,* 1992).

Pseudomonas fluorescens affects freshwater and salt-water fish throughout the world and causes severe economic losses and decrease fish farms efficiencies.(Stoskopf 1993 and Fayed *et al.*1997)

The genus *Pseudomonas* contains five species which have been described as etiological agents of

diseases in fish in Egypt and moreover, it is a septacemic disease characterized by fin rot ,usually associated with stress and improper management (Azza *et al.,* 2002).

Pseudomonas flurescens is widely distributed in aquaculture industries and considered as one of the primary cause of *Bacterial hemorrhagic septicemia* in fish, usually associated with stress conditions such as overcrowding (Allen *et al.,* 1983; Frerichs and Holliman, 1991 and Azza *et al.,* 2002), low temperature and injuries (Aly, 1994 and Abdomenech *et al.,* 1999), as well as secondary invader of damaged fish tissue (Otte, 1963*)* and in chronic virus infection (Roberts and Horne, 1978).Natural mortalities were highest when water temperature at 15 – 20 C (Austin and Austin, 1993).

The aim of this study was isolation and identification of *pseudomonas species* during an outbreaks among *Oreochromus niloticus* last winter in Alexandria Governorate.As well as the pathogencity and antibiotic susceptibility of the isolated bacteria.

MATERIALS AND METHODS

Naturally infected fish

A total number of 50 *O.niloticus* (80± 5 gm) showed scale losses and fin erosion plus signs of septicemia were collected a live from a private fish farm in Alexandria Governorate suffered from high mortality .The farm had bad management in the form of increased organic matter ,high stocking rate (30 000 fish/fadan) and bad water quality .

Clinical signs and post mortem examinations:-

Both freshly dead and sacrificed fish ,either naturally or experimentally infected were examined according to Amlacher (1970) and lucky(1977).

Bacteriological examination

Bacteriological examination was done according to Amlacher (1970) ..Swabs were taken from internal organs under complete aseptic conditions ,inoculated on trypticase soya broth and agar and incubated for 24 hours at 30C. Suspected colonies were picked up and kept on TSA plates for further identification.

Identification of isolated bacteria

Growth and morphological characters of isolated bacteria as well as biochemical characters were done according to Allen *et al*.,(1983) and Austin and Austin (1993).

Antibiogram

The isolated bacteria was tested for their antibiotic sensitivity by using disc diffusion technique according to Cruickshank *et al*., (1982).

Determination of the median lethal dose (LD_{50})

The LD_{50} was evaluated by intraperitonial (i.p) injection of *O.niloticus* (60± 5 gm), with 0.2 ml from serial ten fold dilutions (10^{-1}-10^{-6}) of the isolate at a rate of ten fish /dilution. Moreover, 10 fish were injected with 0.2 ml sterile saline and served as control. The LD_{50} was calculated according to Reed and Munch(1938).

Experimental infection

Experimental infection was carried out by intramuscular injection of 10^3 CFU /0.2 ml /fish. Thirty fish were injected with bacteria and 10 fish were inoculated with sterile saline and kept as a control .

The injected fish were kept under daily observation for 21 days in aeriated glass aquarium (100x50x30 cm) containing dechlorinated tap water at a temperature of 18-20C . The fish were fed on commercial diet containing 25% protein at a rate of 3% of average body weight .

All fish used for injection were previously examined for the presence of injected bacteria and proved to be negative .

RESULTS

Clinical observations

The naturally infected *O.niloticus* showed skin discoloration , scale loss, tail erosion, erythema at the base of fins and some fish showed slight abdominal distention and exophthalmia. Moreover, P.M. lesions were in the form of congestion of all internal organs . Also, there was dullness and loss of reflexes .

Bacteriological examination

A pure culture of isolated bacteria was successfully done from blood ,liver, spleen and kidneys of naturally infected *O.niloticus* .The bacterial growth were creamy, rounded, gram negative and motile . The biochemical patterns of representative of the recovered isolate are summarized in Table (1).

Antibiogram

The isolated bacteria appeared to be highly sensitive only to gentamycin and amikain. Moreover, it was resistance to amoxicillin, erythromycin, flumoquin, cefazolin, ciprofloxacin and sulphamethoxazole +trimethoprim.

Median lethal dose (LD_{50})

The calculated LD_{50} of the isolated bacteria was $10^{4.5}$ viable bacterial cell /ml, (Table 2).

Experimental infection :

Experimental infection was successfully done by I.M injection of 10^3 CFU viable cells of

Table (1) : ***Biochemical characters of the isolated Pseudomonas Sp.***

Biochemical reactions	Results	Biochemical reactions	Results
Indol	-ve	Glucose	+ve(only acid)
Nitrate	+ve	Lactose	-ve
H2S	-ve	Sucrose	-ve
oxidase	+ve	Mannitol	+ve(only acid)
Catalase	+ve	Maltose	-ve
Citrate	+ve	Dulcitol	-ve
Methyl red	-ve	Cytochrome oxidase	+ve
Vorges proskauer	-ve	Pigment production	Fluorescence with U.V.R at 4-40 c
Urease	+ve		

P. aerugenosa / fish. The fish showed sluggish movement , loss of reflexes , erythema at base of fins, scale loss , slight dark discoloration .

Slight exophthalmia and abdominal distention were recorded .

Internally there were congestion of all internal organs and presence of ascetic fluid .

Moreover, mortality rate of 30% was recorded during the period of experiment .

P.aerugenosa was re-isolated from freshly dead fish in case of all experiment to verify the specificity of deaths.

Table (2): LD_{50} and mortality ratio in *Oreochromis niloticus*. *(Reed and Munch, 1938).*

Conc. of bacteria	**Mortality ratio of *O. niloticus***
10^{-1}	**9/10**
10^{-2}	**7/10**
10^{-3}	**6/10**
10^{-4}	**5/10**
10^{-5}	**4/10**
10^{-6}	**3/10**
Control	**0**

§ **LD_{50} in *O. niloticus*:** **P.D.** = $\frac{60-50}{60-40} = 0.5$ **$LD_{50} = 10^{4.5}$**

DISCUSSION

Pseudomonas species is widely distributed in aquaculture industries and considered as one of the primary cause of fin rot in fish and constitutes a part of the normal microflora of the aquatic environment , with maximum and minimum numbers in winter and summer respectively (Adomenech *et al.,* 1999) .

Pseudomonas species affectes freshwater and marine fish through out the world and causes sever economic losses and decreases fish farm efficiencies *(Fayed et al., 1997 ;Olsson et al., 1998).*

In this study we followed the methods of allen *et al.,* (1983) ;Austin and Austin (1993) using the tube methods.

The *pseudomonas arugenosa* isolate, was gram negative aerobic and motile . The isolate was positive for nitrate reduction , oxidase , catalase, urease and glucose and negative for indole ,H2S, methyle red , Vogus prosqeur ,sucrose, manitol and dulcitol.

The obtained results of morphological and biochemical tests were sufficient to presumptively identify isolate as *pseudomonase aerugenosa* .

These results were similar to those reported by Allen *et al.,* (1983) ; Stoskopf(1993).

The clinical signs and P.M lesions were similar in both of natural and experimental infection in the form of absence of reflexes, dark discoloration ,scale loss ,fin rot ,hemorrhage of the body and congestion of the internal organs . These signs has been reported by Miyazaki *et al.,* (1984); Badran (1993) and Azza *et al.,* (2002).

The clinical symptoms , P.M lesions and histopathological changes may be attributed to the action of bacterial toxins.

This explanation supported by the statement of Braude (1964) and Nowotny (1979) . They reported that the nature of pathogenesis caused by all gram negative bacteria was almost similar and the disease process was caused by bacterial toxins .

The presence of bad management and water quality in the farm may act as stress and facilitate the incidence of infection . Kitao *et al.,* (1993) stated that, *pseudomonase* 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.

The same conclusion were reported by Allen *et al.,* (1983) ;

Frerichs and Holliman (1991) and Azza *et al.*, (2002).

The LD_{50} value of isolated *pseudomonas aerugenosa* was $10^{4.5}$. Semons (1994) reported that the LD_{50} of *Pseudomonas* in marine fish was $10^{3.75}$,while *Abd* El-gaber and Samira Rezeka (1999) recorded the LD_{50} value for *Pseudomonas florescence* was 10^{3} . The variation may be due to the differences in fish and *pseudomonas species* as well as environmental conditions.

The results of antibiotic sensitivity of the isolated strain in this study revealed that , it was sensitive to a narrow range of antibiotics namely ,gentamycin and amikin .

While it was resistance to a wide range of recent antibiotics . This finding indicated that these strain has been exposed to un proper dose and/or course .This could be a disadvantage as regards the control of the disease in cultured conditions.

Also, this may be attributed to uncontrolled use of antibiotics in the field by the owner in the treatment and physicochemical properties of the cell wall rather than the antibiotic inhibiting enzymes. (Koncicki and Szubstmaka 1998). Also, El- jakee *et al.*, (1995) found that *P.aerugenosa* strains were highly resistant to many antibiotics and such phenomenon might be due to R-factor.

On conclusion , antibiotic sensitivity test should be carried out before prescription of antibiotics for field application as well as the improving of management in fish farm is a must ,also *P. aerugenosa* can be considered as accountable fish infection under culture condition.

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دراسات على السودوموناس الدموية بين أسماك البلطى النيلى المستزرعة

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السودوموناس سيبتيسيميا تعتبر أحد أنواع البكتيريا الضارة جداً والتى تصيب أسماك المزارع فى مصر فى هذه الدراسة تم عزل السودوموناس أيروجينوزا من أسماك البلطى النيلى ووجد أن العترة المعزولة سالبة الإندول واللاكتوز وموجبة للجلوكوز والنيترات وحساسة للأميكين والجنتاميسين وكانت أهم أعراض الإصابة سواء فى الحالة الطبيعية أو الحقن إسوداد لون الجسم وفقدان القشور وتآكل الزعانف واحتقان الأعضاء الداخلية .