

Some Studies on Some Enterobacterial Diseases Affecting Fish

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ABSTRACT

Enterobacteriaceae species considered the most important bacterial micro-organism which causing server economic fish losses among fish farm in different countries all over the world . We use about natural 50 *Oreochromis niloticus* to isolate the Gram – negative *Enterobacteriaceae* and 50 *O. niloticus* apparently healthy for studying the pathogenecity of 2 isolates of *Enterobacteriaceae* and 30 *O. niloticus* for studying the anti-body titration of these 2 types of bacteria . The results proved that there are 39 strain Gram negative, Catalase negative Oxidase negative which indicate . *Enterobacteriaceae*, the main clinical signs & post mortem lesions are septicemia, ascitis , hemorrhage, exophthalmia, red mouth with congestion and generalized septicemia of all internal organs . The *O. niloticus* give the highest isolated species than any other species .In pathogenicity test the different bacteria give mortalities with different rats according to dilution. *Yersinia*, *E.tarda* re-isolated from different organs by different percent ranged from 0% - 50% and the results of biochemical identification proved both types of bacteria. *E. tarda* was sensitive to Lincospectine and Neomycine while *Yersinia* was sensitive to Erythromycin, Neomycin. Anti-body titration was higher in *Yersenia* than in *E. tarda* from that *Enterobacteriaceae* are highly effective in cultured fish and it can be controlled through antibiotic and vaccination.

Keywords:

INTRODUCTION

Enterobacteriaceae Species either *Edwardsiella tarda*, *E. coli* . *Yersinia ruckeri* or *Proteus retgerri* were considered of the most

important bacterial microorganisms, that causes severe economic losses among fish farms in many countries as it was reported in most of world countries as; North America, Japan,

Taiwan, Thailand and Africa (Bragg, 19991; Durborow et al., 1991; Francis et al., 1993 and Baya et al., 1997). In addition, they are the main causative agents of Enetric Septicemia or Emphysematus Putrefactive Diseases (EPD) and Red mouth disease (RMD) (Baya et al., 1997).

Edwardsiella septicemia is a mild to severe systemic bacterial disease primarily of warm water fish that characterized by the presence of gas-filled malodorous lesions in muscle of fish (Hawke, 1979 and Hawke et al., 1981).

The septicemia caused by *E. tarda* or *E. coli* is the currently accepted name for the disease caused by this pathogen, but other synonyms one are fish gangrene, emphysematous putrefactive disease of catfish (Meyer & Bullock, 1973 and Naglaa Galal, 2002) and red disease of eels (Egusa, 1976). The aim of this study, is the determination of the role of Enterobactereacea in disease problems of cultured fish so, to conduct this aim the following studies was carried out:-

1. Isolation and characterization of *Enterobactereacea* among *O.*

niloticus and losses resulted from infected fish .

2. Sensitivity test of isolated *Enterobactereacea*.
3. The pathogenicity of some isolated species *Enterobactereacea*, in *O. niloticus* via its effect on healthy fishes.
4. Determination of the humeral immune response against some isolated pathogens in a trial to find prevention of *Enterobactereacea*, in cultured fish by vaccination and antibody titration.

MATERIALS AND METHODS

A. Materials

1. Fish

a- Cultured diseased fish

A total of 50 *Oreochromus niloticus*, were collected from fish farms at Behera and Kafr El-Sheikh and Alexandria Provinces. The collected fish showed the clinical signs of different diseases.

b-Fish used for experimental study

I-Fish for pathogenecity study

pathogenicity 50 fish *O. niloticus* apparently healthy were

ENTEROBACTERIAL DISEASES AFFECTING FISH

used for study the pathogenicity of *E. tarda* and *Yersenia ruckeri* isolates. The fish were left for one week under observation for any diseased conditions. Some fishes were scarified and tested for *E.tarda* and *Yersenia ruckeri* micro-organisms.

II- Fish for immunological study

30 fish (*O. niloticus*) divided into 3 groups (aquarium) each one contains 10 fishes, 1 Aquarium injected with *E. tarda* bacterin, one aquarium injected with *Yersinia ruckeri* bacterin and the 3rd one acts as a control injected with sterile saline.

2-Media used

A. Media used for primary isolation of bacteria

Nutrient agar and nutrient broth. Trypticase soya broth, MacConkey agar. (Oxoid Co.).

B. Biochemical characterization

The isolated bacteria was biochemically identified according to (Cruickshank et al., 1975).

The above mentioned media were prepared according to -Gram's stain (Cruickshank et al., 1975).

B-Methods

1-Clinical and macroscopic examination of caught cultured fish

Naturally caught fish were transferred aseptically to laboratory of microbiology in the Fac. of Vet. Med. Alex. Univ. with out delay fish were examined clinically for infection before the bacteriological examination according to **Lucky (1977)**.

2. Primary isolation of Enterobactereaceae

Naturally infected fishes were examined bacteriologically for enterobactereacea according to *Buxton and Fraser (1977)* and *Morrison et al. (1981)*.

Meanwhile *Edwardsiellae* and *Yersinia* species were primarily isolated by inoculation of samples from (liver, intestine, spleen, kidneys and muscles) on the specific media used for their isolation and identification morphologically, culturally and biochemically according to

Buxton and Fraser (1977), Hawke et al. (1981) and Morrison et al. (1981).

3-Antibiotic sensitivity tests (Antibiogram)

The sensitivity of bacterial isolates to different antibiotics was carried out using the disc diffusion technique according to Lennette et al. (1980).

4-The pathogenicity test

The apparently healthy fishes were grouped into 5 groups (Aquarium) each one contains 10 fishes. 2 Aquarium artificially injected with *E.tarda* (10^{-4} , 10^{-2}) respectively, 2 Aquarium injected with *Yersinia ruckeri* (10^{-4} , 10^{-2}). In the same time group number 5 injected with sterile saline in the same dose as a control group, fish kept under observation for 15 days. The symptoms appear on fish and mortalities were recorded. Dead and scarified fish at the end of the experiment were examined clinically and

bacteriologically for reisolation of the injected bacterial isolates.

5- Evaluation of potency of prepared bacterine against *Yersinia ruckeri* bacteria

Bacterin preparation

Yersinia and *Edwardsiella* isolates were used in the bacterin preparation according to the method described by Sakai et al. (1984) and Badran (1990). Preparation of stained antigen was performed according to (Collins et al., 1976).

RESULTS

1. Results of clinical signs and gross lesions in *Oreochromis niloticus* naturally infected with *Enterobacteriaceae* organisms were typified by:-

Loss of scales from some areas of the skin, excessive mucus all over the body surfaces with petechial haemorrhages over the dorsal musculature. Large necrotic lesions extending all over the body with tail rot.

Skin showed superficial ulcers, especially at the caudal

ENTEROBACTERIAL DISEASES AFFECTING FISH

peduncle with extensive hemorrhagic raised areas on the dorsal musculature.

While, the post mortem lesions of naturally infected fish were in the form of generalized septicemia in most internal organs especially kidney, liver, intestinal tract, spleen and severe distension of gall bladder with bile secretion, congested gills and gall bladder .

2. Results of bacteriological examination of naturally infected *O. niloticus* : 13gram – ve bacterial isolates out of 72

isolates were recovered from different examined fish species. The biochemical characters of isolates are shown in Table (1).

Pathogenicity experiment

1. Results of clinical signs and gross lesions of experimentally infected fish: were included redness of the mouth and darkening of the head region especially in case of *Yersinia infection* in some examined fish infected with *E. tarda*. Skin showed abnormal dark color, with slight exophthalmia.

Table (1) : Show the number and percentage of positive samples of *Enterobacteraceae* and the biochemical characters of isolated bacteria:-

Bacterial species	No.	%	Gram stain	Catalase	Oxidase	Indole	MR	V.P	Cit.	Ureas
<i>E.tarda</i>	7	53.3%	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve
<i>E. Coli</i>	1	7.6%	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve
<i>Yerseni ruckeri</i>	4	30.7%	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve
<i>Proteus retgerri</i>	1	7.6%	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve
Total	13	100%								

However, the gross examination of experimentally infected fish were in the form of congestion in most internal organs especially kidney and liver. Area of necrosis are found mainly in liver.

2. The mortality due to bacterial injection Different dilutions give mortalities are shown in Table (2).

3. Results of the bacterial examination from different organs:

The results presented in Table (3) revealed that *E. tarda* was

isolated from (intestine, liver, spleen, kidneys, and muscles) of *O. niloticus* fish at a percentage of 40 %, 50%, 30% , 0% and 20%, respectively.

On the other hand, *Yersinia ruckeri* was isolated from (intestine, liver, spleen, kidneys, and muscles) of *O. niloticus* fish at a percentage of 40 %, 30%, 30% , 30% and 0%, respectively.

4. Antibioticv sensetivity of the isolated *E. Tarda* and *Yersinia species* from fish

Table (2) : Explain the mortality of *O. niloticus* due to bacterial injection by different dilutions:-

Type of bacteria and its dilution	No. of dead fishes	% of mortality
<i>E. tarda</i> 10 -2	7 fish	70 %
<i>E. tarda</i> 10 -4	4 fish	40 %
<i>Yersinia</i> 10 -2	8 fish	80 %
<i>Yersinia</i> 10 -4	3 fish	30 %

ENTEROBACTERIAL DISEASES AFFECTING FISH

Table (3) : Show bacterial species isolated from tissue of fish.

Bacterial species	No.	Site of isolation					No.
		Intestine	Liver	spleen	Kidney	Muscle	
<i>Edwardsiella tarda</i>	14	4	5	3	-	2	10 Fish
<i>Yersinia ruckeri</i>	13	4	3	3	3	-	10 Fish

*Number of examined fish = 10. * The percentage were calculated according to the total number of Oreochromis niloticus .*

Results of antibiotic sensitivity of *E.tarda* revealed that was highly sensitive for Lincospectine and slightly sensitive to Erythromycine , while *Yersinia species* was highly sensitive to Erythromycine and

Oxytetracycline and resistance to Ampiciline.

5.The Antibody titer among both bacterial species Antibody titers are shown in Table (4).

Table (4): Antibody titer among both fish species.

Fish species	Week	N	Antibody titer
			Mean ± SE
<i>Oreochromis niloticus</i> Injected with E. Tarda Vaccine	1	3	3.33 ±0.33 C
	2	3	4.67±0.33 ABC
<i>Oreochromis niloticus</i> Injected with yersinia Vaccine	1	3	5.00±0.58 AB
	2	3	5.33±0.33 A

Means within the same column carrying different letters are significantly different at (P < 0.01) .

DISCUSSION

Bacterial diseases are responsible for mortality in both wild and cultured fish (Roberts, 1989). The effect of bacteria varies from that of primary pathogen to that, the secondary invader in the presence of other disease agents, they may also serve as a stress factors and predispose fish to other diseases (Badran and Eissa, 1991).

The clinical signs and post mortem lesions in naturally infected fish includes : Were loss of scales from some areas of the skin, excessive mucus all over the body surfaces with petechial hemorrhages over the dorsal musculature. Skin showed superficial ulcers, especially at the caudal peduncle with extensive hemorrhagic raised areas on the dorsal musculature.

While in case of experimental infection were redness of the mouth and darkening of the head region. Skin showed abnormal dark color, with slight exophthalmia and eye cataract.

The recorded results were identical with the records of several

authors (Kabata 1985; Post, 1987; Baya et al., 1997 and Miwa and Mano, 2000).

*These symptoms attributed mainly to bacterial infection. (Mona et al., 1997, Danely et al., 1999; Oraic et al., 2002 and Technical Bulletin, 2002).

Enterobacteriaceae was isolated from (liver, kidneys, spleen, intestine and muscles) of *O. niloticus* . A different species of *Enterobacteriaceae* (*E. tarda*) was earlier isolated from Channel catfish in the United States (Meyer and Bullock, 1972) and cultured fish from other parts of the world (Eissa and Yassien, 1994).

Results of biochemical tests were similar to these reported by other investigators including (Romalde and Toranzo, 1993 and Danley et al., 1999).

Results of biochemical tests were similar to those reported by other investigations including (Ling et al., 2001).

Concerning the antibiotic susceptibility patterns of isolated *Edwardsiella tarda* and *Yersinia* in

ENTEROBACTERIAL DISEASES AFFECTING FISH

this study was almost the same to those reported in the literature.

*The isolates of *E. tarda* were highly sensitive to antibiotics especially, Lincospectin, Neomycin, Oxanilic acid, Enrofloxacin, Ciprofloxacin, Oxytetracycline and Ampicillin.

The isolates of yersinia were highly sensitive to antibiotics especially, Neomycin, Penicillin and Erythromycin.

Mona et al. (1997) and Dalsgaard and Madsen (2000) indicated that flumiquine, tetracycline, sulphamerazine, oxytetracycline, tribissen, chloramphenicol, gentamycin, streptomycin and tetramycin, and oxolinic acid are the most effective compounds against all isolates of *Y. ruckeri*.

Also, *Bakal et al. (2004)*, found that all strains of *Y. ruckeri* were sensitive to neomycin, gentamicin, tetracycline, chloramphenicol, cotrimoxazole, nalidixic acid and flumequine.

The Mortality pattern among *O. niloticus*:- was carried out by

injection of different concentration of *E. Tarda* and *Yersinia*. The mortality percentage in *O. niloticus* was higher in high concentration in both species of bacteria.

Mona et al. (1997) reported that there was a high mortality percentage among the experimentally infected fish under different stress conditions. These conditions are overcrowdness, external parasitism and transportation with mortality rates of 83 %, 70% and 60 % in *O. niloticus*, respectively, and all of these stress conditions facilitated the infection with *Enterobactereacea*.

Antibody titer was higher in *yersinia* than *E. Tarda* and increased with increased period of vaccination. This difference is due to the bad the susceptibility of fish with bacterial endotoxins *El-Newashy (2005)*.

These results agreed with those of *Mona et al. (1997) and Berc et al. (1999)* they observed that there are a species differences in its susceptibility to *Yersinia* infection.

The decrease in antibody titer in fish exposed to chronic bacterial infection due to the effect of bacterial infection on (lymphoid T.) leading to immuno-suppression in exposed fish. This supported by depletion of haemobiotic tissue in spleen. *Oraic et al. (2002)*.

Welling et al. (1977) stated that the normal host defense mechanism against disease in fish involve humeral and cell mediated immunity. Macrophages are known to be the important part of cellular

immune system and have function to protect host by phagocytizing foreign materials.

These results similar to those of *Oraic et al. (2002)* as they reported that the pathogenicity of the isolated bacteria was confirmed by challenge experiment. Titers of specific antibodies were determined in the sera of survivors and the antibody titers were highest on the 21st day post-challenge and were detectable until the 28 day.



Fig. (1): *Oreochromis niloticus* naturally infected with Enterobactereacea showing congestion f all body surface , scalloss.

ENTEROBACTERIAL DISEASES AFFECTING FISH



Fig. (2): Oreochromis niloticus naturally infected with Enterobacteriaceae showing hemorrhage and congestion of the external body surface especially mouth and scale loss.



Fig. (3): Oreochromis niloticus experimentally infected with Yersenia 10-2 showing slight tail rot and congestion in head region at first week of infection.



Fig.(4): *Oreochromis niloticus* experimentally infected with *Enterobacteriaceae* showing hemorrhage and congestion of the gills and all internal organs with generalized septicemia . In fish injected with 10-2 yersinia ruckeri

CONCLUSION

At the end we concluded that *Enterobacteriaceae* isolated successfully from cultured fresh water fish by different % and its biochemical characters and sensitivity test done accurately to determine the antibiotic of choice and the pathogenicity test accurate this isolation and the vaccine preparation proved the go prevention of this species.

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بعض الدراسات على البكتيريا المعوية التي تصيب الأسماك

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البكتيريا المعوية تعتبر واحد من أهم أمراض الأسماك التي تصيب المزارع في مصر وخصوصا أسماك البلطي النيلي والقرايط و المبروك و الطوبار و أهم أعراضها تتلخص في الآتي :

اسوداد لون الجسم و فقدان القشور وتآكل الزعانف و احمرار الفم و جحوظ العينينو كذلك تأكل الجلود احمرار الفم.هستوباثولوجيا وجد حالات تنكز و تآكل في كل من الكبد و الطحال المخ والخياشيم ووجد نشاط خلايا إنتاج الميلانين .تم إجراء التجربة على 120 سمكة (50 سمكة بلطي نيلي , 20 سمكة مبروك و 30 قرموط 20 طوبارة) من مزرعة خاصة بمحافظة كفر الشيخ و البحيرة و الإسكندرية , استخدمت لعزل البكتيريا سالبة الجرام.

كما استخدمت 50 سمكة بلطي نيلي لإجراء اختبار الحقن لبعض أنواع البكتيريا المعوية. كما استخدمت 30 سمكة لإجراء اختبار المناعة. حيث تم تسجيل الاعراض والصفات الكيميائية جيدا واتى أظهرت أن 72 عينة سالبة الجرام من بين ال 120 عينة المستخدمة فى الفحص وهناك 39 عينة كانت سالبة الأوكسيداز موجبة الكتالاز (بكتيريا معوية). ثم تم عمل اختبار الحقن الصناعى لتأكيد عزل البكتيريا (الادوارديسيلا و اليرسنيا) وتم تسجيل الاعراض الخارجية والداخلية ثم تم اعادة عزل نوعى البكتيريا من الاعضاء المختلفة(الكبدو الأمعاء و الطحال والكلى والعضلات)بدرجات مختلفة تتراوح من 0% - 40%.

ثم تم عمل اختبار حساسية لكلا النوعين حيث وجد أن (الادوارديسيلا) أكثر حساسية للنيومايسين و الاكسيتتراسيكلين فى حين أن اليرسنيا أكثر حساسية للارثرومايسين والامبسلين.أما نتائج الحالة المناعية فوجد أن التحصين أعطى كفاءة مناعية عالية خصوصا فى اليرسنيا عنه فى الادوارديسيلا.