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MICROBIAL ASSESSMENT OF SOME MARKETED FISH IN DAMANHOUR CITY

(With 4 Tables)

By

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التقييم الميكروبي لبعض الأسماك المعروضة للبيع بمدينة دمنهور

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أجريت هذه الدراسة على عدد 75 عينة عشوائية من أسماك البلطي الطازج والبوري الطازج وأسماك الماكريل المجمد (25 عينة لكل نوع) تم جمعها من أسواق الأسماك المختلفة بمدينة دمنهور وذلك لتقييمها ميكروبياً وأوضحت الفحوص الميكروبية أن نسب تواجد البكتريا الهوائية عند 37° م/48 ساعة والتي تنمو عن د 7° م/10 أيام والبكتريا المعوية والعصيات القولونية والميكروب العنقودي الذهبي وميكروبات السودوموناس والأروموناس والفطريات والخمائر في أسماك البلطي كانت كالآتي : 88% ، 72% ، 64% ، 56% ، 84% ، 36% ، 32% ، 76% و60% بمتوسط عد كلى 2 × 10° ± 1 × 10 ^{4} ، 2 × 10^4 ± 9 × 10 ^{3} ، 2 × 10 ^{4} ± 7× 10 ^{3} ،3 × 10 ^{2} ± 2 × 10 ^{2}، 4×10^4 ± 2×10 ^{4} ، 1 × 10 ^{4} ± 4 × 10 ^{3} ، 7× 10 ^{3} ± 2 × 10 ^{3} ، 1× 10^3 ± 4×10 ^{2} و 1 × 10 ^{3} ± 4 × 10 ^{2} على التوالي. أما في أسماك البوري فكانت النسب كالآتي: 100% ، 96% ، 88% ، 48% ، 88% ، 16% ، 40% ، 60% و 40% بمتوسط عد كلى 1× 10^4 ± 1 × 10 ^{3} ، 1 × 10^4 ± 1 × 10 ^{3} ، 7× 10^3 ± 1× 10^3 ، 2× 10 ^{2} ± 2 × 10 ^{2} ، 6× 10 ^{3} ± 2× 10 ^{3}، 6 × 10 ^{2} ± 2× 10 ^{2}، 2× 10 ^{4} ± 2×10 ^{4} ، 2× 10 ^{2} ± 5× 10 و 6 × 10 ^{2} ± 3× 10 ^{2} على التوالي. بينما في حالة أسماك الماكريل المجمد كانت نسب الميكروبات بترتيب ذكرها كالآتي : 92% ، 60% ، 12% ، 24% ، 88% ، 20% ، 16% ، 60% و44% بمتوسط عد كلى كالآتي: 4× 10 ^{3} ± 1 × 10 ^{3} ، 4× 10 ^{3} ± 2 × 10 ^{3} ، 3× 10 ^{2} ± 9 × 10 ، 3× 10± 1× 10 ، 4× 10 ^{3} ± 9×10 ^{2}، 7 × 10 ^{3} ± 5 × 10 ^{3} ، 4 × 10 ^{3} ± 4 × 10 ^{3} ، 1 × 10 ^{3} ± 1× 10 ^{3} و 5× 10 ^{3} ± 2 × 10 ^{3} على التوالي. هذا وقد تم مناقشة وتقييم النتائج وملاحظة عدم تجاوز غالبية العينات سواء الأسماك الطازجة أو المجمدة للمواصفات القياسية المصرية والعالمية. هذا وقد تم وضع التوصيات اللازمة لجعل هذه الأسماك أكثر أماناً لصحة المستهلك.

SUMMARY

Seventy-five random samples of marketed fish (25 of each fresh *Tilapia nilotica*, fresh *Mugil cephalus* and frozen Mackerel) were collected from different fish marketes in Damanhour city for microbiological examination. The results revealed that the mean values of total counts of aerobic mesophilic bacteria, psychrotrophic bacteria, *Enterobacteriaceae*, Coliforms, *Staph. aureus*, *Pseudomonas*, *Aeromonas*, as well as Moulds and Yeasts of examined fresh samples of *Tilapia nilotica* were $2 \times 10^5 \pm 1 \times 10^4$, $2 \times 10^4 \pm 9 \times 10^3$, $2 \times 10^4 \pm 7 \times 10^3$, $3 \times 10^2 \pm 2 \times 10^2$, $4 \times 10^4 \pm 2 \times 10^4$, $1 \times 10^4 \pm 4 \times 10^3$, $7 \times 10^3 \pm 2 \times 10^3$, $1 \times 10^3 \pm 4 \times 10^2$ and $1 \times 10^3 \pm 4 \times 10^2$ respectively. While that of examined fresh samples of *Mugil cephalus* were $1 \times 10^4 \pm 1 \times 10^3$, $1 \times 10^4 \pm 1 \times 10^3$, $7 \times 10^3 \pm 1 \times 10^3$, $2 \times 10^2 \pm 2 \times 10^2$, $6 \times 10^3 \pm 2 \times 10^3$, $6 \times 10^2 \pm 2 \times 10^2$, $2 \times 10^4 \pm 2 \times 10^4$, $2 \times 10^2 \pm 5 \times 10$ and $6 \times 10^2 \pm 3 \times 10^2$ respectively, and that of examined frozen Mackerel fish samples were $4 \times 10^3 \pm 1 \times 10^3$, $4 \times 10^3 \pm 2 \times 10^3$, $3 \times 10^2 \pm 9 \times 10$, $3 \times 10 \pm 1 \times 10$, $4 \times 10^3 \pm 9 \times 10^2$, $7 \times 10^3 \pm 5 \times 10^3$, $4 \times 10^3 \pm 4 \times 10^3$, $1 \times 10^3 \pm 1 \times 10^3$ and $5 \times 10^3 \pm 2 \times 10^3$ respectively. The results of most of examined fish samples were in compliance with the Egyptian and the international permissible standard limits. The recommended measures for improving the microbial quality of the marketed fish were discussed to become more safety for the consumers.

Key words: *Fish, Tilapia nilotica, Mugil cephalus, Mackerel.*

INTRODUCTION

Fish and shellfish are second to meat and poultry as stable animal protein foods in most of the world. Fish have protein of high biological values as they contain essential amino acids and good source of minerals such as calcium, phosphorus, iron and trace elements like iodine as well as vitamins in addition to the high content of polyunsaturated fatty acids (Sedik *et al.,* 1989).

Fish is subjected to many risks of contamination from different sources either during their aquatic environment, sewage pollution of harvesting areas and/or after being harvested by workers, utensils and equipments during transportation, distribution and food preparation (National Academy of Science, 1985 and El-Leboudi, 2002).

The counts of *Enterobacteriaceae* have the potential indicator for not only the health hazard but also as an indicator of spoilage (Gorczyca *et al.,* 1985). Members of family *Enterobacteriaceae* are of potential

public health importance as it causes diseases for humans during lowering of their resistance. Also this group contains most members of food poisoning microorganisms (Edwards and Ewing, 1972 and Collins, 1984).

The increasing demand of consumers for fish and fish products makes it necessary to assess the microbial contamination of the fresh water fish, *Tilapia nilotica* "Bolti" and *Mugil cephalus* "Bouri" and frozen Mackerel fish marketed in Damanhour city to establish the best utilization at a high quality level and to safe-guard the human health.

MATERIALS and METHODS

A total of 75 random samples of fish (25 each of *Tilapia nilotica* and *Mugil cephalus*) and 25 of imported frozen mackerel fish "*Scomber scombrus*" were collected from different fish markets in Damanhour city. Each sample was wrapped separately in sterile polyethylene bag and transferred directly to the laboratory without delay in an ice box. In the laboratory, each sample was put on a sterile plate and the whole skin surface of each fish sample was sterilized by ethyl alcohol and flaming, the skin surface was removed aseptically and the following examinations were performed.

1. Preparation of samples according to ICMSF (1978):

Ten grams of prepared fish sample were transferred to a sterilized homogenizer flask containing 90 ml of 0.1% sterile peptone water. The contents were homogenized at 14000 r.p.m. for 2.5 minutes to provide a dilution of 10^{-1}. The homogenate was allowed to stand for 5 minutes at room temperature, then 1 ml of homogenate was transferred with a sterile pipette into a sterile test tube containing 9 ml of 0.1% sterile peptone water to obtain a dilution of 10^{-2}. Then further decimal ten fold serial dilutions up to 10^{-6} were prepared.

2. Total aerobic bacterial count according to APHA (1992):

One ml from each dilution was transferred into duplicate sterile *Petri* dishes and mixed with about 10 ml of sterile plate count agar medium "melted and kept at 45ºC". After solidification, cultivated plates as well as control one were incubated at 37ºC for 48 hours in an inverted position. Average count was calculated as a total aerobic count per gram of sample.

3. Total Psychrotrophic bacterial count according to APHA (1992):

The same steps, as in total aerobic bacterial count, were carried out but the incubation was done at 7ºC for 10 days.

4. Total *Enterobacteriaceae* count according to ICMSF (1978):

0.1 ml from each prepared dilution was transferred and distributed over the surface of dried violet red bile glucose (VRBG) agar poured plates, then overlaid by a thin layer of VRBG agar. Inoculated plates as well as control one was incubated at 37ºC for 48 hours. The colonies which showed a purple colour surrounded by a purple zone were counted.

5. Total coliform count (MPN/g):

The multiple tube method recommended by ICMSF (1978) was applied. Most probable numbers (MPN) of coliforms per gram of the examined samples were calculated by using MPN table. A loopful from each positive MacConkey broth tube was picked up and spread over the surface of MacConkey agar plate. Plates were incubated at 37ºC for 48 hours. Suspected colonies were picked up and inoculated into sterile semisolid nutrient agar tubes for further biochemical identification.

6. Total *Staphylococcus aureus* count (ICMSF, 1978):

0.1 ml from each dilution was spread over the surfaces of duplicate dried *Baired Parker* agar plates. The inoculated plates were incubated at 37ºC for 48 hours in an inverted position. The black shiny colonies with narrow white margines and surrounded by a clear zone were counted. Suspected colonies were stabbed in semi-solid agar for further morphological and biochemical identification (catalase, mannitol, coagulase, thermostable nuclease production and oxidation-fermentation of glucose)

7. *Pseudomonas* and *Aeromonas* count: *Pseudomonas-Aeromonas* selective agar base (GSP) recommended by Kielwein (1969):

Suspected colonies were picked from GSP medium then identified according to method recommended by Macfaddin (1980) morphologically (films stained with *Gram's* stain and motility test) and biochemically by the following tests (indole, methyle red, Voges-Proskauer, TSI, citrate utilization, oxidase, O/F of carbohydrates (glucose, mannitol, maltose and sucrose), nitrate reduction and esculin hydrolysis.

8. Total mould and yeast count (Konemen *et al.*, 1994):

One ml from each original dilution was streaked onto Sabouraud dextrose agar an incubated at 25ºC, and examined daily for 5 days.

RESULTS

Table 1: Statistical analytical results of icrobiological counts (cfu/g) of examined fresh fish "*T. nilotica*" and *M.cephalus* Samples. (N = 25 of each)

Microbial counts	*T. nilotica*					*M.cephalus*				
	Positive Samples		Minimum	Maximum	Mean ± SEM	Positive Samples		Minimum	Maximum	Mean ± SEM
	No	%				No	%			
- Aerobic bacterial count :										
Mesophilic	22	88	1×10^2	2×10^6	$2 \times 10^5 \pm 1$ X 10^4	25	100	7×10^2	2×10^4	$1 \times 10^4 \pm 1 \times 10^3$
Psychrotrophic	18	72	8×10^2	2×10^5	$2 \times 10^4 \pm 9 \times 10^3$	24	96	1 x 10	2×10^4	$1 \times 10^4 \pm 1 \times 10^3$
- *Enterobacteriaceae*	16	64	1×10^2	1×10^5	$2 \times 10^4 \pm 7 \times 10^3$	22	88	5 x 10	2×10^4	$7 \times 10^3 \pm 1 \times 10^3$
- Coliforms (MPN/g)	14	56	3	2×10^3	$3 \times 10^2 \pm 2 \times 10^2$	12	48	4	2×10^3	$2 \times 10^2 \pm 2 \times 10^2$
- *Staphylococcus aureus*	21	84	1×10^2	3×10^5	$4 \times 10^4 \pm 2 \times 10^4$	22	88	2×10^2	3×10^4	$6 \times 10^3 \pm 2 \times 10^3$
- Total *Pseudomonas* count	9	36	3×10^2	3×10^4	$1 \times 10^4 \pm 4 \times 10^3$	4	16	3×10^2	1×10^3	$6 \times 10^2 \pm 2 \times 10^2$
- Total *Aeromonas*	8	32	2 x	2 x	$7 \times 10^3 \pm 2$	10	40	1 x	2 x	$2 \times 10^4 \pm 2$

count			10^3	10^4	x 10^3			10^2	10^5	x 10^4
- Total mould count	19	76	1 x 10	8 x 10^3	1 x $10^3 \pm 4$ x 10^2	15	60	1 x 10	8 x 10^2	2 x $10^2 \pm 5$ x 10
- Total yeast count	15	60	4 x 10	5 x 10^3	1 x $10^3 \pm 4$ x 10^2	10	40	1 x 10	3 x 10^3	6 x $10^2 \pm 3$ x 10^2

N = number of examined samples. SEM = Standard error of mean.

Table 2: Statistical analytical results of microbiological counts (cfu/g) of examined forzen Mackerel fish samples. (N = 25)

Microbial counts	Frozen Mackerel				
	Positive Samples		Minimum	Maximum	Mean ± SEM
	No	%			
Total aerobic bacterial count:					
- *Mesophilic*	23	92	2×10^2	2×10^4	$4 \times 10^3 \pm 1 \times 10^3$
- *Psychrotrophic*	15	60	1×10^2	2×10^4	$4 \times 10^3 \pm 2 \times 10^3$
- Total *Enterobacteriaceae*	3	12	2×10^2	5×10^2	$3 \times 10^2 \pm 9 \times 10$
- Total Coliforms (MPN/g)	6	24	3 x 10	9 x 10	3 x 10 ± 1 x 10
- Total *Staphylococcus aureus*	22	88	6 x 10	2×10^4	$4 \times 10^3 \pm 9 \times 10^2$
- Total *Pseudomonas* count	5	20	2×10^2	2×10^4	$7 \times 10^3 \pm 5 \times 10^3$
- Total *Aeromonas* count	4	16	1×10^2	2×10^4	$4 \times 10^3 \pm 4 \times 10^3$
- Total mould count	15	60	3 x 10	2×10^4	$1 \times 10^3 \pm 1 \times 10^3$
- Total yeast count	11	44	3 x 10	2×10^4	$5 \times 10^3 \pm 2 \times 10^3$

N = number of examined samples SEM = Standard error of mean.

Table 3: Incidence of coagulase positive *Staphylococcus aureus* isolated from examined fish samples. (N = 25)

Examined Fish	Samples positive for *Staphylococcus aureus*		Coagulase positive *Staphylococcus aureus*	
	No.	%	No.	%
- *Tilapia nilotica*	21	84	9	43
- *Mugil cephalus*	22	88	7	32
- Frozen mackerel	22	88	11	50

N = number of examined samples.

Table 4: Incidence of isolated microorganisms from examined fish samples.

Microbial species	*T.nilotica*		*M. cephalus*		Frozen Mackerel	
	No.	%	No.	%	No.	%
Coliform group:						
E.coli	2	8	1	4	1	4
E.coli, inactive	2	8	1	4	0	0
Escherichia fergusoni	1	4	1	4	0	0
Citrobacter freundii	1	4	1	4	2	8
Citrobacter diversus	2	8	1	4	2	8
Klebsiella pneumoni subsp						
Pneumoni	3	12	5	20	1	4
Klebsiella oxytoca	3	12	1	4	1	4
Enterobacter aeragenes	2	8	0	0	2	8
Enterobacter intermedium	0	0	2	8	0	0
Pseudomonadaceae:						
P.alcaligenes	2	8	1	4	1	4
P.pseudoalcaligenes	4	16	2	8	2	8
P. aeruginosa	0	0	1	4	1	4
P. fluorescens	1	4	0	0	0	0
P. stutzeri	2	8	0	0	0	0
P. putida	0	0	0	0	1	4
Aeromonadaceae:						
A.hydrophila	2	8	2	8	0	0
A.caviae	2	8	3	12	2	8
A.schubertii	3	12	2	8	1	4
A.sobria	1	4	3	12	0	0
A.veronii	0	0	0	0	1	4

DISCUSSION

Fresh fish:

Table (1) revealed that the mean values of total aerobic, mesophilic and psychrotrophic bacterial counts of the examined *T.nilotica* (Bolti) samples were $2 \times 10^5 \pm 1 \times 10^4$ and $2 \times 10^4 \pm 9 \times 10^3$ while for the examined *M. cephalus* (Bouri) samples were $1 \times 10^4 \pm 1 \times 10^3$ and $1 \times 10^4 \pm 1 \times 10^3$ respectively.

These findings are in agreement with the results obtained by Goda *et al.* (1980), Farouk (1989) and Roushdy *et al.* (1996) while, lower results were reported by Thabet (1972) and Abdel-Hafiez (1991).

Amin (1973) suggested that the spoilage of fish could be detected when APC was reached 10^9/g.

The ice in which the fish are to be preserved, is usually contaminated (10^2/ml of ice melt water) and the holds of the fishing vessels normally have an indigenous flora (FAO, 1996).

The results given in Table (1) showed that the mean values of total *Enterobacteriaceae* counts of the examined *T.nilotica* and *M.cephalus* were $2 \times 10^4 \pm 7 \times 10^3$ and $7 \times 10^3 \pm 1 \times 10^3$ with percentages of 64% and 88%, while that of total Coliform counts (MPN/g) were $3 \times 10^2 \pm 2 \times 10^2$ and $2 \times 10^2 \pm 2 \times 10^2$ with percentages of 56% and 48% respectively.

Nearly similar results were reported by *Ahmed et al.* (1986), Farouk (1989), Roushdy *et al.* (1996), Yehia (1996) and Mousa and Mahmoud (1997), but higher counts were reported by Abdel-Galil *et al.* (1988) and Mahmoud (1990).

Coliforms are intestinal and non intestinal inhabitants, so their presence in food give an index of poor sanitation as well as possible presence of enteric pathogens (Matthes, 1984).

As shown in Table (4) the incidence of isolates of Coliforms group from the examined fresh fish, *T.nilotica* and *M.cephalus* were as follow:

E. coli (8% & 4%), *E.coli*, inactive (8% & 4%), *Escherichia fergusoni* (4% & 4%), *Citrobacter freundii* (4% & 4%), *Citrobacter diversus* (8% & 4%), *Klebsiella pneumoni subsp. pneumoni* (12% & 20%), *Klebsiella oxytoca* (12% & 4%), *Enterobacter aerogenes* (8% & 0%) and *Enterobacter intermedium* (0% & 8%) respectively.

In polluted waters, high numbers of *Enterobacteriaceae* may be found but in clean temperate waters, these microorganioms disappear rapidly and it has been shown that *Escherichia coli* and *Salmonellae* can survive for very long periods in tropical waters and once introduced may almost become indigenous to the environment (Fujioka *et al.*, 1988).

Also, the resulls shown in Table (1) cleared that the mean values of Total *Staph. aureus* counts of the examined samples of *T. nilotica* and *M. cephalus* fish were $4 \times 10^4 \pm 2 \times 10^4$ and $6 \times 10^3 \pm 2 \times 10^3$ cfu/g with percentages of 84% and 88% respectively.

Nearly similar results were reported by Ahmed *et al.* (1986) and Roushdy *et al.* (1996) but lower counts were reported by Hafez (1989) and Yehia (1996).

It is evident from Table (3) that the incidences of the isolated coagulase positive *Staph. aureus* from the examined *T.nilotica* and *M. cephalus* fish samples were 43% and 32% respectively.

Studies indicated that large numbers (usually greater than 1 million cfu/g) of coagulase positive *Staph. aureus* must contaminate the food for producing sufficient enterotoxin to cause food poisoning (Liston *et al.* 1971 and Gilbert *et al.* 1972).

The results recorded in Table (1) showed that the mean value of *Pseudomonas* and *Aeromonas* counts of the examined *T.nilotica* samples were $1 \times 10^4 \pm 4 \times 10^3$ and $7 \times 10^3 \pm 2 \times 10^3$ with percentages 36% and 32%. While of the examined *M.cephalus* samples were $6 \times 10^2 \pm 2 \times 10^2$ and $2 \times 10^4 \pm 2 \times 10^4$ with percentages of 16% and 40% respectively. Similar results were reported by El-Kelish (1995) but slightly lower counts were reported by El-Atabany (1995), Hassan (1998) and El-Mossalami *et al.* (2004).

Table (4) showed that the following species could be isolated from the examined *T.nilotica* and *M.cephalus* samples at percentages as: *Pseudomonas alcaligenes* (8% & 4%), *Pseudomonas pseudoalcaligenes* (16% & 8%), *Pseudomonas aeruginosa* (0% & 4%) *Pseudomonas fluorescens* (4% & 0%), *Pseudomonas stutzeri* (8% & 0%), while *Aeromonas hydrophila* (8% & 8%), *Aeromonas caviae* (8% & 12%), *Aeromoras Schubertii* (12% & 8%) and *Aeromonas sobria* (4% & 12%) respectively. Nearly similar results were reported by Soliman (1988) and Bastawrows and Mohammed (1999).

Aeromonas hydrophila is an apportunistic pathogen in persons with impaired immune function, but it has been detected frequently in stools of patients having diarrhea (Brayan, 1992). Also, it was isolated as causative agent in a case of sever gastroenteritis, including sever abdominal pain, fever and bloody stools, involving a 10 years old girl (Rosner, 1964).

Table (1) showed that the mean values of total mould and yeast counts of examined *T.nilotica* were $1 \times 10^3 \pm 4 \times 10^2$ and $1 \times 10^3 \pm 4 \times 10^2$, while for the examined *M.cephalus* were $2 \times 10^2 \pm 5 \times 10$ and $6 \times 10^2 \pm 3 \times 10^2$ cfu/g respectively. Simlar results were recorded by Hassan and Abdel-Dayem (2004) from the examined fresh local M. cephalus muscles samples.

The mode of handling of fish in the market also contributes to the incidence of fungi on the fish. The possibility of the incidence of toxic fungi or fungal metabolites leading to food poisoning can not ruled out unless proper care is taken (Vishwanath *et al.*, 1998).

Frozen mackerel fish:

Table (2) showed that the mean values of aerobic bacterial counts "mesophilic and psychrotrophic" of frozen mackerel fish, were $4 \times 10^3 \pm$

1×10^3 and $4 \times 10^3 \pm 2 \times 10^3$ cfu/g respectively. Nearly similar results were obtained by Alian *et al.* (1969) and Hassan (1998) but higher results were reported by Lee *et al.* (1967) and Morshidy and Hafez (1986). The comparatively lower aerobic mesophilic counts of frozen fish in comparison to fresh fish could be attributed to that these microorganisms could not adapt to the cold environment. In the meantime the psychrotropic count had been established and began to multiply. In this respect, Frazier and Westhoff (1978) reported that freezing kills some but not all microorganisms present in fish where psychrotrophs can survive freezing and are ready to grow on thawing. Egyptian standard (1991) stated that, the permissible limit for the total bacterial count for frozen fish was no more than 10^6 colonies/g fish muscles.

The microbial activity is one of the main causes of quality deterioration of fish, so the spoilage pattern of fish depends upon the initial bacterial count, in addition to those acquired during handling and storage (Cobb and Vanderzant, 1971).

As regard to total *Enterobacteriaceae* and Coliform counts, Table (2) showed that all the examined frozen mackerel fish samples were negative for total *Enterobacteriacea* count except 3 samples (12%) were exhibited the mean count $3 \times 10^2 \pm 9 \times 10$ cfu/g. Also for the Coliforms count (MPN/g), only 6 samples (24%) were having a mean value of $3 \times 10 \pm 1 \times 10$ cfu/g. Nearly similar results were obtained by lee (1967), Abdel -Hafeez (1991), Awad *et al.* (1993) and Hassan (1998) but higher counts were reported by Gorczyca *et al.* (1985), Morshidy and Hafez (1986), (Hasan 1991) and Mahmoud (1994).

The obtained results were within the permissible limit (100 colonies/g) recommended by the Egyptian Standard (1991) for frozen fish. Using the Coliform count as an index of pollution in frozen food has been criticized because of the susceptibility of this group of microorganisms to freezing injury resulting in gradual disappearance in their numbers in frozen food during continued storage (Licciardello and Hill, 1978).

Table (4) showed that 9 isolates of Coliform group as follow: *E. coli* (1), *Citrobacter freundii* (2), *Citrobacter diversus* (2), *Klebsiella pneumoni subsp. Pneumoni* (1), *Klebsiella oxytoca* (1) and *Enterobacter aerogenes* (2) with incidences 4%, 8%, 8%, 4%, 4%, and 8% respectively. Kosev *et al.* (1990) found that Coliforms and aerobic psychrotrophic bacteria were not detected in muscle tissue at any time of frozen storage at -20ºC for 190 days for samples of carp, trout and fresh

water fish, while these bacteria decreased only in count on fish surfaces by 32-57% during storage.

Table (2) show that the total *Staph. aureus* counts of the examined frozen mackerel fish samples were ranged from 6 x 10 to 2 x 10^4, with a mean value of 4 x 10^3 ± 9 x 10^2 cfu/g and with a percentage of 88%. Similar findings had been reported by *Awad et al., (1993)* but lower counts were reported by Nickelson *et al.* (1980), Morshidy and Hafez (1986) and Hassan (1998). It was cleared from table (3) that *Staph. aureus* can grow best in foods in which the competing microorganisms are present in low numbers.

Table (3) declared that the incidence of coagulase positive *Staph. aureus* isolated from the examined frozen mackerel fish samples marketed at Damanhour city fish markets was 11 (50%).

Enterotoxigenic strains of *Staph aureus* can give rise to food borne intoxication. Hence, such contaminated frozen fish may at times constitute a public health hazard and this agree with the statement reported by Thatcher and Clark (1978).

Results present in Table (2) reveal that the mean values of total *Pseudomonas* and *Aeromonas* in examined frozen mackerel were 7 x 10^3 ± 5 x 10^3 and 4 x 10^3 ± 4 x 10^3 cfu/g, the percentages of positive samples were 20% and 16% respectively.

Hassan (1998) found that the mean values of *Pseudomonas* and *Aeromonas* of the examined frozen mackerel were 3 x 10^3 ± 1 x 10^3 and 3 x 10^2 ± 24 cfu/g in winter and 2 x 10^4 ± 2 x 10^3 and 5 x 10^2 ± 23 cfu/g in summer, respectively.

Pseudomonas alcaligenes (4%), *Pseudomonas pseudoalcaligenes* (8%), *Pseudomonas aeruginosa* (4%) and *Pseudomonas putida* (4%) could be isolated (Table 4). Similar findings were obtained by Edward and Kraszewski (1991) and Mahamoud (1994). *Pseudomonas aeruginosa* produces a variety of extracellular enzymes, including proteases and lipases that are responsible for most of the histopathological effects of the infection (Wilson and Miles, 1975 and Blackweed *et al.*, 1983).

On the other hand, *Aeromenas veronii* (4%), *Aeromonas schubertii* (4%) and *Aeromonas cavia* (8%) could be isolated (Table 4).

Results recorded in Table (2) showed that the mean values of total mould and total yeast counts of examined frozen mackerel were 1 x 10^3 ± 1 x 10^3 and 5 x 10^3 ± 2 x 10^3 respectively.

In moist foods, yeasts and moulds tend to grow slower than other microorganisms (Hobbs, 1983). Low mould count may also be due to

that the moulds are strictly aerobes, while the yeasts can grow in either aerobic or anaerobic environments.

The following bacterial reference values for raw fish for total mesophilic count vary from 10^4 to 10^7 cfu/g (as a rejection limit for spoilage). Reference values for the control of hygienic measures range from 10^2-10^4 cfu/g for *Enterobacteriaceae*, from 0-400 cfu/g for total faecol Coliforms and *E. coli*, and from 100-2000 cfu *Staph.aureus*/g and absence of *Salmonellae* and *Vibrio parahaemolyticus* in 25g in all raw fish (Friedhoff, 1994).

Therefore, good water quality is the key to improve the production and hygiene of fish as a food. An abundant water supply will avoid many problems associated with aquaculture by dilution, in addition to the prevention of pollution of the fish growing farms through the treatment of sewage water and careful determination of the discharge points seems to be a successful strategy to improve fish quality and to protect the consumer from fish borne enteric diseases. With regard to the catched fish, the delay between catching and marketing should be as short as possible and there must be some form of refrigeration to hold the product at 0ºC for fresh fish such as boxing and icing and then the boxes held in an insulated chilled room at between 1 and 2ºC but the temperatare of the cold stores of the frozen fish should ideally be at -18ºC.

REFERENCES

Abdel-Galil, Y.; El-Daly, E. and Mansour, N. (1988): Enteric organisms prevalent in market fish with Special reference to *E.coli*. Bull. High Inst. Publ. Hlth 18: 1123-1129.

Abdel-Hafiez, E.M. (1991): Comparative studies on the keeping quality of some Nile and farm fishes. M.V.Sc. Thesis, Fac. Vet. Med., Alex. Univ.

Ahmed, L.S.; Dosoky, R.M.; Kamel, Y.Y. and Ismail, A.A. (1986): Bacteriological studies of fresh water fish (*Tilapia nilotica*) in Upper Egypt. Assuit Vet. Med. J., 15: 205-209.

Alian, A.M.; Awad, A.A.; El-Wakil, F.A. and Ibrahim, A.A. (1969): Studies on antibiotic treated Egyptian fish. J. Microbiol. 4: 29-69.

American Public Health Association (APHA) (1992): Compendium of methods for the microbiological examination of foods, 3rd Ed. Washington, D.C., USA.

Amin, N.E. (1973): About the bacteriological flora of Silver carp. J.Ribonee Khazaistvo, Kiev, 17:118-124.

Awad, H; Tolba, K.; Niazi-Zinab and Refaai, M. (1993): Quality assurance of imported frozen fishes in Egypt. Vet. Med. J. Giza, 41:137-145.

Bastawrows, A.F. and Mohammed, A.A. (1999): Some microbiological investigations on *Aeromonas hydrophila* group in *Oreochromis niloticus* and *Clarias lazera* in Assiut Governorate. Assiut Vet. Med. J. Vol. 40 No.80: 197-209.

Blackweed, L.; Stone, R.; IgIewski, B. and Penington, J. (1983): Evaluation of *Pseudomonas aeruginosa* exotoxin A and elastase as virulent factors in acute lung infection. Infect. Immunol. 39:198-201.

Brayan, L.F. (1992): Food borne infections and intoxications: Contemporary Problems and Solutions. Proceeding 3rd World Congress, Foodborne infection and intoxication. Berlin, PP.11.

Cobb, B.F. and Vanderzant, C. (1971): Biochemical changes in shrimp inoculated with *Pseudomonas*, Bacillus and Coryneform bacterium. J. Milk Food Technol. 34: 533-540.

Collins, C.H. (1984): Microbiological methods. (5th Microbiology Laboratory Manual). British Library, Butter worth Inc. Co.

Edward, G. and Kraszewski, A. (1991): Identification and characteristics of *Pseudomonas*, *Actinobacter*, *Moraxella* and *Xanthomonas* species isolated from the fish, water and bottom deposits of the Baltic sea. (VI.II) Morstiego Pulku Strzelcaw, 1211: 81-661. Gdynia Medweter, 41: 58.

Edwards, P.R. and Ewing, W.H. (1972): Identification of *Enterobacteriaceae*. 3rd Ed. Burgers publishing, Minneapolis.

El-Atabany, A.I. (1995): Occurance of *Aeromonas hydrophila* group in *Mugil cephalus* fish marketed in Manzala City. Zag. Vet. J. (ISSN. 1110-1458) Vol. 23 No. 5: 8-11.

El-Kelish, H.I. (1995): Incidence of *Aeromonas hydrophila* group in *Tilapia nilotica* marketed in zag. City. Zag. Vet. J. (ISSN. 1110-1458) Vol .23 No. 5: 12-14.

El-Leboudi, S.H. (2002): Quality attributed of some local fresh and salted fishes and their improvement. Ph.D. Thesis, Dept. of food Hygiene, Fac. Vet. Med., Cairo Univ.

El-Mossalami, M.K.; Gehan, M. Kassem and Attallah, O.A. (2004): Quality changes of some ice-stored aquacultured fish. J. Egypt. Vet. Med. Assoc. 64, No. 1: 81-91.

E.O.S. (1991): Egyptian Organization for Standardization and quality control of frozen fish (889-1991).

FAO (1996): Food and Agricultural Organization, quality and quality changes in fresh fish. FAO fisheries Technical paper 348, 135.

Farouk, M. (1989): Microbiological evaluation of some fishes in rural markets. Ph.D. Thesis, Fac. Vet. Med., Alex. Univ.

Frazier, W.C. and Westhoff, D.C. (1978): Growth of microorganisms at low temperature. In "Food Microbiology" 2nd Ed. McGraw-Hill Publisher Co., Inc. N.Y.

Friedhoff, R. (1994): Microbiological spoilage and public health risks of fresh finfish from temperated climate zones. Waren-Chemicus. 24, 142.

Fujioka, R.S.; Tenno, K. and Kansako, S. (1988): Naturally occurring fecal coliforms and fecal *streptococci* in Hawaii's fresh water streams. Toxic Assess. 3, 613-630.

Gilbert, R.T.; Wieneke, A.A.; Lanser, J. and Simkovicova, M. (1972): Serological detection of enterotoxin in foods implicated in *Staphylococcal* food poisoning. J. Food Hyg., 70: 755-762.

Goda, F.; Shauman, T.; Wassef, N. and Farried, A. (1980): A bacteriological study of *Tilapia nilotica* fishes of Naser lake. Egypt Vet. Med. J., Cairo, 28.

Gorczyca, E.M.; Sumner, J.L.; Cohen, D. and Brady, P. (1985): Mesophilic fish spoilage. Food Technol. In Australia 37 (1): 24-26.

Hafez, A.E. (1989): Prevalence of spoilage of fish. In Essays in Agriculture and food Microbiology. "Text book" London.

Hasan, M. (1991): Microbiological status of frozen dressed fish. M.V.Sc. Thesis, Fac. Vet. Med. Moshtohor, Zagazig Univ.

Hassan, A.A. and Abdel-Dayem, R.H. (2004): Prevalence of Fungi and mycotoxins in fresh and salted fish. J.Egypt Vet. Med. Assoc. 64, No. 1: 59-68.

Hassan, N.S. (1998): Studies on quality of Frozen fish. Ph.D. Thesis. Fac. Vet. Med. Beni-Suef, Cairo Univ.

Hobbs, G. (1983): Food poisoning and fish. R. Soc. HPth J. 103: 144.

International Commission on Microbiological Specifications for Foods (ICMSF) (1978): Microorganisms in Food, their significance and method of enumeration. 2nd Ed. Univ. Toronto Press, Toronto and Buffalo, Canada.

Kielwein, G. (1969): Ein Nährboden zur selektiven Zuchtung von Pseudomonaden und Aeromonaden. Arch. F. Lebensmittelhyg., 20: 131-133.

Konemen, E.W.; Allen, S.D.; Janda, M.W.; Schrecken-Berger, P.C. and Winn, C.W. (1994): "Diagnosis microbiology" 6th ed. J.B. Lippincott Philadelphia.

Kosev, D.; Boshkova, K.; Lalov, M.; Donchev, V. and Kuzmanova, E. (1990): Technological and quality indicators in frozen fresh water fish. Khranitelna Promishlenost, 39: 26-29.

Lee, E.H.; Kim, M.C.; Kim, J.S.; Ahn, C.B.; Kim, B.K. and Koo (1967): Studies on the processing of frozen seasoned mackerel. II. Keeping quality of frozen seasoned mackerel during storage. J. Korean Soc. Food Nutrit. 19: 107-114.

Licciardello, J.J. and Hill, W.S. (1978): Microbiological quality of commercial frozen minced fish blocks. J. of food Protection 41 (12): 948-952.

Liston, J.; Matches, J.R. and Baross, J. (1971): Survival and growth of pathogenic bacteria in food. In Food Inspection and Quality Control (ed.) *Kreuzer, R.*; Fishing News Ltd., London, England, P. 246.

Macfaddin, J.F. (1980): Biochemical Tests for Identification of Medical Bacteria. 2nd Ed. Williams and Wilkins, Baltimore, England.

Mahmoud, Y.E. (1990): Studies on the sanitary condition of some Nile fishes marketed in kalyobia Governorate. M.V.Sc. Thesis, Fac. Vet. Med., Moshtohor, Zagazig Univ.

Mahmoud, Y.E. (1994): Studies on frozen fish. Ph.D. Thesis, Fac. Vet. Med. Moshtohor, Zagazig Univ.

Matthes, S. (1984): Diminution of egg quality caused by avian diseases and microbial contamination. J. World's Poult. Sci. 40: 81.

Morshidy, A. and Hafez, A. (1986): Quality evaluation of frozen fish marketed in Zagazig City. Zagazig J. Vet. Sci. 2: 14-17.

Mousa, M.M. and Mahmoud, Y.E. (1997): Hygienic quality of newly caught fresh fish. Assiut Vet. Med. J. 37: 219-233.

National Academy of Science (1985): An evaluation of the role of microbiological criteria for food and food ingredients. National Academy Press, Washington, DC.

Nickelson, R.; Finne, G.; Hanna, M.D. and Vanderzant, C. (1980): Minced fish flesh from non-Traditional Gulf of Mexico finfish sp.: Bacteriology. J. Food Sci. 45: 1321-1325.

Rosner, R. (1964): Aeromonas hydrophila as the etiologic agent in a case of sever gastroenteritis. Am. J. Clin. Pathol. 42: 402-404.

Roushdy, S.A.; El-Sherief, A.A.; Abdel-Aziz, A.A. and Saad El-Din, G. (1996): Sensory and bacteriological quality of some fish in rural areas. Egypt. J. Agric. Res., 74 (4): 1035-1045.

Sedik, M.F.; Safwat, E.E.; Ibrahim, A.M. and Shaaban, A.I. (1989): Studies on some species of locally produced fish. Vet. Med. J. Giza 37 (2): 197-207.

Soliman, M.K. (1988): Pathogenesis of *Aeromonas hydrophila* isolates in fish with special emphasis on their control. Ph.D. Thesis, Fac. Vet. Med., Alexandria Univ.

Sumner, J.L.; Gorczyca, E.; Cohen, D. and Brady, P. (1984): Do fish from tropical waters spoil less rapidly in ice than fish from temperate waters? food Technology in Australia 36 (6): 328-334.

Thabet, F.M. (1972): Studies on the spoilage for some Fish species. Ph.D. Thesis, Fac. Agric., Ain-Shams Univ.

Thatcher, F.S. and Clark, D.S. (1978): Microorganisms in Foods (ICMSF). 2nd Ed., Academic Press, New York.

Varnam, A.H. and Evans, M.G. (1991): Food borne Pathogens. "An illustrated text". Wolfe Publ. Ltd., BPCC Hazel Books Print, Ayes bury, England. Chapter (13) Pp. 267-288.

Vishwanath, W.; Lilabati, H. and Bijen, M. (1998): Biochemical, nutritional and microbiological quality of fresh and smoked mud eel fish "Monopterus albus" – a comparative study. Food chem., 61 (1/2): 153-156.

Wilson, G.S. and Miles, A. (1975): Topley and Wilson's, Principles of Bacteriology, Virology and Immunology. 5th Ed. Edward Arnold Ltd., London.

Yehia, N.Y. (1996): Microbiological evaluation of farm fish in Kafr El-Shikh. M.V.Sc. Thesis, Fac. Vet. Med., Kafr El-Shikh, Tanta Univ.