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INCIDENCE OF AEROMONAS HYDROPHILA IN FRESH WATER FISH (TILAPIA NILOTICUS) AND READY TO EAT FRIED FISH IN ASSIUT CITY

(With 4 Tables)

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مدي تواجد ميكروب الأيروموناس هيدروفيلأ في الأسماك الطازجة (البلطي النيلي) والاسماك المجهزه للاكل في مدينة اسيوط

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اجريت هذه الدراسة علي عدد 80 عينة ،بواقع 50 عينة من اسماك البلطي النيلي الطازج و30 عينة من الاسماك المجهزة للاكل. حيث جمعت هذه العينات من اسواق السمك ومنافذ البيع المحلية المختلفة وكذلك عدد من المطاعم الموجودة في مدينة اسيوط وذلك لفحصها ظاهريا والتعرف علي العدد الكلي لمجموعة ميكروبات الايروموناس باتباع طريقة الانتشار السطحي. ولقد تبين من الدراسة صلأحية هذه العينات ظاهريا للاستهلأك الادمي وان 24 عينة (48%) و3 عينات (10%) من اسماك البلطي النيلي والاسماك المجهزة للاكل تحتوي علي ميكروبات الأيروموناس وان متوسطات عدد الميكروبات كانت x3.7 10^3و1.9x10^2 لكل جرام علي التوالي. وفي هذه الدراسه تم عزل 30 عترة (60%) من اسماك المياة العذبة (البلطي النيلي) ميزت الي مستوي الرتبة مايلي 16(53.33) ايروموناس هيدروفيلا ، 9(30%) ايروموناس كافي ، 5(16.67%) ايروموناس سوبريا. وكذا 16 عترة (35.33%) من الاسماك المجهزة للاكل وميزت هذه العترات الي 7(43.75%) ايروموناس كافي ، 5(31.25%) ايروموناس سوبريا ، 4(25%) ايروموناس هيدروفيلا ولقد تم دراسة بعض من النشاط الانزيمي لهذه العترات. هذا وقد تمت مناقشة الاهمية الصحية لهذه الميكروبات ومدي خطورتها علي الصحة العامة كذلك الطرق المقترحة التي يجب توافرها للحد من هذه الخطورة.

SUMMARY

This study was carried on 80 random samples 50 of fresh water fish (Tilapia niloticus) and 30 samples of ready to eat fried fish these samples were obtained from different shops; represented different localities of different sanitation levels of ○○○Assiut city. All samples were examined for the presence of *Aeromonas hydrophila* group; using enrichment

procedure and surface spread plate technique. The results obtained pointed out that 48% and 10%of the examined Tilapia niloticus fish and ready to eat fried fish samples were positive for the presence of *Aeromonas hydrophlia* organism with an average counts of $3.7x10^3$ and $1.9x10^2$ /g fish respectively. In this study 30(60%) *Aeromonas* strains were isolated from fresh water fish (Tilapia niloticus) and were characterized according to species level as follow; 16 (53.33%) *Aeromonas hydrophlia*; 9(30%) *Aeromonas caviae* and 5(16.67%) *Aeromonas sabria*. On the other hand 16(53.33%) strains were isolated from ready to eat fried fish and were characterized according to species level as follows: 7 (43.75%) *Aeromonas caviae*, 5 (31.25*%) Aeromonas sabria* and 4 (25%) *Aeromonas hydrophlia*. All strains were examined for their ability to produce haemolysin as a virulence factor. The hygienic and public health importance as well as some recommended measures for improving the quality of such products were discussed.

Key words: *Fish, fresh water fish, fried fish, Aeromonas spp.*

INTRODUCTION

Aeromonas hydrophila (*A. hydrophila*), a gram negative bacteria, is widely distributed in aquatic environment (Nakano, *et al.,* 1990; Fiorentini, *et al.,* 1998; Legnani, *et al.,* 1998). *A. hydrophila* has received a particular attention because of its association with infections in a wide variety of hosts including, human, reptiles fish and invertebrates (Kodjo *et al.,* 1997; Pearson *et al.,* 2000; Roux *et al.,* 2000). More ever the bacterium is considered as one of the newly emerging water and food borne pathogens (Merino *et al.,* 1995; Gugnani, 1999). In fish *A. hydrophila* typically causes an exploded haemorrhagic septicemia and has been implicated in different outbreaks associated with heavy losies (Qian *et al.,* 1995; Son *et al.,* 1997).

Species of *Aeromonas* are short, gram negative, faculatively anaerobic, non spore forming, motile bacilli with a single flagellum, and can ferment glucose with or without the production of gas (Andrade, *et al.,* 2006). The most important three motile species associated with human illness are *Aeromonas hydrophila*, *A. caviae* and *A. sobria* (Brooks *et al.,* 1995). Isolation of these bacteria (Aeromonas hydrophila group), have been reported from a variety of food including fishes (Adithepchaikram *et al.,* 2008)

In addition to gastro_enteritis *Aeromonas hydrophila* group infects human causing infections such as septicaemia, acute diarrhea of

short duration, urinary tract infection and ear infection (Koneman *et al.*, 1994). The *Aeromonas hydrophila* group produces a number of potential virulence factors, including enterotoxins, haemolysins, cytotoxins and proteases (Ljungh and Wadstrom, 1983). The Aeromonas microorganisms are normal inhabitant of the intestinal tract of O.niloticus (Akelah, 1978). However, *Aeromonas hydrophila*, *Aeromonas sobria*, and *Aeromonas caviae* has been implicated in some cases of diarrheal disease. *Aeromonas sobria* and *Aeromonas hydrophila* are the primary enteropathognic species, however *Aeromonas caviae* has been implicated in some cases of diarrheal disease (Topic *et al.*, 2000).

In addition, Beta haemolytic strains of *Aeromonas* are assigned to *Aeromonas hydrophlia* and *Aeromonas sobria*, although haemolytic strains of *Aeromonas caviae* have been also found (Deodhar *et al.*, 1991). Burke *et al.* (1981) mentioned that the haemolytic activity is strongly associated with enterotoxin production in members of *Aeromonas* genus.

Rugulska, A., *et al.* (1994) reported that the haemolytic activity of *Aeromonas hydrophila* and *Aeromonas sobria* act as marker of pathogenicity therefore, the initial purpose of this study was to determine the occurrence and level of *Aeromonas* organisms in fresh water fish (Tilapia niloticus) and ready to eat fried fish in Assiut city markets.

MATERIALS and METHODS

1 - Collection of samples:-

Atotal of 80 random samples of fresh water fish (*Tilapia niloticus*) (50 samples) and ready to eat fried fish (30 samples) were collected from fish markets, shops and restaurants of varied sanitary levels at Assuit city.

Each sample was put in a sterile plastic bag. The collected samples were transferred directly to the laboratory under aseptic conditions without any delay where they were organoleptically and bacteriologicaly examined.

2 - Organoleptic examination:-

Ready to eat fried fish were evaluated for their palatability and odour of the flesh, while the fresh water fish were examined for their skin condition, consistency, colour, scales, eyes and gills according to Anon, (1985).

3 - Bacteriological examination:-

The samples were analyzed by using enrichment method as recommended by Okrend *et al.* (1987), where 25gram sample were aseptically transferred to 225 ml of trypticase soy broth containing 10 mg ampicillin / ml and blended for 2 min. The prepared samples were serially diluted up to 10^6 in butterfieds phosphate dilutent, and the count was carried out on the aforementioned dilutions as recommended by Palumbo *et al.* (1985), using MacConky manitol ampicillin agar. The number of colonies which showed red colour in countable plates was enumerated as Aeromonas organisms.

a- Enrichment procedure:

This was done according to the technique adopted by Palumbo *et al.* (1989).

b- Isolation and identification techniques:-

The technique adopted was that used by Okrend *et al.* (1987), Ahmed *et al.* (1991), and Koneman *et al.* (1994).

c- Determination of the haemolytic activity of the isolated strains:

It was carried out using 5% sheep blood agar as recommended by Rogulska *et al.* (1994).

RESULTS

Table 1: Organoleptic examination of fresh and fried fish samples.

Type of samples	No. of Samples	Organoleptic examination	
		Fresh samples	Stale samples
Fresh water fish (*Tilapia niloticus*)	50	50 (100%)	0 (0.0%)
Ready to eat fried fish	30	30 (100%)	0 (0.0%)

Table 2: Statistical analytical results of *Aeromonas* species count/g examined samples.

Type of samples	No. of Examined samples	Positive samples		Count/g of Fish			
		No	%	Min	Max	Mean	SE
Fresh water fish	50	24	48	$1.8x10^2$	$6.4x10^5$	$3.7x10^3$/g	$2.3x10^3$
Ready-to-eat fried fish	30	3	10	2x10	$3.1x10^3$	$1.9x10^2$/g	$1.2x10^2$

Table 3: Incidence of *Aeromonas* species isolated from the examined samples.

Type of Samples	No.of Isolated strains	*Aeromonas hydrophila*		*Aeromonas caviae*		*Aeromonas sobria*	
		NO.	%	NO.	%	NO.	%
Fresh water fish (*Tilapia niloticus*).	30 (60%)	16	53.33	9	30	5	16.76
Ready-to-eat fried fish	16 (53.33%)	4	25	7	43.75	5	31.25

Table 4: Detection of haemolysin activity of *Aeromonas* species isolated from fresh water fish *(Tilapia niloticus)* and ready-to-eat fried fish.

Aeromonas Species	Haemolysin activity					
	Fresh water fish			Ready –to-eat fried fish		
	No. tested	No. positive	%.positive	No. tested	No. positive	%.positive
A. hydrophila	16	11	68.75	4	1	25
A. caviae	9	1	11	7	3	42.86
A. sorbia	5	2	40	5	2	40

DISCUSSION

This study was conducted to investigate the presence of *Aeromonas* species in fresh water fish (Tilapia niloticus) and ready to eat fried fish.

Although the organoleptic examination showed no abnormalities and all the examined samples were fresh and sound, yet *Aeromonas* organisms were recovered from fresh water fish (Tilapia niloticus) and ready to eat fried fish (Table 1), therefore bacteriological examination must be associated with organoleptic examination to give the accurate judgment.

From (Table 2), it is apparent that 24 (48%) and 3(10%) of fresh water fish (Tilapia niloticus) and ready to eat fried fish contained

Aeromonas species with an average count of 3.7×10^3 and 1.9×10^2 /gm respectively.

The obtained incidence are somewhat higher than that reported by Gobat and Jemmi (1992); Abdel. EL-Daym (1999) and Bastawrows and Mohamed (1999).

It was observed that the fresh water fish showed higher incidence and count than that from ready to eat fried fish as *Aeromonas* microorganisms are normal inhabitant of the intestinal tract of Tilapia niloticus (Akelah, 1978).

From Table (3), 30(60%) strains of *Aeromonas* organisms were isolated from the examined fresh water fish (Tilapia niloticus). *Aeromonas hydrophlia* was the most common species isolated (53.33%) followed by *Aeromonas caviae* (30%) strains and *Aeromonas sobria* (16.67%). On the other hand 16 (53.33%) strains were recovered from ready to eat fried fish where *Aeromonas caviae* was the most common species isolated (43.75%) followed by *Aeromonas sobria* (31.25%) and *Aeromonas hydrophila* (25%).

It is evident from the data presented in Table (4) that 11 (68.75%) of 16 *Aeromonas hydrophila* strains, 2(40%) of 5 *Aeromonas sobria* strains and only one (11%) of 9 *Aeramonas caviae* strains, while 3 (42.86%) of 7 *Aeramonas caviae* strains, 2 (40%) of 5 *Aeromonas sobria* and only one (25%) of 4 *Aeromonas hydrophila* strains from fresh water fish and ready to eat fried fish had the ability to produce haemolysin respectively.

Abyta *et al.* (1994) identified *Aeromonas hydrophlila* and *Aeromonas sobria* as the primary enterophogenic species, however *Aeromonas caviae* has been implicated in some cases of diarrheal disease (Topic *et al.,* 2000).

In addition, Beta haemolytic strains of *Aeromonas* are assigned to *Aeromonas hydrophila* and *Aeromonas sobria*, although haemolytic strains of *Aeromonas caviae* have been also found (Deodhar *et al.*, 1991).

Varnam, and Evans, (1991) reported that a number of phenotypic characters have been proposed as a markers of enteropathogenicity of *Aeromonas species* and they added that the most important of these markers was haemolysin production.

The present results disagree, with those reported by Okrend *et al.* (1987); Palumbo *et al.* (1989) and Freitas *et al.* (1992) since these authors pointed out that haemolysin was detected in 100% of *Aeromonas hydrophila* strains recovered from some varities of food.

On the other hand, Bastawrows and Mohammed (1999) found that more of the 12 strains of *Aeromonas caviae* recovered from fresh water fishes lysed the sheep erythrocytes.

In conclusion, the information given by the achieved results revealed that *Aeromonas* species existed in the examined fishes either fresh water fish or ready to eat fried fish and therefore these foods may play a significant role in the epidemiology of gastroenteritis, therefore, strict hygienic measures, good food handling practice at home and finally thoroughly and properly clean and sanitary equipments and contact surfaces should be recommended to avoid contamination with *Aeromonas* organisms.

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