Incidence of *Aeromonas* bacteria in Tilapia Nilotica "Oreochromis Niloticus" fresh water fish, in Egypt.

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Abstract

This study was planned to throw the light on the prevalence of mesophilic bacteria ***Aeromonas*** species in tilapia nilotica "Oreochromis Niloticus" from fresh water and cultured fish, and determine the chemical characterization of fish that may be considered as significant food safety threat. Therefore one hundred and sixty "Oreochromis Niloticus" fish samples. Eighty feral fish collected randomly from Nile, and another 80 net cage cultured fish from Manzala lack in outbreak. The mesophilic ***Aeromonas*** species isolated from feral and net cage cultured fish in a percentage of 8.7% and 26.2 % respectively. Twenty eight strains of mesophilic ***Aeromonas*** were isolated, ***A. hydrophila*** was the highest strain 15(53.5%) followed by ***A. caviae*** 7(25%), ***A. sobria*** 3(10.7%) and ***A. untyped*** 2(7.1%), while the lowest strain was ***A. trota*** one (3.5%). Production of enterotoxin, observed in suckling mouse assay, was detected in 66.6% of ***A. sobria*** strains, in 46.6% of ***A. hydrophila*** strains and in 28.5 % of ***A. caviae*** strains. The results of positive ***Aeromonas*** examined samples showed increase in pH, total volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA) values than normal levels recorded in **Egyptian Organization for Standards and Quality Control (2009)**. These changes may lead to spoilage of fish and may constitute public health hazards, so fish and fish products in markets must be examined frequently. Through these results we suggest that ***Aeromonas*** spp. must be listed in **Egyptian Organization for Standards and Quality Control (2009)** as one of food-borne pathogen. The public health hazard of these microorganisms was discussed.

Key words:

*Microbiological examination, **Aeromonas** spp., Oreochromis Niloticus, Pathogenicity test, chemical evaluation.*

Introduction

The greatest increase in human population with the parallel shortage of animal protein all over the world directed the attention to fish as rapid and healthy compensatory source of good quality animal protein**. (Raafat, 2005).** Fish is a high-protein, low-fat food that provides a range of health benefits. White fleshed fish, in particular, is lower in fat than any other source of animal protein, and oily fish are high in omega-3 fatty acids, or the "good" fats. Since the human body can't make significant amounts of these essential nutrients so the fish are an important part of the diet. Also, fish are low in the "bad" fats commonly found in red meat, while high in good fats called omega-6 fatty acids. Tilapia fish are likely to be the most important of all cultured fish in the 21 century; they are farmed either in semi - extensive or super - intensive farms

(Fitzsimmons, 2000). Tilapia is the major commercial fish species found in Egypt **(Raafat, 2005)**. ***Aeromonas*** spp. is ubiquitous, opportunistic bacterium and normal micro-flora in fish as well as other aquatic mammals. They have been reported in both marine and fresh water environments and can cause diseases in fish under stressful conditions **(Conte, 2004)**.

A. hydrophila and other motile **Aeromonads** are among the most common bacteria in fresh-water habitat throughout the world, and have been recognized as occasional pathogens of cultured and feral fish. However, in fish culture, diseases are often magnified by additional stress factors imposed by husbandry conditions **(González *et al.,* 2002)**. It is one of the most important agent of the outbreak in fresh water fish, in which skin ulcers, hemorrhage and necrosis of the visceral organs (liver, kidney, swim bladder), spleen infracts, fatty liver, ascetic fluid and swollen haemopoietic tissues **(Topić Popovic *et al.,* 2000).** The damage done to both wild and farmed freshwater fish population is extensive, leading to mortality and severe loss of income. ***Aeromonas*** spp. are pathogens that cause food -borne gastroenteritis in human and extra intestinal symptoms such as septicemia, meningitis, endocarditis and osteomyelitis with a high mortality rate in immune-compromised person **(Gold and Salit, 1993)**. The mechanisms by which ***Aeromonas*** spp. cause diarrhea has been known that they produce enterotoxins, certain enzymes, they are able to adhere cell membranes and invade them **(Kirov *et al.,* 1994)**. ***Aeromonas*** species can grow and produce toxins in refrigerated conditions **(Eley *et al.,* 1993).**

The present work aimed to examine the tilapia nilotica "Oreochromis Niloticus" fish for its quality and safety for human consumption through assessment of bacteriological quality with special reference to mesophilic bacterial ***Aeromonas*** species as one of food poisoning microorganisms, as well as to determine hydrogen ion concentration, total volatile basic nitrogen and thiobarbituric acid to assure quality in the aspects of consumer acceptability, degree of freshness, nutritive value and its role as a public health hazard.

Materials and Methods

1-Samples collection.

A total of 160 of **"*Oreochromis niloticus*"** fish samples, 80 of feral fish were collected random from Nile, and another 80 cultured fish net caged in outbreak from Manzala Lake. The samples were transferred to the laboratory in an ice-box to be examined bacteriologically and chemically for mesophilic ***Aeromonas*** species.

2- Bacteriological examination.

-*Aeromonas* spp. isolation.

Twenty-five grams of fish sample were weighed aseptically and homogenized for 2 min in stomacher bags containing 225 ml of TSBA (Tryptic soy broth plus 10 µg/ml ampicillin) Then, the mesophilic ***Aeromonas*** species were isolated using the direct plating method in which spreader onto the surface of Starch-Ampicillin Agar (SAA) plates and incubated at 28ºC for 24 hours, When suspect-ing growing colonies were transferred to tripticase soy agar (TSA) (Difco), triple-sugar-iron slants (TSI) (Difco) and incubated at 28ºC for 24 hours **(Castro *et al.,* 2003)**.

-*Aeromonas* spp. Identification.

The colonies that showed typical reaction in TSI and were oxidase and catalase positive were confirmed as ***Aeromonas*** spp. according to **Popoff (1984)**.

3-Detection of toxin produced by *Aeromonas* spp. (Enterotoxin assay).

The ability to produce enterotoxin was assayed by the infant mouse test according to the tech-nique described by **(Vera *et al.,* 1989).**

-Preparation of culture filtrate.

Strains of ***Aeromonas*** spp. were inoculated in 20 ml of tryptic soy broth (TSB) and incubated at 28ºC for 24 hours. The cultures were centri-fuged at 10,000 rpm for 30 minutes and 0.45 µm filter sterilized (Millipore). The sterile fil-trates were frozen and stored at - 70ºC, till used. A part of the sterile medium was used as a negative control. **(Watson *et al.,* 1985)**.

-Infant mouse assay.
A volume of 0.1 ml of culture filtrate was injected into the stomach of 2-4 day-old suckling mice. Four mice were used for each group (7 groups). After 4 hours at room temperature, the animals were killed with chloroform. The entire intestinal tract of each mouse was removed and immediately weighed. The entire remaining body was weighed as well. The ratio between the intestinal weight and the body weight was determined. The 0.08 ratio was considered positive. A positive control was included in test.

4-Chemical examination.
Eight of examined samples which give positive results for ***Aeromonas*** spp. examined chemically to estimate as an indicator of the degree of fish freshness as cited below:

-**Determination of hydrogen ion concentration (pH).** 10g portions of examined samples were homogenized in 100 ml of distilled water, followed by pH determination in a metrohm pH meter according to **Pearson, (1984)**.

- **Determination of total volatile basic nitrogen (TVB-N)** mg/100gm. It was done according to the methods of **Egyptian Standard Specification (ESS) (1993)**.

- **Determination of thiobarbituric acid (TBA)** mg malonaldehyde / kg sample. It was done according to the methods of **Egyptian Standard Specification (ESS) (1993)**.

Results

Table (1). Incidence of ***Aeromonas*** in "Oreochromis Niloticus" fish.

Source of sample	No. of samples	***Aeromonas*** positive samples	
		No.	%
Feral fish	80	7	8.7%
Net caged cultured fish	80	21	26.2%
Total	160	28	17.5%

[%] calculated according to the total number of the collected samples, (N=160).

Table (2). Prevalence of different ***Aeromonas*** species isolated from examined samples.

Aeromonas species	Number and percent of positive ***Aeromonas*** species	
	No.	%
Aeromonas hydrophila	15	53.5%
Aeromonas caviae	7	25%
Aeromonas sobria	3	10.7%
Aeromonas untyped	2	7.1%
Aeromonas trota	1	3.5%

[%] calculated according to the total number of positive ***Aeromonas*** species (N=28).

Table (3). Frequency distribution of ***Aeromonas*** species in examined samples

Aeromonas species	No. of strain	feral fish		caged net cultured fish	
		No.	%	No.	%
Aeromonas hydrophila	15	5	33.3%	10	66.6%
Aeromonas caviae	7	1	14.2%	6	85.7%
Aeromonas sobria	3	1	33.3%	2	66.6%
Aeromonas untyped	2	0	0%	2	100%
Aeromonas trota	1	0	0%	1	100%

Percentage was calculated based on number of each strain.

Table (4). Frequency distribution of ***Aeromonas*** species from different organs in examined samples.

Aeromonas species	No. of isolated strain	feral fish						caged net cultured fish					
		Body surface		Ascetic fluid+ internal organs		Muscle (Flesh)		Body surface		Ascetic fluid+ internal organs		Muscle (flesh)	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Aeromonas hydrophila	15	1	6.6%	3	20%	1	6.6%	3	20%	5	33.3%	2	13.3%
Aeromona caviae	7	1	14%	0	0%	0	0%	4	57%	1	14%	1	14%
Aeromonas sobria	3	1	33%	0	0%	0	0%	2	66%	0	0%	0	0%
Aeromonas untyped	2	1	50%	0	0%	0	0%	1	50%	0	0%	0	0%
Aeromonas trota	1	0	0%	0	0%	0	0%	1	100%	0	0%	0	0%

Percentage was calculated based on number of each strain

Table (5). Detection of enterotoxin producing by ***Aeromonas*** species from examined samples using infant mouse assay**.**

Aeromonas species	No. of positive strain	Positive of enterotoxigenic ***Aeromonas*** species	
		No.	%
Aeromonas hydrophila	15	7	46.6%
Aeromonas caviae	7	2	28.5%
Aeromonas sobria	3	2	66.6%
Aeromonas trota	1	0	0%

Percentage was calculated based on number of each strain

Table (6). Chemical analysis of (8) examined samples which give high positive results for ***Aeromonas*** species.

Aeromonas strains	PH		TVB-N*		TBA**	
	Feral fish	Net caged cultured fish	Feral fish	Net caged cultured fish	Feral fish	Net caged cultured fish
Aeromonas hydrophila	6.7	7.4	29.8	54.17	0.3	8.28
Aeromonas caviae	6.9	7.3	22.45	85.26	0.33	6.54
Aeromonas sobria	6.8	7.5	18.45	84	0.24	4.84
Aeromonas untyped	-	7.2	-	54.6	-	5.7
Aeromonas trota	-	7.5	-	79.2	-	10.6

*Total volatile bases estimated as mg/100 gm sample.
** Thiobarbituric acid estimates as mg malonaldehyde / kg sample.
Normal permissible limits of chemical composition according to the **Egyptian Organization for Standards and Quality Control number 889-1(2009):**
PH value ---------- 6.5.
Total volatile basic nitrogen (TVB-N) value---------- 30 mg/100gm.
Thiobarbituric acid (TBA) value ---------- not exceed 4.5 mg malonaldehyde /kg.

Discussion

Tilapia fish tolerate adverse water quality and other stressors better than most other commercial aquaculture species. Since stress is a major factor that predisposes fish to **Aeromonas** bacterial infection, tilapia fish is labeled as being very disease resistant and in the presence of pathogens, tilapia fish is the last to break with disease **(Helfman *et al.,* 1997)**. ***A. hydrophila*** and other **Aeromonads** are among the most common bacteria in freshwater habitats throughout the world. Genus ***Aeromonas*** includes prominant microbiota in freshwater reservoirs where they together with other microorganisms act as natural bio-filters and promote self purification of the water body. They are necessarily present in normal microflora and hydrobionats inhabiting fish reservoirs **(Kompanets *et al.,* 1992)**. However, they frequently cause problems in both feral and cultured fish **(Cipriano, 2001).** It is responsible for heavy economic losses caused by both high mortality and deterioration of the product quality **(Groff and Lapatra, 2000; Karunasagar *et al.,* 2003).** Table (1) revealed that the prevalence of bacterial ***Aeromonas*** species in the feral fish was 7 strains with a percent 8.7% less than net caged cultured fish which was 21 strains with a percent 26.2% and that agreement with **(Faisal et al., 1989 and Shayo *et al.,* 2012)** who reported that the overcrowded in net caged cultured , environmental factors act as stressors and can predispose fish to bacterial infections. Table (2) showed that the prevalence of ***A. hydrophila*** infection was high 53.5% followed by ***A. caviae***, ***A. sobria, A. untyped*** and ***A. trota*** in a percent of 25%, 10.7%, 7.1% and 3.5% respectively. This results were difference than **(Abou El-Atta, 2003),** who isolated ***A. hydrophila, A. sobria*** and ***A. caviae*** in a percent of 12.4%, 16.7% and 11.9% respectively. The difference in the rate of isolation of ***Aeromonas*** and species may attribute to difference in localities, methods of sampling, total number of samples and seasons. Table (3) revealed that ***A. hydrophila*** from cultured tilapia in outbreak were 10 strains with a percent (66.6%), and they were 5 strains with a percent (33.3%) from feral fish, this result was higher than **(Ahmed, 2002)** who isolated ***A. hydrophi*** in a percent of 47.3% from diseased culture tilapia in El-

Abbassa, and **(Essa *et al.,* 2009)** who isolated ***A. hydrophi*** in a percent of 53.33% from fresh water fish (tilapia Niloticus) in different localities of Assiut city. The high percentage of ***A. hydrophila*** return to highly polluted condition of water and fish in Manzala lake **(Mahmoud *et al.,* 2010)** and also types of water which representative to human activities and different ecosystems in the lake water environment characterized by brackish water in the south and west, where it is saline water in the north east near to the sea outlets (EL-Gamil outlet) **(Mahmoud *et al.,* 2011)**. While 6 strains of ***A. caviae*** were isolated in a percent of (85.7*%)* from net caged cultured and one strain from feral fish with a percent (14.2*%)*. That agree with **(Araujo *et al.,* 1991)** who mentioned that ***A. caviae*** was the most common species in water with considerable faecal contamination and it was the species most frequently isolated from human faeces. Also **(Ashiru *et al.,* 2011)** said that the water from which the tilapia fish was caught could have faecal contaminant bringing about the presence of ***A. caviae*** in the tilapia fish. On other hand ***A. sobria*** which present name is **(*A. veronii biovarsobria*)** was isolated in a percent of 66.6*%*from net caged cultured and one strain from feral fish in a percent 33.3*%*. ***A. untyped*** and ***A. trota*** were isolated from net caged cultured fish in a percent of (100*%)* and (100*%)* respectively and they didn't detect in feral fish. The obvious results resume to differences in sampling period, water quality, geographical location, the origin of the samples and methodology of analysis, it is difficult to compare the level of ***Aeromonas*** spp. incidence published by different authors. However, the present data clearly confirm the widespread distribution of motile **aeromonads** in tilapia fish.

Table (4) revealed that the prevalence of ***A. hydrophila*** was isolated in high percentage from ascetic fluid+ internal organs and muscles (flesh**)** that agree with **(Newman and Nat-narich 1982)** who found that ***A. hydrophila*** is inhabits as intestinal flora of the healthy fresh and marine water fish. Also **(Rey, *et al.,* 2009)** reported that ***A. hydrophila*** was found in natural and cultured aquatic environment, **(Eisa *et al.,* 1993)** described ***A. hydrophila*** as opportunistic pathogen, and capable of causing disease in weakens fish. **(Company *et al.,* 1999**) who reported that the majority of the infection occurred during the change of water temperature, spawning season, the adverse conditions during intensification. In addition to the increased environmental fluctuation, the cultured fish become more sensitive to stress than the wild population. As a result, the increase in the production of corticosteroid increase the susceptibility of the fish to ***A. hydrophila*** flowed by ***A. caviae***. **(Ashiru *et al.,* 2011)** also reported that ***A. caviae*** was the predominant species of the genus ***Aeromonas*** isolated from the body surface, muscles and body cavity of tilapia fish**.** Table (5) revealed that the Production of enterotoxin, observed in suckling mouse assay, was detected in 2 (66.6%) of ***A. sobria*** strains, in7 (46.6%) of ***A. hydrophila*** strains, in 2 (28.5%) of ***A. caviae*** strains and couldn't detect in ***A. trota***. These results were nearly agreement with **(Vera *et al.,* 1989)** who studied the ability of ***Aeromonas*** spp. to produce enterotoxins by using suckling mouse assay and they found that enterotoxin was detected in 67% of ***A. sobria*** strains, in 60% of ***A. hydrophila*** strains and in 40% of ***A. caviae*** strains.

On reviewing the table (6) the pH results, total volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA) values of feral fish were within the permissible limits according to the **(Egyptian Organization for Standards and Quality Control 2009)**. Meanwhile the PH, total volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA) values of net caged cultured fish were higher than the permissible limits. The increase in pH value indicate bacterial growth and possible spoilage of fish, such increase may be partly attributed to the production of volatile basic compounds such as ammonia **(Galli *et al.,* 1993)**. These results were higher than that of the **(Egyptian Organization for Standards and Quality Control 2009)**. The obtained results were agree with several authors studied the effect of

Aeromonas spp. on the fish and meat, **(Barile *et al.*, 1985**; **Sharma and Goswami, 2010 and Gihan and Hanan, 2012)**. In conclusion, it seems completely necessary to pay even more attention to **Aeromonads** in the field of food health and consumer protection for their abilities to produce toxin, survive under low temperatures and live in a broad spectrum of environments. As the bacteria are able to produce gastroenteritis especially in children, elderly and immune-compromised patients, more focus must be placed on checking the diarrheal stools for **Aeromonads**. The increase of pH value during harvesting time may be partly attributed to the production of the volatile basic compounds and increasing of thiobarbituric acid (TBA) value. The food hygiene laboratories performing the analysis must be accredited according to the ISO standards, so fish must be under hygienic measures, and must be examined frequently. While, the cashed fish and water environment under supervision of veterinary authority and Ministry of health to insure safety for human being in Egypt.

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تحديد مدي تواجد بكتيريا الايروموناس في اسماك البلطي النيلي بمصر

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الملخص العربى

هذه الدراسه ألقت الضوء على تحديد مدي تواجد بكتيريا الايروموناس فى أسماك البلطى وكذلك دراسه تاثير هذه البكتيريا علي الخصائص الكيميائية للعينات الايجابية. حيث تم تجميع 160 عينة من اسماك البلطى النيلي بواقع 80 عينة من الاسماك النيليه و80 عينه من اسماك المزارع من بحيره المنزله بهدف عزل و تصنيف ميكروب الايروموناس . حيث أفضت النتائج إلي أن نسبة العينات الايجابية لبكتيريا الايروموناس كانت اعلى في اسماك المزارع بنسبه 26,2% فى حاله الاصابه بميكروب الايروموناس بينما الاسماك النيليه كانت بنسبه8,7%. و تم عزل 28 عتره من الايروموناس و كان تصنيفها كالآتي 15 عتره من **الايروموناس هيدروفيلا** بنسبة (53,5%) و7عترات من **الايروموناس كاڤي** بنسبة (25 %) و3عترات من**الايروموناس سوبريا** بنسبة (10,7%) و2عتره من **الايروموناس غيرمصنفتين** بنسبه (7,1%) و عتره واحدة من **الايروموناس تروتا** بنسبة (3,5%).أما بالنسبة لنتائج اختبار النشاط الافرازي للسموم داخل الأمعاء وجد ان 2عتره من**الايروموناس سوبريا**، 7 عترات من **الايروموناس هيدروفيلا** و أيضا 2 عترة من **الايروموناس كاڤي** قادرون علي افراز السموم بنسب6,66 %، 46,6%،28,5 % علي التوالي. كما أوضحت الدراسة مدي تأثير ميكروب الايروموناس علي الخصائص الكيميائية للعينات الايجابية حيث أدت إلي زيادة في كل من درجه الأس الهيدروجيني (PH)، كمية النيتروجين الكلي المتصاعد (TVB-N) و حمض الثيوباربتيوريك (TBA) . و تمت مناقشة الأهمية الصحية لهذا الميكروب مع الاهتمام بالمسطحات المائية المصادرة منهاهذه الأسماك و ضرورة الإشراف البيطري و الصحي علي هذه المسطحات لسلامة المنتج بمصر.