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MICROBIOLOGY STUDIES ON THE AFFECTIONS OF SKIN IN SHARP TOOTH CATFISH (CLARIAS GARIEPINUS)

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ABSTRACT

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This study was carried out on 240 Clarias gariepinus fish collected from The River Nile and El- Ibrahemia canal, Assuit city and the around cities (20 fish /month). The period of study was carried out during October 2011 till the end of September 2012. The clinical finding of naturally infected fish included erosions, ulceration of skin, skin darkening, fin rot, petechial hemorrhage at different parts of the body, necrotic foci and growth of the fungl hyphe in different sites on the skin and fins. It's colour was from white to brown.Mycological examination of collected samples resulted in isolation of 1200 isolates from 240 fish in presence of 960 isolates as mixed cases. The incidence of moulds isolated from fish were Fusarium solani (210)17.5%, Aspergillus flavus (184)15.2%, Aspergillus niger (170)14.3%, Mucor hiemalis (162)13.5%, Penicillium chrysogenum (97) 8.1%, Penicillium aurantiogriseum (95) 7.9%, Chladosporium herbarum (85)7.1%, Saprolegnia Sp. (60) 5% , Rhizopus Sp. (54) 4.5%, Chladosporium sphaerospermum (53) 4.4% Acremonium strictum (18)1.5%, Alternaria alternate (12)1%. Bacteriological examination of collected samples resulted in isolation of 370 isolates from 240 fish in the presence of 130 isolates as mixed cases. The incidence of Gram negative bacilli bacterial isolated from fish were *Flavobacterium columnare* (115) 31.1%, *Aeromonas hydrophila* (75) 20.3%, *Edwardsiella tarda* (57) 15.4%, *Pseudomonas sp.* (43)11.6%, *E. coli* (21) 5.7%, *Proteus sp.* (19) *5.1%, Klebsiella* (12) 3.2% . The incidence of Gram postive cocci isolated from fish were *Streptococcus sp.* (15) 4.1%, *Staphylococcus sp.* (13) 3.5%. All fish in this study infected by 1-3 types of bacteria with 3-5 types of fungi at the same time.

Key words: *Skin, sharp tooth catfish, microbes.*

INTRODUCTION

African Sharptooth cat fish is widely accepted by consumers in Upper Egypt as a relatively cheap source of fish protein. Commercial farming of African sharptooth catfish Clarias gariepinus, has significantly increased in Upper Egypt over the past few years.

Marzouk, *et al.* (2003) concluded that in Egypt, the mycotic diseases constitute one of the most important diseases causing troubles in fresh and culture fish with several economic losses. Isolated *Aspergillus flavus, Aspergillus, niger, Penicillium, Alternaria, Cladosporum, Fusarium, Mucor and Saprolegnia* from naturally infected fish, this revealed the presence of clinical abnormalities in the form of skin darkening, necrotic foci with sloughing of tail and body fins with peticheal haemorrhages and cotton wool like growth on various parts of the skin with sloughing of the uppermost layers of skin.

Refai *et al.* (2010) reported that mycologyical examination of *Clarias gariepinus* catfish revealed that isolated moulds belonged to the following genera: *Saprolegnia. Aspergillus. Fusarium, Mucor, Penicillium, Rhizopus* and other genera.

Burgess *et al.* (2000) reported that ulcers and other bacterial lesions are a common fish disease problem. They are one of the most difficult problems to deal with, especially if large numbers of fish are affected.

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Eltallawy, (2008) mentioned that skin abrasion or gill damage act as the main portal of entry to fish and was essential to induce infection. Skin is the target organs for isolation of *Flavobacterium spp*.

Nguyen et al. (2012) concluded that the bacterium *Flavobacterium columnare* was recovered and identified as the aetiological agent causing freshwater columnaris infection in catfish that had suffered high mortality rates.

Analia Murias, (2012) recorded that bacteria of the genera *Aeromonas* and *Pseudomonas* were present in infected fish in Amazonin ponds caused mortality rate 100%. *Hayes & Jon (2007): Fulton& MacDonald, (2008)* detected that *Aeromonas hydrophila* is widely considered a major fish and amphibian pathogen, as will as pathogenic for humans. *Rao et al. (2001), and Mathew et al. (2001)* reported that *Edwardsiella tarda* is a responsible pathogen for Edwardsiella septicemia of catfish, also cause diseases in higher vertebrates including humans.

Huber et al. (2004) isolated and identified *Pseudomonas* and *Proteus* from external and internal lesions in naturally infected fresh water fish.

Kar, (1999) stated that bacteriological examination of the surface lesions and other organs of fishes showing signs of bacterial infection result in isolation of haemolytic strains of *Escherichia coli, Aeromonas 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 skin.

From the above mentioned data, it was important from the bacteriological and mycological point of view to investigate the role of different bacteria and fungi as disease etiology among the sharp tooth catfish in Assuit Governorate and it's around cities.

MATERIALS and METHODS

Tow hundred forty fish samples (catfish Clarias gariepinus) collected from The River Nile and El-Ebrahemia canal and examined for determination of clinical abnormalitis and postmortem lesions according to Stoskopf (1993).

Isolation and Identification of Fungi

Samples of the collected fish (skin, fins, tails and barbel) were squashed and incubated at 25C for 5-10 days on Sabouraud dextrose agar and Czapek Dox agar (Biolife, Italy) during which the developing fungal colonies were counted and morphologicaly identified by lactophenol methylene blue. Wet mount preparation of the samples were commonly made in 10% KOH (Adwic). Fungal identification based on macro and microscopic characteristics following the key of Moubasher, (1993), Noga, (1993) and Ellis D, (2007).

Isolation and Identification of bacteria

Samples of skin surface (skin, fins, tails and barble) were collected from C. gariepinus and streaked on Tryptone soya agar and Brain heart infusion agar (Biolife, Italy), Cytophaga agar medium according to Dian G Ellitt, (2003), Salmonella Shigella broth and Blood agar (Biolife, Italy) which are the most commonly used mediums for isolation and identification of the different bacterial strains in our study. Suspected bacterial colonies were picked up and subcultured for purification and further study. A single colony was inoculated in slant tube of BHI or TSA agar medium for identification by biochemical tests Also, isolates were kept in BHI orTSA broth with 20% (Vol./Vol.) glycerol at -20 °C for further investigations.

Antimicrobial susceptibility testing of isolated strains:

The most frequent bacterial isolates were investigated against 12 antimicrobial agents using the disc agar diffusion technique according to Finegold and Martin, (1982).

The discs of the following antimicrobial agents Neomycin 30µg, Erythromycin 15µg, Enerofloxacin 5µg, Streptomycin 10µg, Oxolinic acid 30µg, Amoxicillin 25µg, Oxytetracycline 30µg, Ampicillin 10µg, Gentamycin 10µg, Chloramphenicol 30µg,, Sulfamethoxazole 100µg, and Nalidixic acid 30µg, (Bioanalyse, Turkey) were used. Interpretation of the zones of inhibition were estimated according to the limits of NCCLS (2011).

RESULTS

Mycological examination revealed the isolation of 1200 fungal isolates from 240 Clarias gariepinus fish**.** The percentage of isolated moulds from skin of Clarias gariepinus fish were detected as *Fusarium solani 210(17.5%) Aspergillus flavus 184(15.2%), Aspergillus niger 170(14.3%), Mucor hiemlis 162(13.5%) isolates, Penicillium chrysogenum 97(8.1%), Penicillium aurantiogriseum 95(7.9%), Chladosporium herbarum 85(7.1%), Saprolegnia 60 (5.0%) isolates, Rhizopus 54(4.5%) isolates, Chladosporium sphaerospermum 53(4.4%) Acremonium strictum 18(1.5%) isolates and Alternaria alternata 12 (1.0%) isolates* (Fig.1).

Bacteriological examination of fish samples resulted in isolation of 370 isolates. According to cultural, morphological and biochemical characteristics. *Percentage of strains isolated were *Flavobacterium columnare (115) 31.1%, Aeromonas hydrophila (75)20.3%, Edwardsiella tarda (57)15.4% Pseudomonas sp. (43)11.6%, Echerichia coli*

(21)5.7%, Proteus sp. (19)5.1%, Klebsiella (12) 3.2%, Streptococus sp. (15)4.1% and Staphylococcus sp. (13)3.5% (Fig.2).

Antibiotic sensitivity test reveled that *F. columnare* was highly sensitive to Enerofloxacin, Oxytetracycline, and Chloramephenicol, while was highly resistant to Erythromycin, Neomycin and Amoxicillin *A.hydrophila* was highly sensitive to Enerofloxacin, Oxytetracycline, Chloramephenicol and Neomycin while was highly resistant to Erythromycin, Ampcillin and Amoxicillin *E.tarda* was highly sensitive to Oxytetracycline, Chloramepheniol and Nalidixic acid, while was highly resistant to Erythromycin, Amoxicillin and Sulfamethazole, *Pseudomonas sp.* was highly sensitive to Enerofloxacin, Oxytetracycline, Chloramephenical and Nalidixic while highly resistant to Erythromycin, Amoxicillin and Neomycin Table (1).

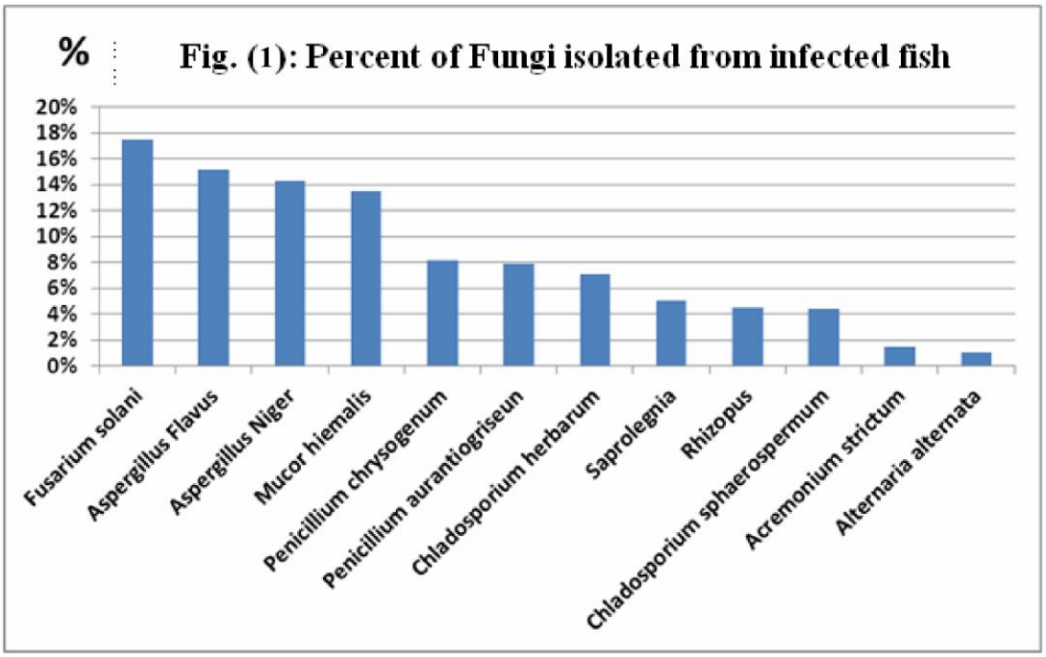

Fig. (1): Percent of Fungi isolated from infected fish

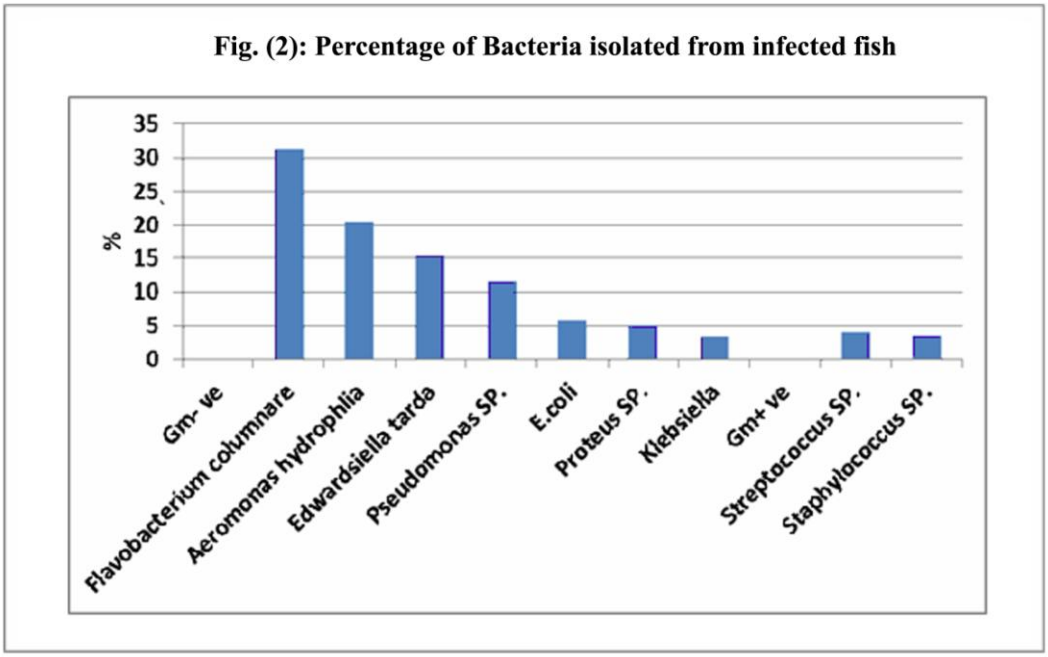

Fig. (2): Percentage of Bacteria isolated from infected fish

Table 1: Antibiotic sensitivity test for the prevelant isolated gram –ve bacteria

Antibacterial aqent	F. colnmnare			Sensitivity rate %	A. hydrophila			Sensitivity rate %	E. tarda			Sensitivity rate %	Pseudomonas sp.			Sensitivity rate %
	S	I	R		S	I	R		S	I	R		S	I	R	
Enero floxacin (5µg)	10	-	-	100%	10	-	-	100%	7	2	1	70%	8	2	-	80%
Oxy tetracycline (30µg)	10	-	-	100%	9	1	-	90%	10	-	-	100%	9	1	-	90%
Chloramephenicol (30µg)	8	2	-	80%	9	1	-	90%	9	1	-	90%	10	-	-	100 %
Erythromycin (15 µg)	-	-	10	0%	-	-	10	0%	-	-	10	0%	1	1	8	10%
Neomycin (30 µg)	1	1	8	10%	8	1	1	80%	1	2	7	10%	-	-	10	0%
Amoxicillin (25 µg)	-	2	8	0%	-	1	9	0%	-	-	10	0%	-	1	9	0%
Gentamycin (10 µg)	2	1	7	20%	6	1	3	60%	1	3	6	10%	4	3	3	40%
Ampcillin (10 µg)	3	1	6	30%	-	1	9	0%	1	2	7	10%	2	1	7	20%
Nalidixic acid (30 µg)	7	2	1	70%	7	2	1	70%	9	1	-	90%	9	1	-	90%
Streptomycin (10 µg)	4	1	5	40%	1	2	7	10%	6	2	2	60%	7	1	2	70%
Sulfamethazole (100 µg)	1	3	6	10%	6	3	1	60%	1	1	8	10%	6	3	1	60%
Oxolinicaid (2 µg)	7	2	1	70%	1	3	6	10%	7	2	1	70%	2	2	6	20%
Total No. of the tested strains	10 strains				10 strains				10 strains				10 strains			

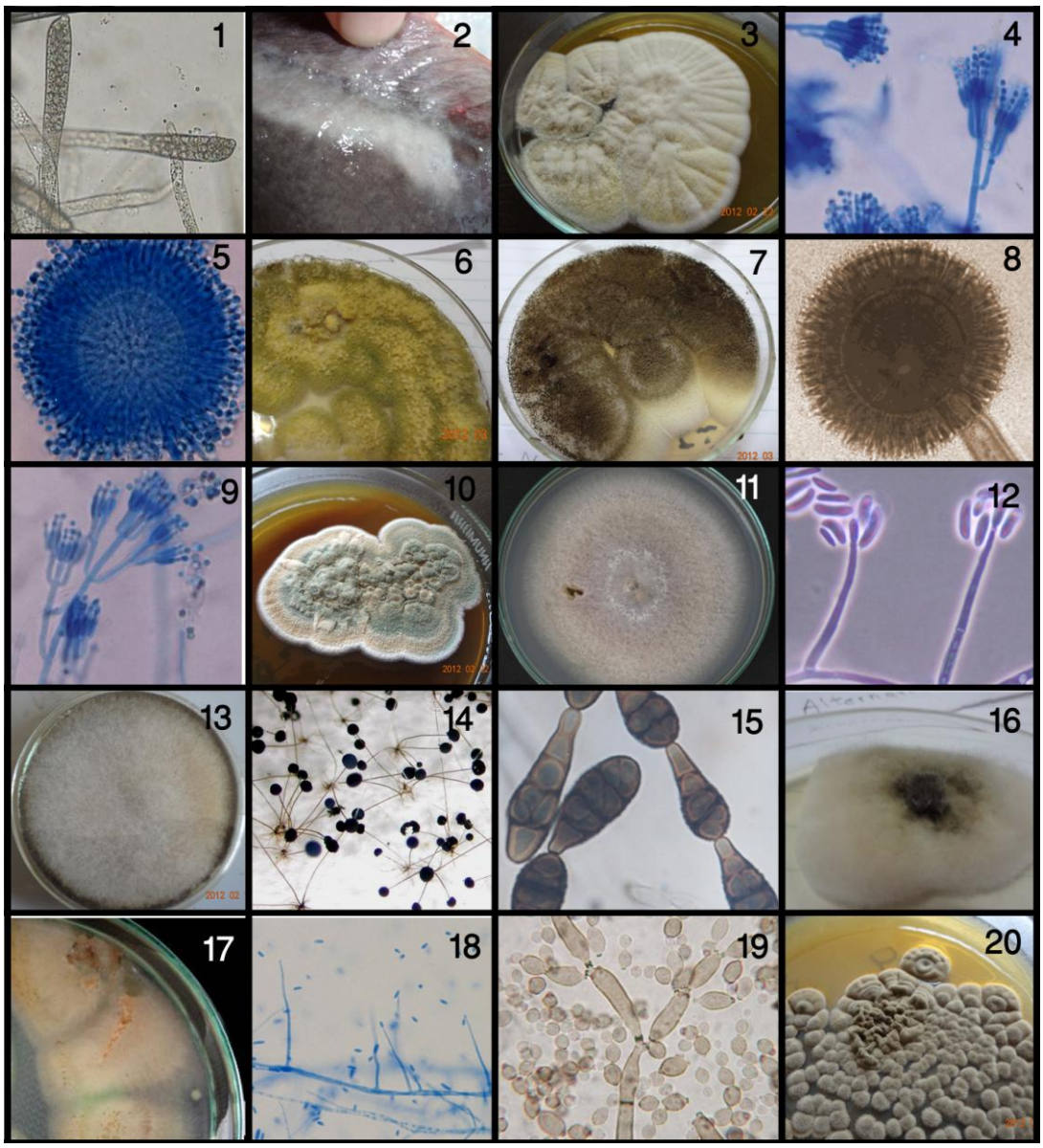

Photo. (1): Non septated broad hyphae of Saprolegnia sp., Photo. (2): Characteristic cotton –wool like growth of Saprolegnia, Photo. (3): P.aurantoigriseum on (SDA), Photo. (4): P.aueantiogriseum showing brush-like arrangement of fruiting head, Photo. (5): Uni and biseriate conidophores with conidia of Aspergillus flavus by lactophenol cotton blue stain, Photo. (6): Colonies of Aspergillus flavus on (SDA), Photo. (7): Colonies of Aspergillus niger on (SDA), Photo. (8): Aspergillus niger showing characteristic round head with black conidia, Photo. (9): Conidiophores and smooth-walled, ellipsoidal conidia, Photo. (10): Penicillium chrysogenum with different colour and texture on (SDA), Photo. (11): Fusaruim solani on (SDA) with the reverse, Photo. (12): Fusaruim solani with characteristic slender, multicelled conidia, Photo. (13): Rhizopus sp. colony on SDA showing dens wooly mycelia, Sporangia was seen as small black dots, Photo. (14): Rhizopus sp. showing long branched sporangiophores and terminate with rhizoids, Photo. (15): Conidiophores, part of a conidial chain, and liberated conidia of Alternaria alternate, Photo. (16): Grey, felty and powdery colonies of Alternaria alternate, Photo. (17): Pink colonies of Acremonium strictum on (SDA), Photo. (18): Conidiophores and conidia of Acremonium strictum, Photo. (19): Conidiophores and conidia of Cladosporium herbarum, Photo. (20): Charactarestic velvety, olive-green to olivaceous brown colonies of Cladosporium sphaerospermum on (SDA)

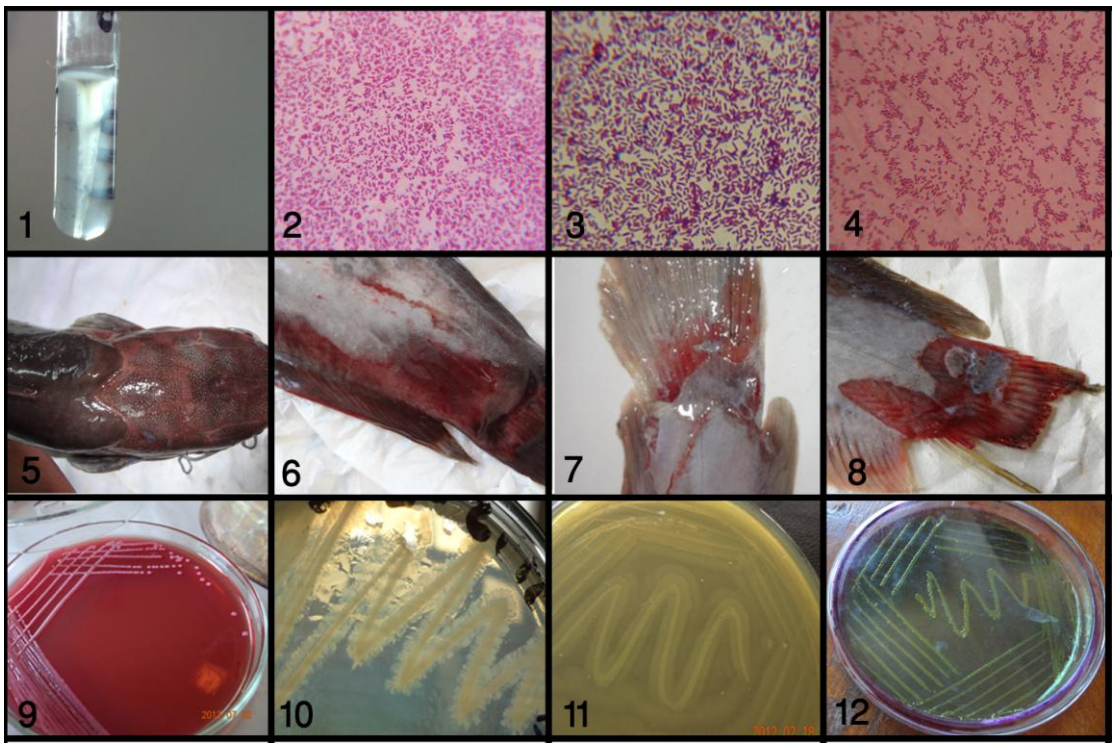

Photo. (1): Motility test +ve , Photo. (2): Gram –ve short rod bacilli E. tarda, Photo. (3): Gm –ve short rod of Flavobacterium columnare, Photo. (4): Gram –ve Areomonas hydrophila, Photo. (5): Sever heamorrhage on head Columnaris infection, Photo. (6): f. columnare infection, Photo. (7): haemorrhage and ulceration oftail, Photo. (8): Fin rot columnaris infection, Photo. (9): Pink colonies of Klebsiella on MacConcy agar, Photo. (10, 11): Swarming with irregular edges of F.columnare on (cytophaga agar), Photo. (12): Blue –black colonies with greenish metallic sheen of E.coli on (EMB),

DISCUSSION

Mycological incidence and distribution:

Mycological examination revealed the isolation of moulds belonged to the following genera: Aspergillus flavus, Aspergillus niger, Fusarium sp., Penicillium aurantiogriseum, Penicillium chrysogenum, Mucor hiemalis, Chladosporium sphaerospermum, Chladosporium herbarum, Saprolegnia, Rhizopus sp., Acremonium strictum and Alternaria sp. are isolated. The nearly same fungal isolates were reported by Abd El- Alim (1992); khalil (1993); Mohamed (1994); Marzouk *et al.* (2003) and Refai *et al.* (2010). The Clinical findings of Clarias gariepinus normally infected with these fungi were exophthalmia, skin darkening on various parts of the body, moderate abdominal distention and haemorrhages all over the body surface and white to brown hyphal growths on skin and fins.

Our study revealed that the main isolates from Catfish on both Czapek Dox agar and Sabouraud dextrose agar media at 25°C. were *Fusarium solani* 17.5%, *Aspergillus flavus* 15.2% and *Aspergillus niger* 14.3% the obtained results agree with Manal (1988); Salem *et al.* (1989); but Mohamed, N. (1994); Marzouk *et al.* (2003) and Refai *et al.* (2010) reported that *Aspergillus* flavus was the first fungi isolated and *Fusarium* came after that in their incidenecs that result are nearly similar to our result. El-Hissy *et al.* (1989) found that *A. flavus* and *A.niger* were the dominant aspergilli in Clarias gariepinus, Also Salem *et al.* (1989) and Bagy *et al.* (1993) found the prevalent aspergilli that were isolated from skin and gills of Nile fishes *A.flavus* and *A.niger*. El-Hissy *et al.* (1989) isolated Penicillium, Fusarium and Mucor from Catfish. Also, members of Mucor including M. hiemalis isolated from skin of catfish by (Bagy *et al.*, 1993). In the current work, some fungi were isolated from skin of catfish *Cladosporium sphaerospermum, Cladosporium herbarum, Alternaria alternata and Acremonium strictum* dominant in fins and skin of fish, They were also isolated by Badran, (1989) and Mohamed, N (1994) *Rhizopus* isolated from samples of fins and skin. This was encountered from fish and Nile water by El-Zayat, (1988) and Badran, (1989) isolated *Rhizopus* species from fish in high incidence 100% from skin, fins and gills.

Alternaria alternate and Acremonium strictum was isolated in low percent in our study but *Alternaria alternate* appeared in high frequency of occurrence

in both fins and skin (62- 80%) . *Saprolegnia* species were isolated from skin 5.0% in combination with other fungal and bacterial infection, in agreement with that reported by Robert, (1989) and Marzouk *et al.* (2003) who recorded that *saprolegnia* is a secondary fungal infection after any predisposing stress factors such as traumatic injury, cold stress and hormonal changes in the body of fish. Also, Refai *et al.* (2010) concluded that *Aspergillius* is the first isolate (43.0%) and *Saprolegnia* (4.2%) was the secondary fungal infecton.

Bacterial inciedance and distribution:

The most frequent bacterial isolates was identified as *Flavobacterium columnare (31.1%), Aeromonas hydrophila (20.3%), Edwardsiella tarda (15.4%) and Pseudomonas sp*. (11.6%). They are nearly similar findings to that reported by Huber *et al.* (2004); Saad El-Deen, (2005); Abd El-Rahman and Elkamel, (2007); Hayes, John. (2007) and Eltallawy (2008).

Intensity of the skin lesions caused by infections depended on the fish species and causative agent(s) involved. The main clinical signs observed on naturally infected samples were erosions and ulceration of skin at the base of the dorsal fin and on the head, paleness, sloughing, erosion, petechial haemorrhages and loss of the tips of the fins and fin rot with separation of fin rays as previously reported by Durborow *et al.* (1998) and Tripathi *et al.* (2005).

Our result in the present study concluded that *F. columnare* was the first major isolates with incidence of 31.1%, in accordance with those reported by Eltallawy (2008) who concluded that *F. columnare* is the main cause of skin lesions and fin rot with incidence of 32.1% in *sharptooth catfish.*

Although in some cases, Bader *et al.* (2003) and Welker *et al.* (2005) can be isolated *F. columnare* from inner organs but skin and gills are the tissues of choice for isolation Abd EL-Rahman and Elkamel., (2007) concluded that the first bacterial isolate from skin of *sharptooth catfish* was *F. columnare* and the second cause was *A. hydrophila* which were in accordance with our result. But Atallah *et al.* (1997) reported that *A. hydrophila* was the first bacteria isolated from skin and fins of sharptooth catfish followed by *Pseudomonas fluorescens*. Also Plumb (1994) recorded that *A. hydrophila* was the most predominant bacteria isolated from fish species suffering from fin and tail rot. Some researchers believe that A. hydrophila is a primary fish pathogen while others consider it only a secondary invader of already weakened fish, this reported by Hayes John. (2007). In our result all A. hydrophila infected fish already infected with fungi. Eissa and Yassien (1994) reported that *E. tarda* one of the main causative agent of skin affections in *sharptooth catfish*. Moreover Saad El-Deen (2005) reported that *E .tarda* is the main isolate from skin of infected *sharptooth catfish*. Abd EL-Rahman and Elkamel (2007) reported that *Pseudomonas.sp* was the third major isolates from affected skin of *sharptooth catfish*. In the present study *Pseudomonas sp.* was the fourth major bacteria isolated so, this lower rate may be due to the fact that the fish had a generalized condition of septicemia rather than a confined case of bacterial skin infection as the clinical signs of diseased fish described support this suggestion.

Antibiotic test:

Antibiotic Disc agar diffusion reveled that *F. columnare* was sensitive to Enerofloxacin, Oxytetracycline, and Chloramephenical, while was resistant to Erythromycin, Neomycin, Amoxicillin *A. hydrophila* was sensitive to Enerofloxacin, Oxytetracycline, Chloramephenical and Neomycin, while was resistant to Erythromycin, Ampcillin and Amoxicillin *E. Tarda* was sensitive to Oxytetracycline, Chloramephenicol, Oxolinic acid and Nalidixic acid and was resistant to Erythromycin,Amoxicillin and Sulfamethazole *Pseudomonas sp.* was sensitive to Enerofloxacin, Oxytetracycline, Chloramephenical and Nalidixic acid while resistant to Erythromycin, Ampcillin, Amoxicillin and Neomycin These results are nearly similar to those reported by Kar, (1999); Dalsgaard and Madsen, (2000); Abd El-Rahman, (2002); Saad El-Deen,(2005); Abd EL-Rahman and Ahmad Elkamel (2007) and Eltallawy (2008).

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دراسات على الاصابات الميكروبية الجلدية فى اسماك القراميط النيلية

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فى هذا العمل تم اجراء الفحص البكتريولوجى والفطرى لعينات من الاصابات والالتهابات الجلدية السطحية و الزعنفية فى الاسماك القطية (القراميط النيلية) أجريت هذه الدراسة على عدد 240 سمكة من القراميط جمعت كلها من نهر النيل وترعة الابراهيمية فى محافظة أسيوط وكذلك بعض المدن المجاورة للمحافظة بمعدل 20 سمكة/ شهر وامتدت فترة الدراسة من شهر أكتوبر 2011 حتى سبتمبر 2012. الاعراض الاكلينيكية الظاهرة تتمثل فى تآكلات وتقرحات جلدية وتعفن فى الجلد ووجود نزف تحت الجلد فى مناطق مختلفة من الجسم والزعانف والشاربين مع نمو ظاهر للفطريات على الجلد والزعانف يتراوح لونة من الابيض الى البنى. كانت نسبة العزل الفطرى من الاعلى الى الاقل كالاتى : فطر اسبرجيلوس بنوعية اسبرجيلوس فلافوس واسبرجيلوس نايجر 29.5% فطر فيوزيريوم 17.5% فطر بنسيليوم بنوعية اورينتوجريزيوم وكريزوجينوم 16% فطر ميوكر هيمالز 13.5% فطر كلادوسبوريوم بنوعية سفيروسبيريم وهيرباريم 11.5% فطر سابروليجنيا 5% فطر ريزوبس 4.5% فطر اكريمونيوم استيريكتوم 1.5% فطر التيرناريا 1%. كانت نسبة العزل البكتيرى من الاعلى الى الاقل كالاتى: عصويات سالبة الجرام ميكروب الفلافو بكتيريم كولومينارى بنسبة 31.1% وميكروب ايرومونس هيدروفيلا 20.3% يلية ميكروب إيدورزيلا تاردا 15.4 ثم ميكروب سيدومونس 11.6 % يليها ميكروبات اقل نسبة عزل وهى إشرشيا كولاى 5.7% بروتيس 5.1% كلبسيلا 3.2% وأيضا كرويات موجبة الجرام ستربتوكوكس 4.1 % استافيلوكوكس 3.5%. وقد تم إجراء إختبار الحساسية للميكروبات البكتيرية الاكثر انتشارا ووجد ان الميكروبات شديدة الحساسية لكلا من المضادات الحيوية الاتية : إنروفولوكساسين اوكسيتتراسيكلين كلورومفينيكول وحمض النالديكسيك ومتوسطة الحساسية لكلا من نيوميسين جاراميسين وحامض الاوجزالينك0

هذا البحث مستخرج من رسالة دكتوراة خاصة للمؤلف الرابع