ISSN 110-2047.ALEX.J.VET.SCIENCE.2007.VOL 25 NO 1

# BACTERIAL CAUSES OF SKIN LESIONS IN SOME FRESHWATER FISH

BY

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## SUMMARY

The aim of this study was to isolate and identify the bacteria associated with skin lesions and ulcerations and its frequent distribution in the most cultured fish species in Upper Egypt as ***Nile tilapia (Oreochromis niloticus), Common carp (Cyprinus carpio)*** and Sharp tooth catfish ***(Clarias gariepinus).*** This study was carried on 150 fish showing skin lesions that were collected from different fish farms in El-Minia and Assiut provinces, of which 30 ***Oreochromis niloticus***, 40 ***Common carp*** and 80 ***Sharp tooth Catfish.*** Also, pathogenicity of the predominating strains isolated from ***Oreochromis niloticus*** and ***Sharptooth catfish*** and their antibiogarm were investigated.

Results revealed that ***Flavobacterium columnare, Aermonas hydrophila*** **and** ***Pseudomonas sp.*** were the predominant bacteria isolated from skin lesions of the three fish species. ***F. columnaris*** was the predominant bacteria isolated from skin lesions of ***Nile tilapia, common carp and sharptooth catfish*** at a rate of 36.8%. 37.3% and 36.3%, respectively. On the other hand, ***A. hydrophila*** was the second leading bacteria that caused skin lesions in ***Nile tilapia, common carp*** and ***sharptooth catfish*** and isolated at a rate of 26.3%, 29.4% and 20.6%, respectively. Furthermore, ***Pseudomonas sp.*** was the third major cause of skin bacterial infection and was isolated at a rate of 18.4%, 19.6% and 14.7% from ***Nile tilapia, Common carp and sharptooth catfish***, respectively.

Also, ***F. columnare, A. hydrophlia*** **and** ***Pseudomonas sp.*** isolated from skin lesions of naturally infected fish were able to induce infections in challenged fish showing nearly the same clinical signs observed on naturally infected fish. The antibiogram of such strains were, also, discussed.

## INTRODUCTION

Causes of skin lesions in fish are diverse and include trauma, poor water quality, stress, parasitism and viral, fungal and bacterial infections. Fish skin is a primary target for a number of common infections ***(Noga, 1996).*** Bacterial ulcers are a common fish disease problem, particularly with ornamental and pond fish. They are one of the most difficult problems to deal with, especially if large numbers of fish are affected ***(Burgess et al., 1998).***

Large hemorrhages, epidermal degeneration followed by sloughing of scales, necrosis and cutaneous ulcers are the principal symptoms of skin bacterial diseases that had been responsible for large-scale mortality in many species of fish ***(Burgess et al., 1998).*** ***Angka et al., (1988)*** isolated ***Micrococcus sp.*** **and** ***Aeromonas hydrophila*** from ulcerated ***Clarias lazera,*** while **Okaeme et al., (1988)** isolated **Myxobacteria** from ulcerated ***C. lazera.*** **Kar (1999)** stated that bacteriological examination of the surface lesions and other organs of fishes showing signs of bacterial infections result in isolation of haemolytic strains of ***Eschericha coli, A. hydrophila, Pseudomonas aeruginosa, Staphylococcus epidermitis and Klebsiella sp. Also, Bakeer et al., (1991)*** reported that ***Flavobacterium columnare, Pseudomonas sp., Aeromonas sp., Staphylococcus sp. and Proteus sp.*** caused ulceration in tilapia, while ***Atallah etal(1997)*** concluded that ***F. Columnaris,*** A.hydrophila and ***Pseudomonas fluorescence*** were the most common.

Since clinical signs of skin bacterial infections are rarely pathognomonic or diagnostic for any specific pathogen, definitive diagnosis of the cause requires the culture of the pathogen(s) from skin lesions. Also, the culture of the pathogen(s) is essential to determine antibiotic sensitivity which often varies widely between isolates ***(Noga, 1996).***

Present study was conducted to investigate the predominant bacteria associated with skin lesions and ulcerations in ***Nile tilapia (Oreochromis niloticus),Common carp (Cyprinus carpio) and sharptooth catfish (Clarias gariepinus),*** the three most cultured fish species in Upper Egypt. Also, pathogenicity of bacteria isolated and their antiobiogram were determined.

## MATERIAL AND METHODS

### 1-Fish

150 fish showed skin lesions were collected from different fish farms in El-Minia and Assiut provinces and transferred to the laboratories on ice for clinical and bacteriological examination. Eamined fish were 30 ***Nile tilapia (Oreochromis niloticus), 40 common carp (Cyprinus carpio) and 80 sharptooth catfish (Clarias gariepinus).***

### 2- Clinical examination:

Clinical examination of natural and experimental infected fish was performed according to ***Stoskopf (1993).***

### 3- Bacteriological examination:

Bacteriological isolation from skin lesions was conducted according to ***Sherbina (1973).*** Samples from skin lesion were inoculated into 10% sterile peptone water, and incubated at 25°C for 18 hrs. A loopfull from each broth culture was streaked on brain heart infusion (BHI) agar, blood agar, MacConkey agar, Hsu-Shotts

agar, Rimler-Shotts agar, and Cytophaga agar at 25°C for 24 or 48 hrs. Suspected colonies were picked up and subculture for purification. A loopfull of each pure culture was inoculated on two tubes of nutrient agar slopes for further identification. Pure isolates were identified based on colony morphology, Gram-stain and biochemical characters according to ***Anacker and ordal, 1959, Shott and Rimler (1973), Lucky (1977), Cruickshank et al., (1982), Shotts (1991), Inglis et al., (1993), Chen et al., (1995).***

**4- Detection of isolates pathogenicity in *Oreochromis niloticus and sharptooth catfish*:**

Three isolates of bacteria, ***A. hydrophlia, Pseudomonas sp.*** **and** ***F. columnare*** were used. A total number of 50 apparently healthy ***Nile tilapia*** with an average weight of 45±5g and 50 apparently healthy ***sharptooth catfish*** with an average weight of 75±5g were used. Fishes were subdivided into groups and inoculated by scarification of the skin (Lucky, 1977) with 0.5ml of bacterial suspension containing $2x10^7$ colony forming units (cfu)/ml or sterile broth (control), while a control group of each species remained un-scarified as per the table below. All groups were observed daily for 10 days post inoculation to record clinical changes and mortalities. Re-isolation of bacteria from the inoculated fishes was done as described above. The same procedures were used in case of catfish.

| Isolate | Fish | No. of fish | Route | Dose |
|---|---|---|---|---|
| *Aeromonas hydrophlia* | *Tilapia* | 10 | S/S | 0.5ml of $2x10^7$ cfu/ml |
| | *Catfish* | 10 | S/S | 0.5ml of $2x10^7$ cfu/ml |
| *Pseudomonas sp.* | *Tilapia* | 10 | S/S | 0.5ml of $2x10^7$ cfu/ml |
| | *Catfish* | 10 | S/S | 0.5ml of $2x10^7$ cfu/ml |
| *Flavobacterium columnare* | *Tilapia* | 10 | S/S | 0.5ml of $2x10^7$ cfu/ml |
| | *Catfish* | 10 | S/S | 0.5ml of $2x10^7$ cfu/ml |
| *Sterile broth (control)* | *Tilapia* | 10 | S/S | 0.5ml |
| | *Catfish* | 10 | S/S | 0.5ml |
| *Un-scarified control* | *Tilapia* | 10 | - | - |
| | *Catfish* | 10 | - | - |

*** S/S means scarification of the skin**
*** CFU means colony forming units**

**6) Antibiogram test:**

The antibiogram of random 10 isolates of ***A. hydrophlia, Pseudomonas sp.*** **and** ***F. columnare*** was investigated against 10 antimicrobial agents using the disc diffusion technique as described by Finegold and Martin (1982). Antibiotic sensitivity was determined based on the diameter of clearance

zone around the discs according to the manufacturer recommendations (Oxoid, Basingstoke, Hampshire, England). Used antibiotics were Neomycin (30ug), Gentamycin (10ug), Chloramphenical (30ug), Oxytetracycline (30ug), Nalidixic acid (30ug), Ampicillin (10ug), Erythromycin (15ug), Amoxicillin (25ug), Streptomycin (10ug) and Enerofluxacin (10ug).

## RESULTS

### 1-Clinical signs:

Intensity of the skin lesions caused by infections depended on the fish species and causative agent(s) involved. Clinical signs of infection with ***F. columnare in Nile tilapia*** and ***Common carp*** were confined to fin erosions and rot and presence of areas of erosions on the body scales, while in ***Sharptooth catfish*** were fin rot, local loss of pigment and presence of large dull area at the base of dorsal fin. External signs of infection with ***A. hydrophila in Nile tilapia (O. niloticus)*** were hemorrhages on all body surface, skin erosion, scales loss, fin rot, and ulcers (fig.1), while in ***Common carp (C. carpio)*** were shallow white erosions, hemorrhages, sloughing of scales and cutaneous ulcers. ***Sharptooth catfish (C. gariepinus)*** infected with ***A. hydrophila*** showed scattered haemorrhages with local loss of pigmentation and ulcers. Infection with ***Pseudomonas sp.*** in the three fish species caused haemorrhages on body surface and erosions and, sometimes, ulcers.

### 2-Bacteriological examination:

Bacteriological examination of fish samples resulted in isolation of 225 isolates. According to cultural, morphological and biochemical characteristics, 191 isolates were identified as fish pathogens while the 34 unidentified strains were considered water-born non-specific organisms that contaminated skin samples according to morphology and Gram staining characters and were excluded. Strains isolated were ***Flavobacterium columnare (70 isolates), Aeromonas hydrophila (46 isolates), Pseudomonas sp. (32 isolates), Edwardsiella tarda (12 isolates), Proteus sp. (11 isolates), Echerichia coli (10 isolates), Streptococus sp. (6 isolates) and Staphylococcus sp. (4 isolates)*** from all fishes examined (table 1).

Frequent distribution of the total number of bacteria isolated showed that ***F. columnare, A. hydrophila*** and ***Pseudomonas sp.*** were the three predominant isolates (table 1). Frequent distribution of bacteria isolated from each fish species revealed that ***F. columnare*** was the predominant strain in ***Nile tilapia, Common carp and Sharptooth catfish*** and isolated from skin lesions at a rate of 36.8%. 37.3% and 36.3%, respectively. On the other hand, ***A. hydrophila*** was the second leading bacteria that caused skin lesions in ***Nile tilapia, Common carp and sharptooth catfish*** and isolated at a rate of 26.3%, 29.4% and 20.6%, respectively. Furthermore, ***Pseudomonas sp.*** was the third major cause of skin bacterial infection and

was isolated at a rate of 18.4%, 19.6% and 14.7% from ***Nile tilapia, common carp and sharptooth catfish,*** respectively. Other bacteria species isolated, ***Edwardsiella tarda, Proteus sp., Staph. sp. and E. coli,*** were considered minor causes of skin lesion in the three fish species(table 1).

Results revealed that there were a total of 41 cases of mixed infection where two or more different species of bacteria isolated from a single fish. The combinations were quite diverse with no specific or frequent incidences. ***F. columnare, A. hydrophila*** **and** ***Enterobacteriaceae,*** however, were major components of those combinations.

**3-Pathogenicity of *F. columnare, A. hydrophlia,* and *Pseudomonas sp.* in *Nile tilapia and Sharptooth catfish:***

Fishes experimentally infected with ***F. columnare, A. hydrophlia*** **and** ***Pseudomonas sp.*** showed nearly the same clinical signs that are observed on naturally infected fishes. ***Nile tilapia*** inoculated with ***F. columnare*** developed skin erosions and dorsal fin rot near the site of inoculation and 2 out of 10 inoculated fish died. ***Sharptooth catfish*** inoculated with ***F. columnare*** showed loss of skin pigmentation and erosions at the base of dorsal fin where bacteria were inoculated (fig.2), and 4 fish died. The main clinical signs observed on ***Nile tilapia*** inoculated with ***A. hydrophlia*** were loss of scales and skin erosions. Four out of ten Nile tilapia inoculated with ***A. hydrophlia*** developed general septicemia and skin ulcers and died within the first week of the experiment. The main clinical signs noticed on ***sharptooth catfish*** inoculated with ***A. hydrophlia*** were erosion and ulcers at site of inoculation and 3 out of 10 inoculated fish died. ***Nile tilapia and Sharptooth catfish*** inoculated with ***Pseudomonas sp.*** showed redness and ulcerations at site of inoculation. Data are summarized in table 2.

**4- Antibiogram test:**

In the present study it was evident that ***F. columnare*** was sensitive to Enerofloxacin, Oxytetracycline, and Chloramephenical, while was resistant to Erythromycin, Neomycin, Amoxicillin, Gentamycin and Ampcillin. *A. hydrophila* was sensitive to Enerofloxacin, Oxytetracycline, Chloramephenical and Nalidixic acid, while was resistant to Erythromycin, Ampcillin, Amoxicillin and Streptomycin. ***Pseudomonas sp.*** was sensitive to Enerofloxacin, Oxytetracycline, Chloramephenical, Nalidixic acid and Streptomycin, while resistant to Erythromycin, Ampcillin, Amoxicillin. Table (3) summarizes the results of sensitivity to antimicrobial agents used in this study.

**Table 1**. Frequent distribution of bacteria isolated from skin lesions in ***Nile tilapia (Oreochromis niloticus), common carp (Cyprinus carpio) and sharptooth catfish (Clarias gariepinus)***

| Isolate | Fish sp. | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | *Nile tilapia* | | *Common carp* | | *catfish* | | |
| | No. | % (n=38) | No. | % (n=51) | No. | % (n=102) | |
| **Gram-negative bacilli** | | | | | | | |
| ***Flavobacterium columnare*** | 14 | 36.8 | 19 | 37.3 | 37 | 36.3 | 70 |
| ***Aeromonas hydrophlia*** | 10 | 26.3 | 15 | 29.4 | 21 | 20.6 | 46 |
| ***Pseudomonas sp.*** | 7 | 18.4 | 10 | 19.6 | 15 | 14.7 | 32 |
| ***Edwardsiella tarda*** | 1 | 2.6 | 0 | 0.0 | 11 | 10.8 | 12 |
| ***Proteus sp.*** | 1 | 2.6 | 1 | 2.0 | 9 | 8.8 | 11 |
| ***E. coli*** | 2 | 5.3 | 2 | 3.9 | 6 | 5.9 | 10 |
| **Gram-positive cocci** | | | | | | | |
| ***Streptococcus sp.*** | 2 | 5.3 | 2 | 3.9 | 2 | 1.9 | 6 |
| ***Staphylococcus sp.*** | 1 | 2.6 | 2 | 3.9 | 1 | 1.0 | 4 |
| **Total** | 38 | 100% | 51 | 100% | 102 | 100% | 191 |

"n" refers to number of isolates

**Table 2**. Experimental infection of ***Nile tilapia (Oreochromis niloticus)*** and ***sharptooth catfish (Clarias gariepinus) with Flavobacterium columnare, Aeromonas hydrophlia and Pseudomonas sp.***

| Type of isolate | Fish species | No. of fish | Death / Day | | | | | | | | | | Mortality rate | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | No. | % |
| ***Flavobacterium columnare*** | ***Tilapia*** | 10 | - | - | 1 | - | 1 | - | - | - | - | - | 2 | 20% |
| | ***Catfish*** | 10 | - | 1 | 1 | - | - | - | 1 | - | 1 | - | 4 | 40% |
| ***Aeromonas hydrophlia*** | ***Tilapia*** | 10 | 1 | - | 1 | - | 2 | - | - | - | - | - | 4 | 40% |
| | ***Catfish*** | 10 | - | 1 | - | 1 | 1 | - | - | - | - | - | 3 | 30% |
| ***Pseudomonas sp.*** | ***Tilapia*** | 10 | - | 1 | 1 | - | 1 | - | - | - | - | - | 3 | 30% |
| | ***Catfish*** | 10 | - | - | 1 | 1 | - | - | - | - | - | - | 2 | 20% |
| ***Sterile broth*** | ***Tilapia*** | 10 | 1 | - | - | - | - | - | - | - | - | - | 1 | 10% |
| | ***Catfish*** | 10 | - | - | - | - | - | - | - | - | - | - | 0 | 0% |
| ***Un-scarified control*** | ***Tilapia*** | 10 | - | - | - | - | - | - | - | - | - | - | 0 | 0% |
| | ***Catfish*** | 10 | - | - | - | - | - | - | - | - | - | - | 0 | 0% |

**Table 3**. Antibiogram for ***Flavobacterium columnare, Aeromonas hydrophlia*** **and** ***Pseudomonas sp.*** isolated from examined fish.

| Antibacterial agent | Isolates | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *A. hydrophlia* | | | *Pseudomonas sp.* | | | *F. columnare* | | |
| | S | I | R | S | I | R | S | I | R |
| Ampcillin (10ug) | - | 1 | 9 | - | 1 | 9 | 3 | 1 | 6 |
| Neomycin (30ug) | 8 | 1 | 1 | - | - | 10 | 2 | 1 | 8 |
| Enerofloxacin (5ug) | 10 | - | - | 8 | 2 | - | 10 | - | - |
| Amoxicillin (25ug) | - | 1 | 9 | - | 1 | 9 | - | 2 | 8 |
| Erythromycin (15ug) | - | - | 10 | - | - | 10 | - | - | 10 |
| Oxytetracycline (30ug) | 9 | 1 | - | 9 | 1 | - | 10 | - | - |
| Streptomycin (10ug) | - | 2 | 8 | 7 | 1 | 2 | 4 | 1 | 5 |
| Nalidixic acid (30ug) | 9 | 1 | - | 9 | 1 | - | 7 | 2 | 1 |
| Chloramephenical (30ug) | 9 | 1 | - | 10 | - | - | 8 | 2 | - |
| Gentamycin (10ug) | 6 | 1 | 3 | 4 | 3 | 3 | 2 | 1 | 7 |

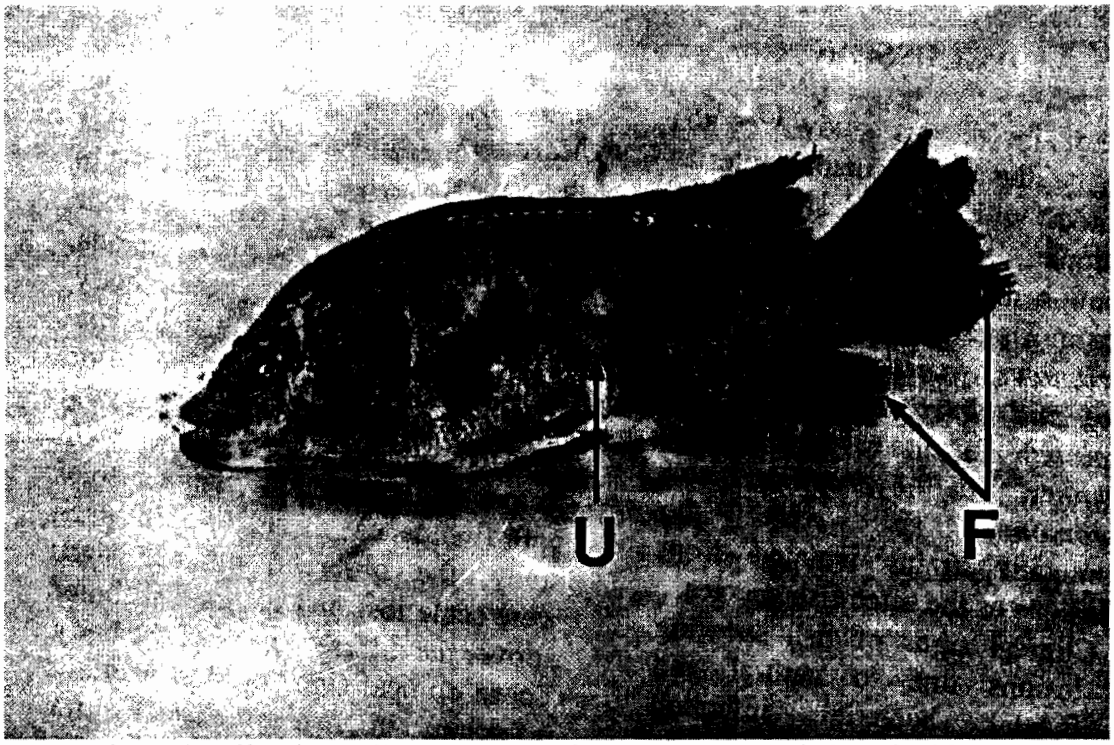


**Figure1**. ***Nile tilapia (Oreochromis niloticus)*** naturally infected with ***Aeromonas hydrophila*** showing ulceration on the skin (U) and fin rot (F).

**Figure2.** ***Sharptooth catfish (Clarias gariepinus)*** experimentally inoculated with ***Flavobacterium columnare*** showing skin erosions and ulceration at site of inoculation.

## DISCUSSION

The aim of this study was to investigate the predominant bacteria associated with skin lesions and ulcerations and their frequent distribution in the most cultured fish species in Upper Egypt, ***Nile tilapia (O. niloticus), common carp (C. carpio) and sharptooth catfish (C. gariepinus).*** Results revealed that ***F. columnare, A. hydrophila and Pseudomonas sp.*** were the predominant strains isolated from skin lesions of the three fish species.

Clinical signs of fish showing skin lesions due to natural and experimental bacterial infection described in the present study nearly similar to those reported by ***Post (1987), Plumb (1994), Enany (1995) and Durborcw et al., (1998). Moreover, Inglis (1993) stated*** that the main signs of bacterial skin infections in fish include development of reddened lesions, sores, or ulcers on the body, reddening of the base of the fins, and dullness or darkening of skin color. Furthermore, ***Eissa et al., (1996)*** reported ulcer diseases and skin lesions that affected all tilapia species with incidence of 12%. In such study, the observed clinical signs were scale loss and ulcers distributed allover the different parts of the body. Those clinical signs of skin bacterial infections in fish are usually not pathogen-specific, hence diagnosis of the causative agents depends on their

isolation and identification. Diagnosis of the causative agents can, also, be more complicated by the presence of several organisms. Any water-born organism can colonize open skin lesions of fish, thus it is important, but difficult, to isolate the initiating or primary cause of the skin lesions. Alternatively, the predominant bacteria should be identified as suggested by ***Noga (1996).*** In the present research 34 isolates were discovered.

Such findings are in accordance with those reported by ***Atallah et al., (1997)*** who concluded that ***F. columnare, A. hydrophila and Pseudomonas florescence*** are the main causes of skin lesions and fin rot in ***Nile tilapia, common carp and sharptooth catfish. Eissa et al., (1996),*** however, concluded that that ***F. columnare, A. hydrophila, Vibrio sp.*** are the predominant bacteria isolated from skin of different species of diseased tilapia.

***Decostere et al., (1999)*** conclude that ***F. columnare*** is an important cause of gills and skin disease in fresh water fish. In the present study, *F. columnare*, was considered the main bacteria causing skin lesions in ***Nile tilapia, common carp and sharptooth catfish.*** Similar findings were reported by ***Atallah et al., (1997)*** who isolated ***F. columnare*** from skin and fins of fish showing skin and fin lesions at a higher rate than all other bacteria isolated. In addition, ***Thune (1991)*** found that out of 53 cases involving ***F. columnare*** in channel catfish, only 11% showed external lesions, 17% were internal infections and 72%, however, were a combination of the two. Also, ***Eissa et al., (1996)*** showed that ***F. columnare*** was the predominant bacteria isolated from skin lesions of tilapia with incidence of 56.7% compared to the total number of bacteria isolated from skin.

Results showed that ***A. hydrophila*** is the second leading cause of skin bacterial lesions in ***Nile tilapia, common carp and sharptooth catfish.*** Nearly similar findings were reported by ***Bakeer et al (1991). Moreover, Eissa et al., (1996)*** had showed that ***A. hydrophila*** is the second most frequent bacteria isolated from skin lesions of different species of tilapia relative to the total number of bacteria isolated from skin. ***Atallah et al. (1997),*** also, reported that ***A. hydrophila*** was the second leading bacteria isolated from skin and fins of ***sharptooth catfish;*** however, it was the third leading bacteria isolated from ***Nile tilapia and common carp.***

***Pseudomonas sp.*** was the third major cause of skin bacterial infection in the three fish species examined in the current study. ***Atallah et al., (1997),*** isolated ***Pseudomonas florescence*** from ***tilapia, common carp and sharptooth catfish*** showing skin lesions and fin rot at a rate higher than that in the present study and concluded that ***Ps. florescence*** was the third major bacteria isolated from skin lesions in ***sharptooth catfish,***

while it is second in case of ***Nile Tilapia and common carp. Eissa et al., (1996),*** however, isolated ***Pseudomonas aurognosa*** from skin of different species of diseased tilapia at a lower rate (4.05%) than that in the present study. In such study, the lower rate of could be due to the fact that the fish had a generalized condition of septicemia rather than a confined case of bacteria skin infection as the clinical signs of diseased fish described support this suggestion.

In the present study, ***F. columnare, A. hydrophila, and Pseudomonas sp.,*** the three predominant strains isolated from skin lesions of the three fish species, were used to challenge fish. Results the pathgenicity test of ***F. columnare*** showed that ***Nile tilapia*** inoculated by S/S with $1x10^7$ cfu/ml developed nearly the same clinical signs observed on naturally infected fish. Such findings were met by ***Bakeer et al (1991)*** who stated that S/C inoculation of $4x10^7$ cells/ml ***F. columnare*** resulted in loss of scales, excessive mucous, loss of pigment and skin ulcers of infected fish. Experimental infection of ***catfish*** with ***F. columnare, A. hydrophila, and Pseudomonas sp.*** produced erosions and ulcers on the skin beside petechial hemorrhages on tail and fin rot as reported by ***Atallah et al. (1997).*** Also, ***El-Bouhy (1985)*** recorded that typical signs and gross lesions had appeared on ***O. niloticus*** inoculated with 0.5 ml of ***Ps. fluorescence*** suspension as observed on naturally infected fish.

Results of the antibiogram of ***F. columnare*** presented in this study are in partial concurrence with those of ***Aly (1984), Kar (1999)*** and ***Abdel-Rahman (2002),*** while those of ***A. hydrophila*** met with those recorded by ***Angka and Lioe (1982), Kar (1999), Eissa (1994, 1996).*** Results of the antibiogram of ***Pseudomonas sp.*** nearly consent with those found by ***Faisal and Eissa (1987), Kar (1999), Enany et al., (1995) and Eissa et al., (1996)*** found that ***Pseudomonas putida*** was sensitive to Oxytetracycline, Chloramephenical Nalidixic acid, while resistant to Ampcillin and Amoxycillin.

From that results we concluded that the present study clearly revealed that ***F. columnare A. hydrophila and Pseudomonas sp.*** are the leading cause for skin lesions in ***Nile tilapia, common carp and sharptooth catfish.*** To be economically efficient, any management or treatment approaches to control skin bacterial infections must be based on accurate identification of the actual cause(s).

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