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ENTEROBACTERIACEAE IN LOCALLY PRODUCED FISHES

BY

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INTRODUCTION

Fish play an important role not only in human food diets but also in animal and poultry rations. It is considered a cheap source of protein. Many investigators discussed the fish freshness and the hygienic measures applied during fish catching, transportation, preparation and consumption (Shewan, 1971; Ghittino, 1972 and Shewan, 1972). However, Lotfi et al.,(1974), Amin et al.,(1977), Morshady (1978), Mosa (1986) and Shaban (1988) studied the sanitary condition and the microbial status of fishes caught fresh water, brackish and marine fishes. In this respect, Khalil (1936) recorded the survival of bacteria in the fish muscles after cooking, pickling and smoking. This is of special importance in Egypt where fishes are not refrigerated until they reach the market. Griffiths (1937) Markoff, (1939) and Brunner (1949) stated that the exposure of fishes to polluted water during catching contaminate them with enterobacteriaceae organisms. The examination of fish for the existence of enterobacteriaceae organisms is an index of the sanitary conditions under which it is produced. Therefore,this study was planned to estimate the total enterobacteriaceae count as well as the isolation and identification of its different members in locally produced fish collected from Cairo fish markets.

MATERIAL AND METHODS

Collection of samples

Four hundred fish samples were collected from Cairo fish markets, including 100 individuals each of Tilapia nilotica and Clarias lazera as fresh water fishes, Pagrus spp. and Solea spp. as marine fishes. Every 25 samples of each fish group collected every season allover one year. Samples were packed individually in sterile nylon sacs and transported to the laboratory where examined as soon as possible.

Preparation of samples:

A) Fish surface:

Swab samples taken over a prescibed area (20 cm^2) on fish surface, each swab was then suspended separately in a test tube containing 20 ml of 0.1 % sterile peptone water. Further ten-folds serial dilutions were prepared.

B) Fish muscles:

Ten gram of muscles were obtained from each fish samp under aseptic condition and transferred to a sterile nylon sac containing 90 ml of 0.1 % sterile peptone water then homogenized by means of stomacher (Stomacher Lab-Blender 400) to get a dilution of 10^{-1}, then 10 folds serial dilutions were prepared.

Bacteriological examination:

1) Determination of the total enterobacteriaceae cou

The drop technique applied was that recommended by ICMSF (1978) using violet red bile glucose agar media

2) Isolation and identification of enterobacteriacea organisms:

The original dilutions were pre-enriched at 37°C for

24 hours. A loopfull from pre-enriched dilutions were streaked onto Eosin Methlene blue agar and Mac-conkey's plates then incubated at 37°C for 24 hours. Suspected colonies were identified biochemically according to Edwards and Ewing (1972) and Cruickshank et al.(1975).

3) Isolation and identification of salmonellae:

One ml of each pre-enriched culture was transferred aseptically into 10 ml of Rappaport's broth, then incubated at 37°C for 48 hours. A loopfull from enriched broth was streaked onto S.S. agar and then incubated at 37°C for 24 hours. Suspected colonies were identified biochemically according to Edwards and Ewing (1972) and Cruickshank et al. (1975) and typed serologically according to Kauffman (1974).

RESULTS AND DISCUSSION

The results obtained regarding the total enterobacteriaceae count were given in table (1).

Table (1): Average count of Enterobacteriaceae organisms in the examined fish samples

Season	Tilapia nilotica		Clarias lazera		Pagrus species		Solea species	
	Surface/cm²	Muscles/g	Surface/cm²	Muscles/g	Surface/cm²	Nuscles/g	Surface/cm²	Muscles/g
Autumn	$1x10^5$	$3x10^3$	$7x10^6$	$2x10^3$	$6x10^3$	$>1x10^2$	$2x10^4$	$>1x10^2$
Winter	$2x10^3$	$2x10^3$	$4x10^4$	$1x10^3$	$4x10^3$	$>1x10^2$	$3x10^3$	$>1x10^2$
Spring	$1x10^6$	$8x10^3$	$2x10^6$	$4x10^3$	$2x10^4$	$>1x10^2$	$1x10^5$	$>1x10^2$
Summer	$3x10^7$	$7x10^3$	$1x10^8$	$7x10^3$	$3x10^5$	$8x10^2$	$2x10^6$	$2x10^2$

It is noticed that during summer the enterobacteriaceae counts were comparatively higher as compared with other

seasons. Meanwhile Clarias lazera and Tilapia nilotica gave the highest counts followed by solea species and then pagrus species.

The count of enterobacteriaceae organisms were higher during summer then decreased during spring, outumn and winter due to the change of temperature. This is in agreement with the results obtained by Goda et al. (1980).

The high enterobacteriaceae counts on the surface of Tilapia nilotica and Clarias lazera when compared with pagrus and solea species could be attributed to the habitat of such fresh water fishes. Fresh water is of high organic loads and farourable for the survival of many genera of bacteria while the salinity of marine habitat inhibits the growth of such bacteria (Robert, 1978).

On the other hand, higher counts of enterobacteriaceae recorded from the surface of Clarias lazera then that of Tilapia nilotica as fresh water fishes and the higher counts recorded from the surface of solea species then that of pagrus species as marine fishes; can be attributed to the fact that Clarias lazera and solea species are nacked, as the abscence of the scales give a wide chance for surface contamination to penetrate rapidly through fish muscles (Boulenger, 1907).

The presence of enterobacteriaceae in the muscles may be attributed to the fact that bacteria penetrate the gill tissues and proceed along the vascular system into the flesh or through the intestine into the body cavity and belly walls or through the skin into the flesh.

Results recorded in table (2, 3, 4 and 5) showed the incidence of enterobacteriaceae isolated from the surface and muscles of Tilapia nilotica, Clarias lazera, pagrus and solea species during autumn, winter, spring

Table 2 : Incidence of isolated Enterobacteriaceae organisms from Tilapia nilotica.

Isolates	Autumn				Winter				Spring				Summer			
	Surface		Muscle.		Surface		Muscles		Surface		Muscles		Surface		Muscles	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Edwardsiella tarda	-	-	-	-	-	-	-	-	-	-	-	-	1	4	-	-
Salmonella typhimurium	-	-	-	-	-	-	-	-	-	-	-	-	1	4	-	-
Arizonae	-	-	-	-	-	-	-	-	-	-	-	-	2	8	-	-
Citrobacter freundii	5	20	1	4	5	20	1	4	8	32	2	8	9	36	5	20
Citrobacter diversus	2	8	-	-	1	4	-	-	3	12	-	-	3	12	-	-
Citrobacter amalonaticus	1	4	-	-	1	4	-	-	2	8	-	-	2	8	-	-
Klebsiella pneumoniae	1	4	-	-	1	4	-	-	2	8	-	-	3	12	-	-
Klebsiella oxytoca	1	4	-	-	-	-	-	-	2	8	-	-	3	12	-	-
Enterobacter cloacae	10	40	3	12	5	20	5	20	12	48	5	20	18	72	8	32
Enterobacter aerogenes	1	4	1	4	1	4	1	4	1	4	1	4	5	20	2	8
Enterobacter agglomerans	1	4	-	-	-	-	-	-	1	4	-	-	2	8	-	-
Enterobacter sakazakii	-	-	-	-	-	-	-	-	-	-	-	-	2	8	-	-
Enterobacter gergoviae	-	-	-	-	-	-	-	-	-	-	-	-	1	4	-	-
Serratia marcescens	-	-	-	-	-	-	-	-	-	-	-	-	2	8	-	-
Serratia liquefaciens	-	-	-	-	-	-	-	-	1	4	-	-	2	8	-	-
Serratia rubidaea	1	4	-	-	1	4	-	-	1	4	-	-	3	12	-	-
Proteus vulgaris	-	-	-	-	-	-	-	-	1	4	-	-	3	12	-	-
Proteus mirabilis	5	20	1	4	1	4	1	4	9	36	5	20	9	36	7	28
Morganella morganii	4	16	-	-	-	-	-	-	-	-	-	-	5	20	-	-
Providencia rettgeri	5	20	1	4	3	12	1	4	5	20	2	8	8	32	5	20
Providencia alcalifaciens	8	32	1	4	3	12	2	8	9	36	3	12	9	36	3	12
Providencia stuartii	5	20	2	8	2	8	2	8	6	24	3	12	10	40	3	12
Yersinia enterocolitica	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	22	88	5	20	20	80	3	12	23	92	3	12	24	96	5	20

Table 3 : Incidence of isolated Enterobacteriaceae organisms from Clarias lazera.

Isolates	Autumn				Winter				Spring				Summer			
	Surface		Muscle		Surface		Muscle		Surface		Muscle		Surface		Muscle	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Citrobacter freundii	8	32	1	4	5	20	1	4	10	40	2	8	15	60	3	12
Citrobacter diversus	5	20	-	-	5	20	-	-	5	20	-	-	7	28	-	-
Citrobacter amalonaticus	2	8	-	-	1	4	-	-	3	12	-	-	5	20	-	-
Klebsiella pneumoniae	2	8	-	-	1	4	-	-	2	8	-	-	3	12	-	-
Klebsiella oxytoca	1	4	-	-	1	4	-	-	1	4	-	-	2	8	-	-
Enterobacter cloacae	15	60	2	8	15	60	2	8	18	72	3	12	20	80	5	20
Enterobacter aerogenes	1	4	-	-	1	4	-	-	1	4	-	-	1	4	-	-
Enterobacter agglomerans	1	4	-	-	1	4	-	-	1	4	-	-	1	4	-	-
Serratia rubidaea	1	4	-	-	-	-	-	-	1	4	-	-	1	4	-	-
Proteus vulgaris	-	-	-	-	-	-	-	-	-	-	-	-	3	12	-	-
Proteus mirabilis	8	32	1	4	5	20	-	-	10	40	3	12	15	60	5	20
Providencia rettgeri	8	32	2	8	5	20	1	4	9	36	2	8	9	36	3	12
Providencia alcalifaciens	9	36	1	4	8	32	1	4	9	36	3	12	10	40	5	20
Providencia stuartii	10	40	1	4	8	32	1	4	10	40	3	12	10	40	5	20
Escherichia coli	23	92	2	8	23	92	2	8	24	96	3	12	24	96	5	20

Table 4 : Incidence of isolated Enterobacteriaceae organisms from Bagrus sp.

Isolates	Autumn				Winter				Spring				Summer			
	Surface		Muscle		Surface		Muscle		Surface		Muscle		Surface		Muscle	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Citrobacter freundii	2	8	-	-	2	8	-	-	6	24	-	-	8	32	2	8
Enterobacter cloacae	3	12	-	-	2	8	-	-	5	20	-	-	6	24	5	20
Enterobacter aerogenes	-	-	-	-	-	-	-	-	1	4	-	-	1	4	1	4
Enterobacter agglomerans	-	-	-	-	1	4	-	-	2	8	-	-	1	4	-	-
Proteus mirabilis	6	24	-	-	5	20	-	-	8	32	-	-	8	32	2	8
Providencia rettgeri	3	12	-	-	2	8	-	-	7	28	-	-	9	36	-	-
Providencia alcalifaciens	5	20	-	-	3	12	-	-	6	24	-	-	8	32	5	20
Providencia stuartii	5	20	-	-	1	4	-	-	7	28	-	-	8	32	5	20
Escherichia coli	15	60	-	-	10	40	-	-	18	72	-	-	20	80	2	8

Table 5 : Incidence of isolated Enterobacteriaceae organisms from Sallen sp.

Isolates	Autumn				Winter				Spring				Summer			
	Surface		Muscle		Surface		Muscle		Surface		Muscle		Surface		Muscle	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Citrobacter freundi	3	12	-	-	1	4	-	-	8	32	-	-	10	40	1	4
Enterobacter cloacae	5	20	-	-	2	8	-	-	8	32	-	-	9	36	1	4
Enterobacter aerogenes	-	-	-	-	-	-	-	-	1	4	-	-	1	4	-	-
Enterobacter agglomerans	-	-	-	-	-	-	-	-	5	20	-	-	8	32	-	-
Proteus mirabilis	8	32	-	-	5	20	-	-	9	36	-	-	9	36	1	4
Providencia rettgeri	5	20	-	-	3	12	-	-	8	32	-	-	9	36	-	-
Providencia alcalifaciens	5	20	-	-	3	12	-	-	9	36	-	-	9	36	5	20
Providencia stuartii	7	28	-	-	5	20	-	-	8	32	-	-	9	36	3	12
Escherichia coli	18	72	-	-	15	60	-	-	20	80	-	-	23	92	3	12

and summer seasons. It was clear that Salmonella typhimurium and arizona organisms could be isolated the surface of Tilapia nilotica only during summer. Such findings agree with various investigators(Lotfi et al. 1972; Morshady, 1978 and Mosa, 1986). On the other hand Sedik (1971); Ahmed (1976) and Ez-El-Din (1978) failed to detect Salmonellae or arizona organisms during their studies.

Isolation of Salmonella typhimurium from the surface of Tilapia nilotica agrees with the findings of Heuschmann and Brunner (1974) who stated that fish can become carrier or reservoir for Salmonellae and can play a role in their epidemiology.

Isolation of Salmonellae from fish is of great public health importance. Moreover, fish meal is added to animal and poultry rations.

Salmonella food-borne infections are characterized by vomiting, abdominal pain, diarrhoea, headache, general body pain and shivering (Sojka and Field, 1970).

Escherichia coli is the clasical indicator of the possible presence of enteric pathogens in foods. E.coli in food has been associated with outbreaks of gastrointestinal disturbances (Delepine, 1903).

E.coli may also cause colibacillosis in adults, peritonitis, meningitis, enteritis, cystitis, pyelonephritis, appendicitis, otitis and puerperal sepsis (Pyatkin and Krivoshein, 1980).

Klebsiella organisms are responsible for food borne outbreaks of gastroenteritis (Bailey and Scott, 1974).

K.pneumoniae is responsible for pneumonia, broncho-pneumonia involves long lobes, producing fused foci and

lung abscesses and may be responsible for meningitis, appendicitis, pyaemia, cystitis and pyelonephritis (Pyatkin and Krivoshein, 1980).

Citrobacter freundii organisms have been found among intestinal, urinary and other pyogenic infections (Krieg and Holt, 1984).

Proteus species have been incriminated in cases of gastroenteritis following the ingestion of contaminated food as well as the infections of the urinary tract (Cooper et al. ,1941, Cherry et al. 1946; Wassef, 1969; and ICMSF, 1978).

Providencia organisms are not highly pathogenic but they have been incriminated in sporadic cases of human diarrhoea and urinary tract infections (Bailey and Scott, 1974).

SUMMARY

Hundred fish samples each of Tilapia nilotica, clarias pagrus and solea species; were collected from fish markets, at Cairo, 25 samples from each species were collected during each season of the year.

Samples were subjected to bacteriological examinations for the determination of the total enterobacteriaceae counts per cm^2 surface and per gram muscles and for the isolation and identification of enterobacteriaceae Obtained counts and identified enterobacteriaceae organisms were recorded.

Inconclusion, the counts of enterobacteriaceae were higher in fresh water fishes than in marine fishes and also during summer than other seasons of the year. This could be attributed to the effect of temperature on the bacterial growth.

The public health singificance of the isolated enterobacteriaceae organisms was discussed in details.

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