Animal Health Research Institute
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STUDIES ON *AEROMONAS HYDROPHILA* IN FRESHWATER FISH (*OREOCHROMIS NILOTICUS* AND *LABEO NILOTICUS*) AND SMOKED FISHES (HERRINGS) IN ASSIUT GOVERNORATE

(With 3 Tables)

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(Received at 6/6/2000)

دراسات عن ميكروب الايروموناس هيدروفيلا في الأسماك الطازجة (البلطي النيلي والليبس النيلي) والأسماك المدخنة (الرنجة) في محافظة أسيوط

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أجريت هذه الدراسة على عدد ٨٠ سمكة طازجة (٥٠ من اسماك البلطي النيلي و ٣٠ من أسماك الليبس النيلي) بالاضافة الى عدد خمسون عينة من الأسماك المدخنة (الرنجة) (٣٣ من الأسماك المدخنة الغير مغلفة , ١٧ من الأسماك المدخنة المغلفة) والمعروضة بأسواق السمك المختلفة والمحلات ذات المستويات الصحية المختلفة بمدينة أسيوط وذلك لفحصها ظاهريا واستبيان العدد الكلى لمجموعة ميكروبات الأيروموناس هيدروفيلا باتباع طريقة الانتشار السطحى ولقد تبين من الدراسة صلاحية هذه العينات ظاهريا للاستهلاك الآدمى وأن ٤٨% , ٣٦.١٧% , ٣٠.٣٠ % , ١٧.١٥ من اسماك البلطي النيلي ,الليبس النيلي ,اسماك الرنجة الغير مغلفة واسماك الرنجة المغلفة تحتوى على ميكروبات مجموعة الايروموناس هيدروفيلا وان متوسطات عدد الميكروب كانت ٣.٢×10^{3} , ١.٢ × 10^{2} , ٢.١ × 10^{3} , ١.٩ ×٢١٠ لكل جرام على الترتيب وكانت الدراسة تهدف أيضا إلى عزل ميكروبات هذه المجموعة بطريقة الأغناء والأخصاب ثم الزرع على المستنبتات الخاصة بهذه المجموعة ولقد تم عزل ٤٧ عترة من أسماك المياه العذبة [البلطي , الليبس النيلي] ميزت إلي مستوى الرتبة كما يلي ٢٤ ايروموناس هيدروفيلا , ١٥ ايروموناس كافي , ٨ ايروموناس سوبريا وكذا عدد ٢٣ عترة من الاسماك المدخنة المعبأة والغير معبأة وميزت هذه العترات إلى ٥ ايروموناس هيدروفيلا , ١٢ ايروموناس كافى , ٦ ايروموناس سوبريا ولقد تم دراسة بعض من النشاط الأنزيمي لهذه العترات هذا وقد تم مناقشة الأهمية الصحية لهذه الميكروبات ومدى خطورتها على الصحة العامة والطرق المقترحة الواجب اتباعها لدرء خطرها .

SUMMARY

80 random samples of fresh water fishes were collected and they included "50 *Oreochromis niloticus* and 30 Labeo niloticus". In addition, 50 random samples of smoked herring fish (33 unpackaged and 17 packaged) were collected. These samples were obtained from different markets and shopes of varied sanitary levels at Assiut City. All the samples were examined organoleptically and bacteriologically to enumerate *Aeromonas hydrophila* group microorganisms. All the examined samples were accepted organoleptically. Bacteriologically, by using the surface plate technique, the results pointed out that 48%, 36.67%, 30.30% and 17.15% of the examined *O.niloticus*, *Labeo niloticus*, unpackaged and packaged smoked fish samples were positive for the presence of *Aeromonas hydrophila* organism with an average counts of 3.2×10^3, 1.2×10^2, 2.1×10^3 and 1.5×10^2/g fish respectively. In this study, 47 *Areomonas hydrophila* strains were isolated from *O.niloticus* and *Labeo niloticus* and were characterized according to species level as follow: 24 *Aeromonas hydrophila*, 8 *Aeromonas sobria* and 15 as *Aeromonas caviae*. On the other hand, 23 strains were isolated from smoked fishes either unpackaged or packaged and were characterized according to species level as follow: 12 *Aeromonas caviae*, 6 *Aeromonas sobria* and 5 as *Aeromonas hydrophila*. All strains were examined for their ability to produce haemolysin enzyme. The hygienic and public health importance as well as some recommended measures for improving the quality of such products were discussed.

Key words: *Aeromonas, freshwater fish, smoked fishes.*

INTRODUCTION

The *Aeromonas hydroiphila* group is collectively referred to as motile aeromonas mesophilic Aeromonas (Anon, 1992). The most important three motile species associated with human illness are *Aeromonas hydrophila*, *A. caviae* and *A. sobria* (Brooks et al., 1995).

In recent years Aeromonas has received increasing attention as an agent of foodborne diarrhoeal disease in otherwise healthy people (Palumbo et al., 1985). The fatality rate of patients affected with *Aeromonas hydrophila* group may reach of to 61% (Davis et al., 1978). The isolation of these bacteria have been reported from a variety of food

including fishes (Pin et al., 1994) and smoked herring fishes (Bill Horner, 1992; Gobat and Jemmi, 1993 and Hudson and Mott, 1993).

The quantitive data on the incidence and extent of *Aeromonas hydrophila* in freshwater and smoked fishes is generally lacking. Therefore, the intial purpose of this investigation was to study the occurrence of Aeromonas organisms in fresh water and smoked herring fishes sold in Assiut City markets.

MATERIAL and METHODS

Collection of samples:

Eighty random samples of fresh water fishes in addition to fifty random samples of smoked fishes were collected from some markets and shops of varied sanitary levels at Assiut City. The samples included 50 *Oreochromis niloticus*, 30 *Labeo niloticus*, 33 unpackaged and 17 packaged smoked fishes. Each sample was put in a sterile plastic bag while the packaged samples were collected in its retail sealed plastic bags. The samples after collection were transferred directly to the laboratory under aseptic conditions with a minimum of delay, where they were subjected to organoleptically and bacteriological examination.

Organoleptic examination:

Fresh water and smoked fishes were evaluated for their skin condition, consistency, colour and odour of the flesh, while scales, eyes and gills of fresh water fishes were examined organoleptically according to Anon, (1985).

Preparation of samples:

The samples were prepared according to the technique adopted by Anon, (1978).

Determination of Aeromonas organisms count:

The count of Aeromonas organisms was determined by using the surface spread plate technique, where 10g. of each sample were aseptically transferred to 90 ml. of peptone water 1.0% and blended for 2 min.. The prepared samples were serially diluted up to 10^{-6} using 1.0% peptone water, and the count was carried out on the aforementioned dilutions as recommended by Palumbo et al. (1985) using MacConkey manitol ampicillin agar. The number of colonies which showed red colour in countable plates was enumerated as Aeromonas organisns.

Isolation of Aeromonas species:

(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

The results are tabulated in Tables 1, 2 & 3.

DISCUSSION

Although the organoleptic examination showed no abnormalities and all the examined samples were fresh and sound, yet Aeromonas organisms were recovered from fresh water and smoked fishes (Table, 1). Therefore, bacterilogical examination must be associated with organoleptic examination to give the accurate judgement.

From Table (1), it is apparent that 24 (48%) and 11 (36.67%) of *O.niloticus* and *Labeo niloticus* contained Aeromonas species with an average count of 3.2×10^3 and 1.2×10^2/g respectively while these organisms were present in packaged and unpackaged smoked fishes in 3 (17.65%) and 10 (30.30%) with an average count of 1.9×10^2 and 2.1×10^3/g respectively. The obtained incidences and counts are somewhat higher than that recorded by Gobat and Jemmi (1993); Abdel El-Daym (1999), and Bastawrows and Mohammed (1999).

It was observed that the incidence and count recovered from *O.niloticus* were higher than those from *Labeo niloticus* as Aeromonas microorganisms are normal inhabitant of the intestinal tract of *O.niloticus* (Akelah, 1978).

It is worth mentioning that the presence of *Aeromonas hydrophila* microorganisms in herrings is not surprising because the action of smoking and dehydration is not sufficient to reduce the bacterial counts significantly (Deng et al., 1974). Furthermore, the smoke components such as formaldehyde, acetic acid and cresol would

penetrate the interior of the food slowly and therefore, do not affect the microorganism in deeper regions (Duan, 1979).

Locally produced smoked fish in Egypt are mainly prepared from imported raw material of frozen herrings fish (Kasem et al., 1985). Meantime, it should be noted that the presence of Aeromonas microorganisms in frozen herrings fish is not surprising because these organisms can survive at -17°C for 18 months even in adverse conditions (Saad, 1991).

From Table (2), 47 strains of *Aeromonas* organism were isolated from examined freach water fish samples 30(63.83%) from *O.niloticus* and 17 (36.17%) from *Labeo niloticus*. *Aeromonas hydrophila* was the most common species isolated 24 strains (51.06%) followed by *Aeromonas caviae* 15 strains (31.91%) and *Aeromonas sobria* 8 strains (17.02%). On the other hand 23 strains were recovered from smaked herring fish samples and included 16 (69.57%) from unpackaged and 7 (30.43%) from packaged smoked herrings fishes. *Aeromonas caviae* was the most common species isolated 12 strains (52.17%) followed by *Aeromonas sobria* 6 strains (26.09%) and *Aeromonas hydrophila* 5 strains (21.74%).

It is evident from the data presented in Table 3 that 15 (51.72%) of 29 *Aeromonas hydrophila* strains, 4 (28.57%) of 14 *Aeromonas sobria* strains and only one (3.70%) of 27 *Aeromonas caviae* strains had the ability to produce haemolysin. Varnamm and Evans (1991) reported that a number of phenotypic characters have been proposed as 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. (1993) since these authors pointed out that haemolysin was detected in 100% of *Aeromonad hydrophila* strains recovered from some varities of food. On the other hand, Bastawrows and Mohammed (1999) found that none of the 12 strains of *Aeromonas caviae* recovered from fresh water fishes lysed the sheep erythrocytes.

Abeyta et al. (1994) identified *Aeromonas hydrophila* and *Aeromonas sobria* as the primary enteropathogenic species, however *Aeromonas caviae* has been implicated in some cases of diarrhoeal disease (Nammdari and Bottone, 1990). In addition, Beta haemolytic strains of Aeromonas are assigned to *Aeromonas hydrophila* and

Aeromonas sobria, although haemolytic strains of *Aeromonas cavaie* have been also found (Deodhar et al., 1991).

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

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Table 1: Frequency distribution of the examined samples based on organoleptic and count of Aeromonas species/g

Type of samples	No. of samples	organoleptic examination		positive samples		Count/g. of fish		
		fresh samples	stale samples	No.	%	Min	Max	Aver age
Oreochromis niloticus	50	50 (100%)	0 (0.0%)	24	21.8	1.6×10^2	5.2×10^5	3.2×10^3/g
Labeo niloticus	30	30 (100%)	0 (0.0%)	11	36.67	2.3×10	8.1×10^3	1.2×10^2/g
Unpackaged smoked fish	33	33 (100%)	0 (0.0%)	10	30.30	4×10	5.3×10^4	2.1×10^3/g
Packaged smoked fish	17	17 (100%)	0 (0.0%)	3	17.65	3×10	3.3×10^3	1.9×10^2/g

Table 2: Frequency distrbution 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 fishes							
O.niloticus	30 (63.83)	16	53.33	9	30	5	16.67
Labeo niloticus	17 (36.17%)	8	47.06	6	35.29	3	17.65
Total	**47**	**24**	**51.06**	**15**	**31.96**	**8**	**17.02**
Smoked fishes							
Unpackaged	16 (69.57%)	4	25	7	43.75	5	31.25
Packaged	7 (30.43%)	1	14.29	5	71.43	1	14.29
Total	**23**	**5**	**21.74**	**12**	**52.17**	**6**	**26.09**

Table 3: Detection of haemolysin activity of Aeromonas species isolated from fresh water and smoked fishes

Aeromonas species	No. of isolates from			Haemolysin positive strains	
	freshwater fishes	smoked fishes	Total	No.	%
Aeromonas hydrophila	24	5	29	15	51.72
Aeromonas Caviae	15	12	27	1	3.70
Aeromonas sobria	8	6	14	4	28.57