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CLINICAL AND BACTERIOLOGICAL STUDIES ON MYXOBACTERIA INFECTION IN SOME AQUARIUM FISHES

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Abstract

Flavobacterium columnare was isolated from five freshwater diseased ornamental fish spp., Guppy (*Poecilia spp*.), Mollies (*Poecillia* spp.), Goldfish (*Carassius auratus*), Koi (*Poecilia spp.*) and Swordtail (*Xiphophorus spp.*) in Egypt. The main clinical signs of infected fish were respiratory manifestation, yellowish brown or white lesions on the gills and skin, fin and tail rot. Flavobacterium columnare is a thin Gram-negative yellow rod bacterium belong to genus Flavobacterium characterized by spreading colonies on Anacker and Ordal,S media. The isolated Flavobacterium columnare proved its sensitivity to Nalidixic acid; oxytetracyclin and Erythromycin while it was resistant to Neomycin and Ampicillin.

Experimental infection using immersion method by 24 hr. old broth culture of isolated Flavobacterium columnare proved 100%, 90%, 20%, 40% and 60% mortalities with the ornamental fish spp. Guppy, Mollies, Goldfish, Koi and Swordtail respectively.

Treatment trail with Potassium permanganate at 2 ppm bath or Oxytertracyclin proved effectiveness with Koi spp. in preventing infection spread.

INTRODUCTION

Nowadays, bacterial diseases create a common problem facing ornamental fish industry.

The term Myxobacteria is commonly used to describe the group of gliding bacteria associated with a number of fish diseases, all of which belong to the genus Flexibacter. The organisms can be recognized by the characteristic yellow, spreading colonies and gliding motility on low nutrient media (Anacker and Ordal, 1959).

Flavobacterium columnare is a thin Gram-negative rod bacterium of the genus Flavobacterium. It's name was derived from the way in which the organism grows in rhizoid columnar formations where it was first described by Davis (1922), and validated by Bernardet and Grimont (1989). The species has been known previously as Bacillus columnaris, Flexibacter columnaris, and Cytophaga columnaris. Durborow *et al.* (1998).

Flavobacterium columnare being one of the oldest known bacteria among warm water fish, its infection was commonly known as Columnaris which was first reported by Davis in 1922, and it remains one of the most frequently encountered and devastating bacterial diseases of freshwater fishes Durborrow *et al.* (1988), Decostere (2002) and Tripathi *et al.* (2004)

In addition, Durborow *et al.* (1998) had showed that Columnaris occurred frequently among fish raised intensively in cages and in closed recirculation systems and is attributed to crowding and cage abrasions. Once established, the infection can spread quickly and cause high mortality rates. Decostere *et al.* (1999) had proved the ability of a high virulence strain (AJS 1) and a low virulence strain (AJS 4) of Flavobacterium columnare (*Flexibacter columnaris*) to attach to the gills of black mollies (*Poecilia sphenops*). However, Staroscik and Nelson (2008) referred the presence of lesions on the gills, skin and fins of diseased fish to the ability F.columnare to utilize fish skin mucus as a substrate for growth. While Tripath *et al.* (2005) reported that the pathogenesis of columnaris disease is not well understood. Recently, Musa *et al.* (2008) had isolated 14 spp. of Flavobacterium columnare from freshwater ornamental fish from retail pet shop in Malaysia.

Outbreaks occur following environmental stress the bacteria attach to the gills where they multiply, eventually cover and destroy the entire gill filament - leading to fish death. Once established the infection will

spread through the water column, and potentially affecting most fish, with which it comes into contact, Durborow *et al.* (1988). The unfortunate problem with this disease is that the infected fish rapidly becomes ill and stops eating therefore, treating with medicated food may not be an option. Kanamycin daily in the water is a great first choice for treating Flexibacter also Cephalexin can be used at the rate of 250 mg per 10 gallons daily preceded by a 25% water change also disinfectant dips, with prolonged immersion in potassium permanganate have been advocated (Noga, 2000).

Therefore, the present work was carried out to detect the prevalence of Flavobacterium columnare infection in naturally infected freshwater ornamental spp. with a trail for treatment of such bacteria either by using potassium permanganate or antibiotic in water.

MATERIAL AND METHODS

A: Naturally infected fish:

A total of 50 naturally infected ornamental fishes were collected from private commercial fish farm and pet shops in Cairo.The collected collected fishes belonged to Guppy (*Poecilia spp*.), Mollies (*Poecillia spp*.), Goldfish (*Carassius auratus*) Koi (*Poecilia spp*.) and Swordtail (*Xiphophorus spp*.). The were transported alive in plastic bags to the Fish Diseases Department, Animal Health Research Institute Dokki, Giza. Fish were subjected to:

1- Full clinical and post mortem examination according to (Schäper-claus *et al.* 1992).

2 - Microscopic examination of wet preparation of scraping from eroded area in infected fish as a rapid presumptive diagnosis of myxobacterial infections to observe the characteristic "columnar" according to Shotts and Starliper (1999).

3- Bacteriological examination: Swabs taken under aseptic condition from skin lesions and from gills streaked on low levels of both nutrients and agar. Cytophaga Agar (Anacker & Ordal 1959) composed of tryptone 0.5, sodium acetate 0.2, beef extract 0.2 and agar 9.0 (g per liter of distilled water) provides a suitable medium for the isolation of freshwater strains. Pure colonies were streaked onto soft agar to be used for further studies. Bacterial isolates were identified by colonial morphology, growth characters on specific media and microscopically appearance as well as phenotypic characteristics (Buller, 2004).

4- Detection of virulence factor: All isolates of Flavobacterium columnare were tested for the ability to take up Congo red (C.R) dye which is one of the indicators for testing virulence gram negative bacteria according to (Berkhoff and Vinal, 1986)

5- Growth in different temperature degrees: All isolates of Flavobacterium columnare were tested for the ability to grow at different temperature degree (12°C, 15°C, 20°C, 25°C, 30°C and 35°C) for 24 hrs.

6- In-vitro sensitivity test for isolated Flavobacterium columnare: It was carried out against various chemotherapeutic agents and judgment of the obtained results in comparison to interpretive standards was applied as described by Quinn *et al* (2002).

B: Experimentally infection: The isolated Flavobacterium columnare was examined for its ability to produce the disease in apparently healthy ornamental freshwater fishes under experimental condition. One hundred ornamental freshwater apparently healthy fishes were collected from a farm, twenty fish from each sp. (Guppy, Mollies, Goldfish, Koi and Swordtail). All fishes were stocked in well aerated glass aquaria supplied with decolorinated tap water for two weeks to be acclimatized prior to the experimental infection and Fed once a daily with commercial pellets as 1 % of body weight. Scraping from skin were taken randomly and

examined under microscope to insure that they free from natural infection.

Fish were scarified and divided into 10 groups each containing 10 (every sp. into two groups) Group no 1,3,5,7,9 were immersed for one hour in a bath containing 10^6 CFU/ml of F. columnare while group 2,4,6,8,10 kept as a control without infection Experimentally fish groups were continuously investigated throughout two weeks (experimental period). Clinical signs were observed and mortality rate was recorded. Samples from moribund fish were taken aseptically and bacteriologically examined.

A: Trials of treatment: 30 samples of Koi apparently healthy fish were collected from a farm, and stocked in well aerated glass aquaria supplied with decolorinated tap water. Fish were immersed in a bath containing 10^6 CFU/ml of F. columnare. After 24 hr fish were divided into 3 groups, 2 mg/l of Pot. Permanganates is added to the aquarium water of group 1 ensure that the water retains the resulting 'purple/red' for at least 4 hours. If the colour begins to fade, then more P. Permanganates will have to be added**.** 50 mg of Oxytertracycline was add to water of group 2 while group 3 remind as a control clinical signs were observed and mortality rate was recorded in the three groups according to Noga (2000).

RESULTS AND DISCUSSION

Examination of infected ornamental freshwater spp. revealed the presence of excessive mucus formation, necrotic lesions on the skin, which often were white/gray colored with an edging of red or ulcers. This picture was observed in Goldfish while Guppies fish suffered from fin and tail rot, loss of scale with excessive mucus formation which also observed on Mollies spp. while discoloration of skin was observed in Koi and Swordtail spp. This pictures observed also by Decostere, (2002) and Musa *et al* .(2008).

Microscopic examination of wet preparation from scraped eroded areas of skin, gills and fins of infected ornamental freshwater spp. revealed presences of Flavobacterium columnare which was recognized as long slender rods exhibiting a characteristic flexile motility. These results agree with Austin & Austin (2007), Shotts and Starliper 1999 and Noga (2000).

Microbiological examination of samples from skin, gills and fins revealed isolation of Flavobacterium columnare from infected examined ornamental freshwater spp. as reported by Decostere *et al.* (1998) who isolated 4 strain of Flavobacterium columnare from black mollies and platies and also with Musa *et al.* (2008) who isolated Flavobacterium columnare from 14 spp. of freshwater ornamental fish from retail pet shop in Malaysia.

Our results revealed that Flavobacterium columnare cells are long, flexible, Gram-negative aerobic rods, with rounded, motile by gliding movement. Colonies of the bacteria are flat, with yellow (cream to orange) colonies on agar (Fig. 1 A) as reported by Austin & Austin (2007) and Musa *et al.* (2008). The isolated Flavobacterium columnare has rhizoidal pattern of growth on low agar plates This agree with Griffin (1992) and they tight adherence to the agar surface as reported by Decostere *et al.* (1998)and Altinok and Grizzle (2001)

The isolated Flavobacterium columnare take up Congo red (C R) dye (Fig. 1 B) as reported by Bertolini & Rohovec (1992) and Durborrow *et al.* (1988). Up take of Cogo red dye has been shown to be a virulence marker for several pathogens as reported by Statner and George (1987). Our results indicated that all isolates of Flavobacterium columnare light or no growth at temperature less than 20^{o}C, greater growth of yellow convoluted centered colonies with rhizoid edges at 25^{o}C will at 30^{o}C scanty growth. This agrees with Woodland (2004).

Biochemical tests of Flavobacterium columnare was recorded in table (1) which shown that Flavobacterium columnare are gram -ve bacteria, motile, +ve to oxides, nitrate, H_2S production and gelatin hydrolysis while it -ve to starch hydrolysis, indole and catalse. These results agree with Woodland (2004).

The sensitivity of the isolated Flavobacterium columnare to different antibiotic discs revealed that it is highly sensitive to Erythromycin, Spiromycin , Oxytetracyclin and Nalidixic acid while it less sensitive to Neomycin and Oxolonic acid, also it is resistant to Ampicillin, Gentamycin, Colistin and Cephalothin table (2). Our results go hand to hand with Noga (2000) and Austin and Austin (2007).

Experimentally infected ornamental freshwater fishes by immersion in 24 hr old broth culture of isolated Flavobacterium columnare proved, 100%, 90%, 20%, 40% and 60% mortalities with Guppy, Mollies, Goldfish, Koi and Swordtail spp. respectively table (3). The result indicated that Guppy was highly sensitive to Flavobacterium columnare with 100% mortality without any signs as reported by Noga (2000). The highly virulent strain will kill fish in 24 hours and these fish often die so quickly, that there may not even be enough time for them to show any outward signs of infection.

Experimentally infected ornamental freshwater fishes were suffered from variety of clinical signs as sluggish movement, loss of appetite, swimming near the water surface, gasping air and showing an increase in the breathing frequency. All these signs grow faster in Guppies and Swordtail fishes than other spp. Also it appears slowly after time in Goldfish. Moreover, pale color, discoloration slimy skin and detachment of scales were observed clearly in Mollies and Koi after several days while tail and fin rot and detachment of scales were showed in Guppies and Swordtail fishes. Fig. (2-6) showed clinical signs of experimentally infected fish. Our results go hand to hand with Durborrow *et al.* (1988),

Bernardet *et al.* (1996) and Decostere (2002), and Musa *et al.* (2008). The changes of clinical signs in different fish spp. agree with Tripath *et al.* (2005) who reported that the pathogenesis of columnaris disease is not well understood.

P.M examination revealed that gills showed pale appearances at the extremities of the filaments with sever erosion giving the gills a ragged appearance especially in Guppy and Swordtail fish. Some fishes had normal appearance of the internal organs. Hemorrhagic enteritis with serous fluid accumulations in the abdominal cavity and pale internal organs were showed in some fish. The clinical and P / M examination go hand to hand with that reported by Decostere *et al.* (1998).

Treatment of experimentally infected koi fish revealed that Pot. Permanganates is succeeded to reduce mortality in group 1 while 4 fish from 10 fish suffered from tail rot loss of scale mortality rate is 10 % while fish in group 2 while treated with Oxytetracycline is very active, no mortality was recorded . Fish in group 3 suffered from superficial ulcer and respiratory manifestation mortality rate reached 50 % in the end of experiment the observed results agree with Noga (2000).

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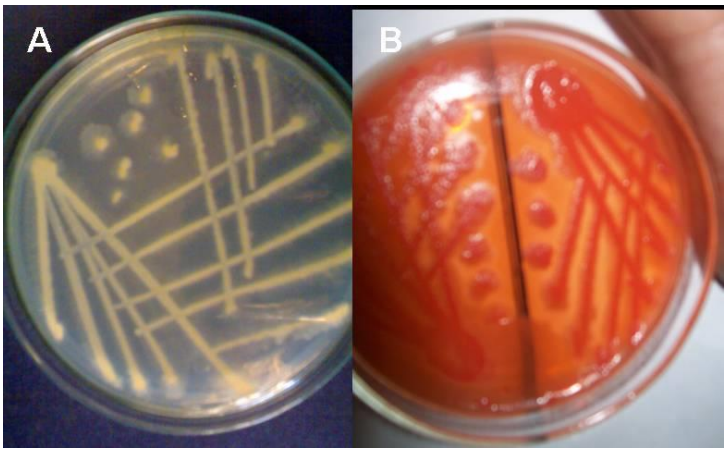

Fig.1: A. showed F. columnare in Ordal´s medium.
B. showed F. columnare take red color on congo red.

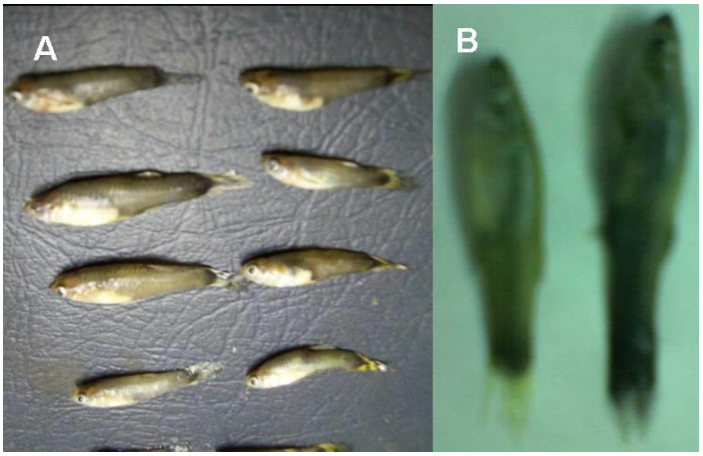

Fig. 2: A. Experimentally infected guppies fish with F. columnare 24 hrs post infection without clinical signs.
B. Naturally infected guppies fish with F. columnare showing tail rot and excessive mucus.

Fig. 3: Experimentally infected Goldfish fish with F. columnare showing skin ulceration tail rot with loss of scale.

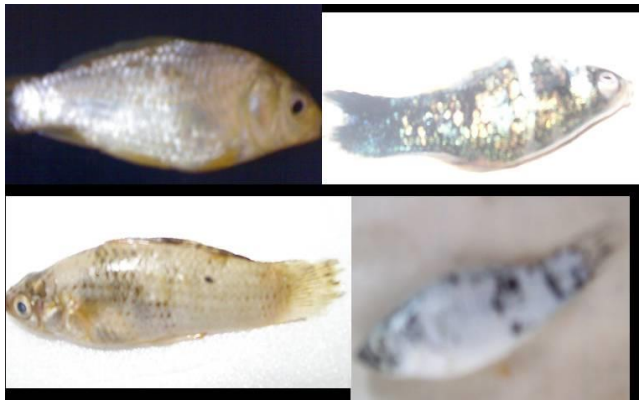

Fig. 4: Experimentally infected koi fish with F. columnare showing skin ulceration tail rot with loss of scale.

Fig. 5: Experimentally infected Swordtail fish with F. columnare showing skin discoloration with loss of scales, skin ulceration at head fin and tail rot.

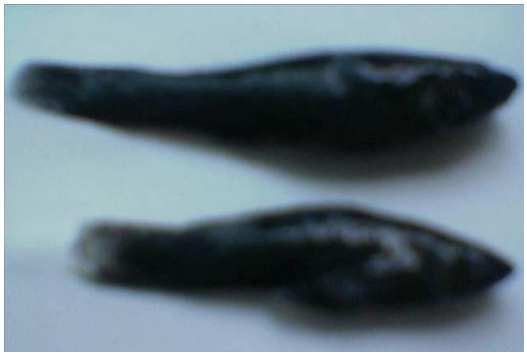

Fig. 6: Experimentally infected black molly with F. columare showing tail rot and excessive mucus

Table (1): Results of phenotypic and biochemical identifecation of the isolated Flavobacterium columnare**.**

Test	Reaction of *F. columnare*	Test	Reaction of *F. columnare*
Gram stain	Gram -ve	Methyle red	-
Shape	Long thin bacillus	Vogaus-Proskauer	-
Motility	Motile by gilding	Gelatine hydrolysis	+
Growth on : Cytophaga agar	Yellowish flat with rhizoidal edges	Indole test	-
Growth on 0.5% NaCl	+	Fermentation of:	
Growth on 1.0% NaCl	-	Glucose	-
Cytochrome Oxidase	+	Sucrose	-
Catalase	-	Maltose	-
		Mannitol	-
		Lactose	-
Nitration reduction	+	Hydrolysis of starch	-
H_2S on TSI	+		

Table (2): Showed in-vitro sensitivity tests of F. columnare to different chemotherapeutic agents.

Chemotherapeutic agents	Concentration per disc	F. columnare
Ampicillin (Am)	10 µg	R
Erythormycin (E)	15 µg	S
Gentamycin (CN)	10 µg	R
Neomycin (N)	30 µg	I
Nalidixic acid (NA)	30 µg	S
Spiromycin (SP)	100 µg	S
Oxolonic acid (OA)	2 µg	I
Oxytetracyclin (OT)	30 µg	S
Colistin (CT)	10 µg	R
Cephalothin (C)	30 µg	R

S: Sensitive I: Intermediate R: Resistant

Table (3): Showed mortality rate of ornamental fish experimental infected with F. columnare.

Group no.	No. of fish	Mortality rate	Species of fish	Type of infection	Morbidity rate	Beginning of fish death
1	10	100%	guppy	bath with bacteria	100%	24 hours
2	10	90%	mollies	bath with bacteria	60%	2 day
3	10	20%	fintail	bath with bacteria	50%	5 day
4	10	40%	koi	bath with bacteria	60%	3 day
5	10	60%	Swordtail	bath with bacteria	50%	2 day
6	10	-	guppy	Control group without infection	-	-
7	10	-	mollies	Control group without infection	-	-
8	10	-	Goldfish	Control group without infection	-	-
9	10	-	koi	Control group without infection	-	-
10	10	-	Swordtail	Control group without infection	-	-

دراسات اكلينيكية وبكتيريولوجية على العدوى بالميكروبكتيريا فى بعض اسماك الزينة

سعاد صبرى سلامه

قسم أمراض الأسماك- معهد بحوث صحة الحيوان- الدقى

تمت هذه الدراسة على بعض انواع اسماك الزينة فى مصر حيث تم عزل ميكروب الفلافوبكتيريا كولمنارى من اسماك الجوبى والمولى والفانتيل والكوى والسوديتال وكانت اهم الاعراض الاكلينيكية هى تآكل فى الذيل والزعانف مع صعوبة فى التنفس وتقرحات جلدية.

ميكروب الفلافوبكتيريا من البكتيريا السالبة لصبغة الجرام ويتميز بالحركة الزاحفة وينمو بطريقة مميزة على ميديا الاودالس.

اثبتت الدراسة ان الميكروب له حساسية عالية للاوكسى تتراسيكلين ومقاوم للامبيسللين

ادت العدوى التجريبية عن طريق الغمس بنسبة نفوق ١٠٠ % فى اسماك الجوبى ، ٤٠% لاسماك الكوى ، ٩٠ % لاسماك المولى ، ٢٠% لاسماك الفانتال اما اسماك السوديتال فكانت نسبة النفوق ٦٠%

ادى استخدام البوتاسيوم برمنجانات الى انخفاض نسبة النفوق فى اسماك الكوى الى ١٠% أما الاوكسى تتراسيكلين فقد أوقف نسبة النفوق.