STUDIES ON SOME BACTERIAL DISEASES IN SOME CULTURED FRESHWATER FISHES IN KAFR EL-SHEIKH GOVERNORATE

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ABESTRACT

Bacterial diseases affecting fresh water fishes are numerous causing high economic losses in Egypt, therefore, this study was conducted for two main goals: isolation and identification of bacterial isolates affecting cultured freshwater fishes as well as the incidence of these diseases in Kafr El-Sheikh Governorate.

180 cultured freshwater fishes (120 Oreochromius niloticus& 60 Cyprinus carpio) were collected from both private and governmental farms in Kafr El-sheikh Governorate.

The most common clinical signs were congestion and haemorrhages on body surface and base of fins. Post-mortem findings revealed pale anaemic liver in some cases and in other cases, liver were haemorrhagic and congested. Kidney and spleen were enlarged and congested.

80 bacterial isolates from all naturally examined fish species were obtained (46 Oreochromius niloticus isolates from & 3460 Cyprinus carpio) isolates from).

Biochemical identification of these isolates revealed 12 bacterial serotypes; Aeromonas hydrophila, Pseudomonas spp., Edwaradsiella

spp., Yersinia ruckeri, Proteus ssp., Provedencia rettegri, Citrobacter freundii, Alkaligene faecalis, Klebsiella pneumonia, Flavobacterium columnare, Streptococcus spp. and Enterococcus faecalis.

Results of antibiogram screening that ciprofloxacin is the drug of choice for treatment of Aeromonas hydrophila.

INTRODUCTION

Aquaculture has an important role in the development and meeting the increased demand for aquatic animal production *(Haylor and Bland 2001)*. Aquaculture industry gradually developed in the world as well as in Egypt to meet the increased needs of human consumption *(Asaad 2008)*.

Fish is an essential source of high nutritive value with good digestibility and cheap source of animal protein. Fish is susceptible to wide variety of biological hazards *(Garrett et al. 1997; Hanjra et al. 2012; Huss 1994; Reilly et al. 1997)*.

Bacterial diseases are the most common diseases in intensive fish rearing facilities *(Ibrahim et al. 2013; Kusuda and Salati 1999)*. Outbreaks of diseases attributed to bacterial Pathogens are devasting to both cultured and wild fish populations *(Austin and Austin 2007)*.

This study was planned to fullfil the following objectives:

1. Isolation & identification of some predominant bacterial pathogens from some freshwater fishes.

2. The prevalance of bacterial isolates between two species s of freshwater fish.

3. Recording the clinical signs and post-morten lesions associated with such bacterial isolates in naturally infected fishes.

4. Serotyping of bacterial isolates in naturally infected fishes.

5. Application of antibiotic sensitivity test for one of the isolated bacteria to be a helping tool in control of the disease originated from such bacterial isolate.

MATERIALS AND METHODS

1. Fish:

A total number of 180 fish samples (120 *Oreochromius niloticus* and 6 *Cyprinu*s *carpio* 0) were randomly collected from freshwater fish farms in Baltim, El-hamoul, Sedi-Salem at Kafr El-Sheikh.governorate .

Collected samples were transferred alive in oxygenated plastic containers to fish diseases and management lab., Faculty of Veterinary medicine, Kafr El-Sheikh University and subjected to clinical and post-mortem examination as described by *(Schäperclaus 1992)*.

2. Bacteriological Examination:

Samples were aseptically collected from liver, kidney and spleen of collected naturally infected fishes. Collected samples were cultured on liquid media (Tryptic soya broth oxoid), solid media (Tryptic soya agar oxoid , TCBS oxoid and MacConkey,s agar oxoid) and semisolid media Nutrient agar and incubated at 25-30C°for 24-48 hr.

Purified isolated were identified by standard biochemical tests according to *(Austin and Austin 2007; Holdeman et al. 1984; Schäperclaus 1992)*.

3. Antibiogram:

The Sensitivity of isolate was carried out against 12 bacterial pathogen *A. hydrophila* to different antibiotics was estimated according to *(Panta et al. 2013)*. The antimicrobial susceptibility testing was applied according to the guidelines stipulated by National Committee for Clinical Laboratory Standards (NCCLS-2006).

4. Histopathological Examination:

Specimens for histopathological techniques were freshly taken from liver, kidney, spleen and skin of naturally infected fishes. Histopathological techniques were carried out according to *(Roberts 2001)*.

RESULTS

Clinical Signs:

The most common clinical signs of naturally infected fishes were congestion and haemorrhages on the body surface, sunken aye, tail detachment, fin erosion, shallow to deep necrotizing ulcers, dermatitis and abdominal distention with sero-haemorrhagic fluids exudating from the vent (Figure 1).

Post-mortem Findings:

The post-mortem findings naturally infected fishes exhibited sero-sanguinous fluid in the abdominal cavity, enlarged liver, kidney, speen and gall bladder (Fig 2).

Histopathological Findings:

Histopathological evaluation revealed marked congestion of the hepatopancrease and hepatic sinusoids (Fig 3) with multifocal focci of necrosis of hepatocytes especially in centrolobular area (Multifocal Necrotic Hepatitis). In some cases, massive liver necrosis and degeneration of hepatopancrease with absence of inflammatory reaction were observed.

Renal acute tubular degeneration as well as interstitial heamorrhage was also observed). The spleenic parenchyma showed marked lymphoid depletion, massive necrosis in melanomacrophage centers and some cases showed focal necrosis abscess-like lesion. Skin showed degeneration of epithelial layer with marked sub-epithelial congestion, oedema and diffuse leucocytic and infiltrations (Fige 4).

Subcutaneus musculature showed marked fragmentation of sarcoplasm, atrophy showed marked fragmentation of sarcoplasm, atrophy as well as separation of the muscle bundles and inflammatory cells infiltration.

Results of Bacteriological Examination:

bacteriological examination of governorate, revealed 80 bacterial isolates (46 isolates from Nile tilapia & 34 from common carp).

Fig. (1): Naturally diseased (*O. niloticus*) showing extensive skin hemorrhage, fin and tail hemorrhage.

Fig. (2): Naturally diseased (*O.niloticus*) showing Hepatomegally and palness in liver, the intestine is filled sero-hemorrhagic fluid and congestion of internal organs.
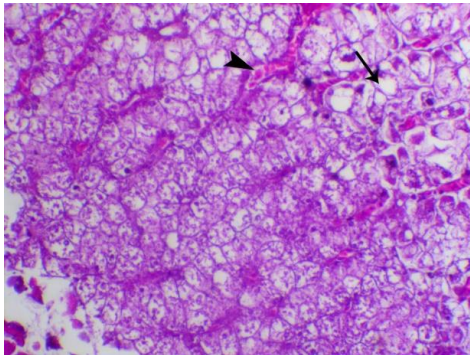

Fig. (3): liver of Naturally diseased showed marked congestion of the hepatic sinusoids, H&E, X200.
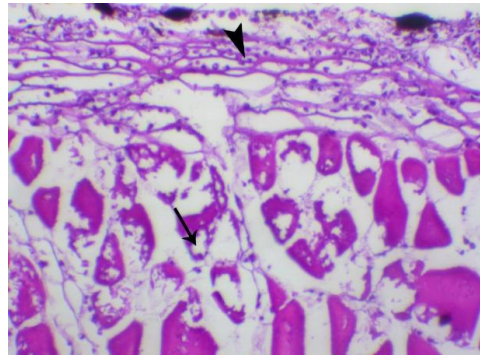

Fig. (4): Subcutaneous musculature showed oedema and inflammatory cells infiltrations (arrowhead), marked fragmentation of sarcoplasm (arrow). H&E, X200.

Biochemical reactions for these obtained isolates revealed ten gram negative bacterial isolates (*A. hydrophila, Pseudomonas fluorescence, Pseudomonas pseudoalkaligenes, Edwardsiella tarda , E. ictaluri, Yersinia ruckeri, Flavobacterium columnare, Alkaligene faecalis, Klebsiella pneumonae, Citrobacter freundii, Proteus vulgaris* and *Proteus mirabilis*) and two gram positive bacteria (*Streptococcus iniae, St. agalactia and Enterococcus faecalis*).

The biochemical reactions was done according to **Holdeman et al. (1984)**.

The results of biochemical reactions for obtained bacterial isolates is shown in (Table 1 & 2). However, the prevalence of different bacterial isolates among positive cases was described in (Table 3&4).

The incidence of bacterial pathogens in common carp were much more than in tilapia.

The cultural and morphological characters of the most prevalent gram negative bacteria (*Ps. Fluorescence*) and gram positive bacteria (*S. iniae*) was clearly reported in Table 5.

Table (1): Biochemical tests for identification of Gram negative bacteria

Test	Edwardsiella		Proteus		Pseudomonas			A. hydrophila	Y. ruckeri	F. clum.	P. rettgeri	K. pneumoniae	E. aerogenes	C.freundii
	E. tarda	*E ictaluri*	*P. mirabilis*	*P. vulgaris*	*Ps. fluorescen*	*Ps. auroginoa*	*Ps. pseudoalc*							
Motility	+	+	+	+	+	+	+	+	-	-	+	-	+	+
Indole	+	+	-	+	-	-	-	+	-	+	+	V	-	-
Methyle red	+	+	+	+	-	-	-	V	+	V	+	-	-	+
Voges Proskuaer	-	-	V	-	-	-	-	V	-	V	-	+	+	-
Citrate utilization	-	-	V	-	+	+	V	V	-	+	+	+	+	+
Urease	-	-	+	+	V	V	-	-	V	-	-	+		V
H2S	+	+	+	+	-	-	-	+	-	+	-	-	-	V
Nitrate reduction	-	-	+	V	+	+	-	+	V	-	V	V	+	-
Gelatin liquefaction	-	-	+	+	+	+	-	+	-	+	-	-	V	-
ODC	+	+	+	-	-	-	-	-	-	V	-	-	+	V
LDC	+	-	-	-	-	-	-	+	-	V	-	+	+	-
Arginine dihydrolase	-	-	-	-	+	+	+	+	-	-	-	-	-	V
ONPG	-	-	-	-	-	-	-	V	+	V	-	V	V	+
Sugar fermentation Lactose	-	-	-	-	-	V	-	V	-	+	-	+	+	+
Sucrose	V	-	-	+	+	+	-	V	-	-	V	+	+	-
Dulcitol	-	+	-	-	V	V	V	-	+	-	-	V	-	V
Salicin	-	-	-	V	-	-	-	-	+	-	-	+	+	-
Arabinose	V	-	-	-	V	V	-	V	+	-	-	+	+	+
Inositol	-	V	-	-	V	V	V	-	-	V	+	+	+	-
Xylose	-	-	+	V	V	V	-	V	V	-	-	+	+	+

+ = positive   - = negative   V = variable

Table (2): Biochemical tests for identification of Gram positive cocci

Test	*S. iniae*	*S.agalactiae*	*E. faecalis*
Catalase	-	-	-
Growth at pH 9.6	-	-	+
Growth at 6.5 % salt	-	-	V
Growth at 10 ℃	+	-	+
Growth at 45 ℃	-	-	V
Esculin hydrolysis	+	-	+
Arginine dihydrolase	V	+	+
Hippurate hydrolysis	-	+	+
Sugar fermentation: Lactose	-	V	+
Mannitol	+	-	+
Arabinose	V	-	-
Ribose	+	V	V
Sorbitol	-	-	+
Raffinose	V	-	V

+ = positive   - = negative   V = variable

Table (3): Prevalence of gram negative bacterial isolates among positive cases

Bacteria	Fresh water fish species	
	Tilapia	Common carp
No. of positive cases	46	34
A. Hydrophila	7	5
%	15.21	14.7
Ps. Fluorescene	8	8
%	17.39	23.5 3
Ps. Pseudoalkaligenes	2	2
%	4.35	5.88
Ps. aeruginosa	0	1
%	0	2.94
Edwardsiella tarda	3	0
%	6.52	0
E. ictaluei	0	5
%	0	8.82
Yersinia ruckeri	0	2
%	0	5.88
Flavobacterium columnare	1	0
%	2.17	0
Proteus vulgaris	4	2
%	8.69	5.88
P. mirapilis	2	2
%	4.35	5.88
Providencia rettgeri	3	4
%	6.52	11.76
Alcaligenes faecalis	1	2
%	2.17	5.88
Klebsiella pneumonia	2	0
%	4.35	0
Citrobacter freundii	1	0
%	2.17	0

Table (4): Prevalence of gram positive bacterial isolates among positive cases

Bacteria	Fresh water fish species	
	Tilapia	Common carp
No. of positive cases	46	34
S. iniae	6	0
%	13.04	0
S. agalactiae	1	0
%	2.17	0
Enterococcus faeca	3	3
%	6.52	8.82

Table (5): Culture and morphological characters of isolated bacteria

Bacterial isolates / Media	S. iniae	P. Flu orescene
TSA	White creamy colored cocci	Wide spreading colony rods with fluorescent pigment
TCBS	Pale color	Pale color
MacConkey	Pale color nlf	Red color lf

Nlf = non-lactose fermenter   lf = lactose fermenter

Results of sensitivity test:

Sensitivity of isolated bacterial pathogen *A. hydrophila* to different antibiotics revealed that *A. hydrophila* is highly sensitive to ciprofloxacin and highly resistant to **Neomyeine as shown in (Table 5).**

Table (6): Percentages of Antimicrobial susceptibility of *A. hydrophila* (n=12)

Antimicrobial agent	S		I		R	
	NO.	%	NO.	%	NO.	%
Neomycin (N)	-	-	-	-	12	100
Penicillin (P)	-	-	1	8.3	11	91.7
Streptomycin (S)	-	-	1	8.3	11	91.7
Ampicillin (AM)	-	-	2	16.7	10	83.3
Kanamycin (K)	1	8.3	2	16.7	9	75.0
Oxytetracycline (T)	2	16.7	3	16.7	7	58.3
Amikacin (AK)	4	33.3	1	8.3	7	58.3
Netilmicin (Net)	3	25.0	3	25.0	6	50.0
Erythromycin (E)	5	41.7	2	16.7	5	41.7
Nalidixic acid (NA)	6	50.0	2	16.7	4	33.3
Chloramphenicol (C)	5	41.7	3	25.0	4	33.3
Sulphamethoxazol (SXT)	8	66.7	1	8.3	3	25.0
Gentamicin (G)	9	75.0	2	16.7	1	8.3
Ciprofloxacin (CP)	11	91.7	-	-	1	8.3

DISCUSSION

In the present study, we focus on the clinical and post-mortem lesions of the most predominant bacterial pathogense affecting some freshwater fishes in Kafr El-Sheikh governorate. Moreover, isolation and identification of these bacterial pathogens by traditional biochemical tests.

Concerning the clinical signs and post-morten lesions of naturally examined freshwater fishes, the clinical picture of *streptococcosi*s in this study were nearly similar to that obtained by *(Colorni et al., 2002)* where they mentioned that S. iniae cause severe bilateral exophthalmia and internally haemorrhages in the abdominal cavity.

In concern the clinical picture and pm lesions of pseudo moniasis, the result obtained picture in this study were nearly similar to that reported by *Masbouba, (2004)* and *(Toranzo et al., 2005)* where they illustrated that main clinical signs were abdominal distention and haemorrhagic petechiae in the skin and internal organs.

Concerning the culture, morphological and biochemical characteristics of the bacterial isolates, the obtained result in this study, for S. iniaa were in agreement with those reported by *(Colorni et al., 2002; Whitman, 2004)*.

However, the culture, morphological and biochemical characteristics of *Pseudomonas fluorescence* noticed in this study were similar to those reported by *(El-Moghazy, 2004; Masbouba, 2004)*.

In regard to the incidence of the bacterial isolates among naturally infected freshwater fishes, our results revealed that common carp was the most affected species (34/60)followed by Nile tilapia (46/120).

the prevalence percentages of the isolated bacterial groups, our results revealled that *Pseudomonas fluorescence* were the most prevalent bacteria among common carp (23.53%) followed by *Aeromonas hydrophila* (14.7%). However, in Nile tilapia *Ps. fluorescence* was the prevalent bacteria representing (17.39%) followed by *A. hydrophila* (15.21%). This result was similar to ***Masbouba, (2004)*** where they stated that *Pseudomonas fluorescence* is the predominant Spp. among all *Pseudomonasi*s Spp. and its predominant representing (63.9%). But, in contrast to ***El-Galagel (2015)***, where they stated that *A. hydrophila* represented 60% and *pseudomonas* Spp. isolates represented 24.4%.

CONCLUSION

From the present study, it can be concluded that *Pseudomonas fluorescence* is the most prevalent bacterial pathogen among freshwater fishes in Kafr El-Sheikh Governorate followed by *Aeromonas hydrophila*. The incidence of bacterial pathogen amogen carp is much more prevalent than in Nile tilapia. Ciprofloxacin is the main drug of choice for treatment of the disease resulting from *Aeromonas hydrophila diseased fish.*

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