

## Bacteriological Studies on *Flavobacterium columnare* in Fish

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### Abstract

Columnaris is one of the oldest known fish diseases caused by *Flavobacterium columnare*. The wide spreading of the causative agent in freshwater environments and the susceptibility of fish to be attacked by it after mechanical and/or environmental factors makes *F. columnare* among the most prevalent pathogens in cultured, ornamental, and wild fish populations. One hundred fishes of tilapia (*Oreochromis niloticus*) were collected randomly from ponds of Central Laboratory for Aquaculture Research of Abbassa, Abou Hammad, Sharkia, Egypt for *Flavobacterium columnare* isolation. *F. columnare* was isolated from (150) out of the (350) examined samples. The bacteriological examination revealed that the prevalence of *F. columnare* was (42.8%) in *Oreochromis niloticus*. The highest prevalence of *F. columnare* was in gills (33.3%) followed by skin (30%) then fins (24.6%) and kidney (12%). These isolates were further characterized by PCR that revealed positive result for 16S RNA gene with specific amplicons size 675bp. The result of antimicrobial sensitivity testing of the isolates revealed that doxycycline, erythromycin, tetracycline, trimethoprim, nalidixic acid and streptomycin were the most effective drugs against *F. columnare*.

### Introduction

*Flavobacterium columnare* is thin, Gram-negative, rod-shaped bacteria discreted from the other comparable bacteria in morphology by their distinctive rhizoid pattern of growth on a low nutrient medium, and by their growing ability on media containing neomycin and polymyxin B. **Griffin (1992)**.

Columnaris disease considers the second leading killer of commercially raised channel catfish, *Ictalurus punctatus* next to

enteric septicemia (ES) caused by *Edwardsiella ictaluri*. **Durborow et al. (1998)**

Columnaris disease or mixed infections of columnaris and ES were listed as the first cause of the greatest economic losses on catfish farms by 70% of farmers from the four leading catfish producing states (**USDA, 2003**), with losses estimated in the millions of dollars. This study aimed to investigate the propagation of *Flavobacterium columnare* in *Oreochromis*

*niloticus*, characterize representative *F. columnare* isolates recovered from *Oreochromis niloticus* and summarize the resistance pattern of isolated *F. columnare* against various antibiotics.

### Material and Method:

#### Fish:

Total number of (100) clinically diseased tilapia fish of the genus *Oreochromis niloticus* were transferred alive in oxygenated plastic containers to the laboratory and subjected to full clinical and postmortem examination.

#### Bacteriological examination:

Samples were taken under complete aseptic conditions from gills, skin, fins, kidney, liver, spleen and muscle. The samples were inoculated onto Ordal's broth for 28hrs at 37°C then subcultured on Ordal's agar. The obtained pure colonies were identified according to colonial morphology, slide film was stained with gram stain for

microscopical examination and description of the shape and arrangement of bacteria was done according to *Collins et al; (1989)*.

#### Polymerase Chain reaction:

For accurate identification of *Flavobacterium columnare* universal primers for 16S rRNA gene were used **Table (1)**. DNA extraction had been done by following manufacturer's instructions of QIAamp DNA mini kit. PCR products were electrophorized using 1% Agarose gel using Gel casting apparatus (Biometra). The gel was photographed by a gel documentation system and the data analyzed through computer software according to *Sambrook et al. (1989)*.

#### Antimicrobial sensitivity test:

The antimicrobial sensitivity test of *F. columnare* isolates were performed by disc diffusion test according to *Bauer et al. (1966)* and interpreted according to *NCCLS/CLSI (2007)*.

**Table (1) Oligonucleotide primers sequences:**

Primer	Sequence	Amplified product	Reference
<i>F.COLUMNARE</i> 16SrRNA	F: 5'-CAGTGGTGAAATCTGGT-3'	675	<i>Darwish et al., 2004</i>
	R: 3'-GCTCCTACTTGCGTAGT-5'		

### Results and Discussion:

#### clinical examination of fish:

Naturally infected *Oreochromis niloticus* were clinically examined and revealed loss of scales with presence of grey-white discoloration on some parts of the

head, tail, around the mouth, fins and other areas (**Figure 1**). In some cases, ulceration, and degeneration of underlying muscle fibers around dorsal fin observed (saddle back lesion) (**Figure 2**). The gill filaments showed presence of

congestion, necrosis and covered with excessive mucus.

### **Bacterial isolation and identification:**

The present study revealed that *F. columnare* can grow only on the selective media (cytophaga agar media) with addition of antimicrobial agents including polymyxin B and neomycin sulfate in order to inhibit growth of other bacteria. Colonies appeared on selective media as smooth flat golden yellow colonies with circular edges measuring 3-4mm in diameter and adhered to the agar. Microscopically, isolates were gram-negative bacterium, long rods and scattered in arrangement. This result was harmonious with those recorded by *Bullock et al., (1986), Griffin (1992)* who stated that selective media must be used to isolate *F. columnare* and differentiate it from other yellow pigmented gliding bacteria. They described the colony on solid media as yellow to orange in color, translucent, circular, smooth and shiny with entire edges.

The present investigation revealed that the total prevalence of *F. columnare* among naturally infected *Oreochromis niloticus* was (42.8%) **Table (1)**, this finding was higher than recorded by *Elgamal (1995)* who recorded that prevalence of *F. columnare* among naturally infected *tilapia* was (16.6%). also higher than result obtained by *Eltalaway (2008)* who found that that prevalence of *F. columnare* infection in examined

*Clarias guriepinus* was (22.9%) and *Kamel (2013)* who recorded (4.16%) among infected *Oreochromis niloticus*.

The isolation of *F. columnare* occurred from gill with the highest prevalence (33.3%) followed by skin (30%), tail and fins (24.6%) and kidney (12%) while, there is no isolation found from other internal organs **Table (2)**. This agrees with that stated by *smith (1982)* who mentioned that the gill is the largest tissue for presence of *F. columnare*, *Elgamal, (1995) and Eltalaway (2008)* who isolated *F. columnare* from skin, gill and kidney.

### **Genotypic characterization:**

The molecular identification of *F. columnare* by PCR produced unique and clear bands corresponded to the 675 base pair (bp) by internal fragments of 16S rRNA gene confirmed without doubt that the isolated strains were *F. columnare* (**Figure 3**). These results were similar to those recorded by *Darwish et al., (2004)*.

### **Antibiotic sensitivity test:**

The present findings showed that *F. columnare* strains were sensitive to tetracycline, nalidixic acid, trimethoprim, doxycycline, streptomycin and erythromycin. While moderately sensitive to colistine and kanamycin and highly resistant to neomycin, **Table (3)**. These results are in agreement with *Hesami et al. (2010)* who recorded that all strains of *F. columnare* were susceptible to ampicillin, erythromycin, streptomycin,

tetracycline, trimethoprim- sulphat but displayed acquired resistance to neomycin and polymyxin B. and partially agree with the result recorded by *Elgamal (1995) and Varga et al. (2016)*.

On conclusion, this study indicated that *F. columnare* is one of the most common fish pathogens which cause mortalities and high economic losses. High prevalence of *F. columnare* among *Oreochromis niloticus* collected from ponds of Central Laboratory

for Aquaculture Research of Abbassa, Abou Hammad, Sharkia, Egypt reaches to (42.8%). The distribution of organism in tissue and organs of infected *Oreochromis niloticus* were high in gills (33.3%) followed by skin (30%) then fins (24.6%) and kidney (12%). Nalidixic acid, tetracycline, streptomycin, erythromycin, doxycycline and trimethoprim were the most effective drugs against *F. columnare*.

**Table (1):** Prevalence of *F.columnare* in examined *Oreochromis niloticus*

Fish species	Total No	Total samples	Positive growth samples	%of isolation of <i>F.columnare</i>
<i>Oreochromis Niloticus</i>	100	350	150	42.8%

**Table (2)** Prevalence of *F.columnare* among various organs of *Oreochromis niloticus* samples.

Bacteria	Total No	Organ													
		gill		skin		Tail&fin		kidney		liver		spleen		muscle	
		No	%	No	%	No	%	No	%	No	%	No	%	No	%
<i>F.Columnare</i>	150	50	33.3	45	30	37	24.6	18	12	0	0	0	0	0	0

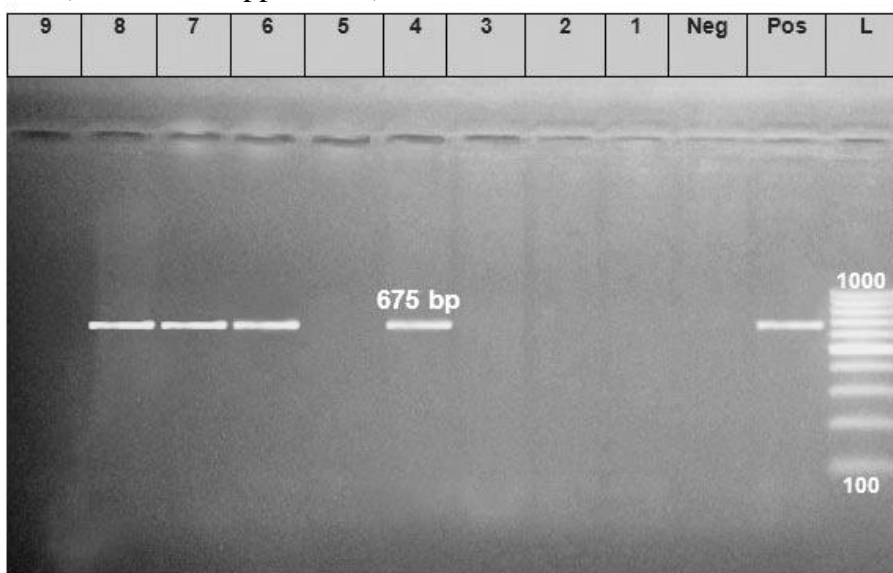
**Table (3)** Results of antibiotic sensitivity test of isolated bacteria

Antibiotic agent	Disc conc (µg)	<i>F.columnare</i>	
		Inhibition zoon(mm)	Reaction
Colistine sulfate	10	7	I
Streptomycin	10	23	S
Kanamycin	30	17	I
Erythromycin	15	23	S
Neomycin	30	0	R
Doxycycline	30	30	S
Nalidixic acid	30	33	S
Tetracycline	30	23	S
Trimethoprim	1.25	30	S



**Figure (1):** *O. niloticus* showed white necrotic tissue, rough scales and scale less area behind the head.

**Figure (2):** *O. niloticus* showed erosion & ulcer of the back reached to the muscle (saddle back appearance)



**Figure (3):** Electrophoretic pattern of 16S r RNA PCR assay of *F. columnare* isolated from fish.

L=ladder

Pos=positive control

Neg=Negative control (saline)

Sample: *F. columnare* isolates from *Oreochromis niloticus* (lanes: 4, 6, 7, 8).

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## دراسات بكتريولوجية علي الفلافوباكتريم كولمنار في الأسماك

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جُمعت ١٠٠ سمكة (بلطي نيلي) عشوائياً من المزارع التابعة للمعمل المركزي لبحوث الثروة السمكية بالعباسة , محافظة الشرقية لمعرفة مدى تواجد الفلافوباكتريم كولمنار بها. ووضحت الدراسات البكتريولوجية ان نسبة انتشار مرض الكولمنارس في البلطي النيلي كانت (٨.٤٢%) وان اعلي نسبة تواجد كانت في الخاشيم بمعدل ٣٣,٣% يليها الجلد بمعدل ٣٠% , الزعانف ٢٤.٦% والكلية ١٢% . واكد اختبار انزيم البلمرة المتسلسل وجود الرابط المخصص ليكتريا الفلافوبكتريا كولمنار في سمك البلطي النيلي وذلك عند ٦٧٥ قاعدة مزدوجة الذي ينفرد بوجود بكتريا الفلافوبكتريا كولمنار وذلك بناء علي الجين المخصص لها ١٦SrRNA. كما اوضحت نتائج اختبار الحساسية ان دوكسيسيكلين, اريثروميسين, سلفاترايمثوبريم, حمض النالديكسيك وتتراسيكلين هما المضادات الحيوية الاكثر فاعلية لمقاومة وعلاج مرض الكولمنارس.