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BACTERIOLOGICAL SAFETY OF FRESH FISH FROM URBANAND RURAL AREAS SOLD AT MANSOURA CITY

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ABSTRACT

Two hundred freshwater fish samples including 100 Tilapia fish (50 from urban and 50 from rural areas) and 100 *Mugilcephalus* fish (50 from urban and 50 from rural areas) were collected from different fish markets at Mansoura City, Dakahlia Governorate. The collected samples were examined bacteriologically for determination oftotal aerobic plate count (APC), Coliforms count, anaerobic bacterial count in addition to isolation and serotyping of *Staph. aureus*; *Salmonellae*; *E. coli* and *Listeria monocytogenes*. The obtained results showed that there were a significant difference (P<0.05) in bacterial counts between rural and urban areas in the examined samples of *Tilapia nilotica* and *Mugilcephalus,* these results were in accordance with the Egyptian Organization for Standardization and Quality Control (EOS) No. 3494 (2007) for APC while some higher results were recorded in anaerobic counts, Coliforms and *Staph. aureus* which were unacceptable. Meanwhile, the results of coagulase positive *Staph. aureus* were negative which were acceptable. In addition to the incidence results of *E. coli, Salmonellae* and *L. monocytogenes* which give indication of sewage pollution, mishandling during transportation, distribution and storage conditions as well as marketing. Hence fish should be chilled as quickly as possible to lowest temperature from the harvesting point up to consumption with periodical cleaning and disinfection for containers used for fish transportation.

Key words: *Tilapia nilotica, Mugilcephalus, Staph. aureus, E. coli, Salmonellae , Listeria monocytogenes*

INTRODUCTION

Fish can contribute to a higher level of food safety and security by providing protein of high quality, essential fatty acids, vitamins and minerals. Italso plays an important role in the economy of many countries by increasing employment opportunities. The whole world fish production reached 52.5 million tons in 2008, responsible for 45.5% of the world food fish consumption (FAO, 2012). Furthermore, fish is eaten in many ways including smoked, cooked and raw. However, it has been shown that fish may be a source of food borne illness, causing outbreaks, this has made consumers more aware and has therefore become an important public health issue, which in many cases were neglected (EFSA, 2010).

Fish could be spoiled from both outer and inner surfaces as fish stomach and intestine. After fish is being caught and dying, the immune system collapses and bacteria allowed to proliferate freely on the skin surface and viscera, penetrating the intestinal walls to move into the flesh through the muscle fiber where the intestinal microflora is the main causative agent of fish spoilage (Kaneko, 1971), besides in 2004, Novotny *et al.* found many listed pathogenic bacteria in fresh water fish including *Staph. aureus, E.coli,* Salmonella, *Cl. Botulinum, L. monocytogenes*.

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MATERIALS AND METHODS

A-Collection of samples: A total number of 200 freshwater fish samples including 100 *Tilapia nilotica* fish (50 from rural and 50 from urban areas) and100 *Mugil cephalus* fish (50 from rural and 50 from urban areas) were collected from different fish markets at Mansoura city. The collected samples were kept in an insulated ice-box and transferred to the laboratory without delay, where they directly exposed to the following examination.

B-Sensory evaluation of the examined fish samples: Fish samples were washed using potable water and examined physically for general appearance of the skin, Consistency of flesh, odor and color of gills, color and condition of eyes and slime formation following the scheme provided by FAO (1995).

C-Preparation of fish samples for bacteriological examination according to APHA (2001): all the

examined samples were apparently normal. The scales and fins were removed. The skin was sterilized by alcohol and flamed under complete aseptic conditions then25 g of fish flesh from each sample were dessicated in a sterile flask with 225 ml sterile peptone water (0.1%) which added and thoroughly mixed using sterile blender for 1-1.5 minutes, followed by six fold serial dilutions.

D-Bacteriological examination: the prepared samples were subjected to the following analysis:

1-Determination of total aerobic bacterial count: according to APHA (2001).

2-Determination of total Coliforms count: was carried out according to the procedures recommended by FDA (2001) using Violet Red Bile agar medium.

3-Determination of total anaerobic bacterial count according to Savvaidis *et al.* (2001) and identification of anaerobic bacteria according to Koneman *et al.* (1992): All samples were inoculated into cooked meat broth medium in duplicate tubes. One of the two tubes was heated at 80^0C for 10 min. in a water bath to eliminate vegetative organisms while the other inoculated medium was kept without heating and both were anaerobically incubated at 37^0C for 48 h. A loopful from the inoculated heated broth was streaked onto 10% sheep blood agar plates while that from unheated media was streaked onto the same media containing 75 ug/ ml neomycinsulphate blood agar and the inoculated plates were incubated anaerobically at 37^0C for 48h. The growing surface colonies which showed catalase negative reaction were picked up inpure form and reinoculated into cooked meat broth for further identification.

4-Isolation of *Staph. aureus*: on Baird parker agar according to **FDA (2001)**. The presumptive *Staph. Aureus* colonies were confirmed by Coagulase test.

5-Isolation of Salmonellae: according to **FDA (2007)** enrichment in rappaport vassiliades broth at 35^0C for 24h., platting on XLD agar at 42^0C for 24h. The presumptive colonies were confirmed biochemically and serologically.

6-Isolation and serotyping of *E.coli*: were carried out according to ICMSF (1996).

7- Isolation of *Listeria monocytogenes*: 25 g of each sample were homogenized separately in 225 ml of UVM I (University of Vermont Listeria enrichment broth Ryser *et al.* (1996). Aliquots of 1 ml of primary enrichments were transferred to 20 ml of UVM II (UVM I with 0.025 g. of acriflavine hydrochloride in 10 ml of sterile distilled water, pH 7.2) and incubated again at 37^0C for 24-48 h Jorgensen and Huss (1998). Aliquots of 0.1 ml of secondary enrichments were plated in duplicate PALCAM Listeria selective agar (Merck) supplemented with PALCAM Listeria selective supplement (Merck). Suspected colonies were confirmed by Gram staining, motility test (hanging drop), catalase and B-haemolysis tests and sugar fermentation tests for rhamnose, xylose and mannitol APHA (2001) and Harrigan (1998). Serotypes were determined using Bacto-Listeria-O polyvalent antiserum and Bacto-Listeria-O antisera types 1 and 4 (Difco). The obtained results were statistically evaluated by using t test according to Feldman *et al.* (2003).

RESULTS

Table1: Statistical analytical results of bacteriological counts (log_{10}cfu/g) for the examined fresh *Tilapia nilotica* and *Mugil cephalus* fish samples (N=50 of each).

Types of examined fish	Types of count	Source of samples (Log mean ±SE)		*
		rural areas	urban areas	
Tilapia nilotica	APC	4.5±3	4.8±3.5	$<10^6$
	Coliform count	2.5±1.3	2.7±1.7	$<10^2$
	Anaerobic count	2±1.8	2.3±2	**
	Staph.aureus count	2.6±1.4	2.7±1.8	$<10^3$
Mugil cephalus	APC	3.9±2	4.4±3.2	$<10^6$
	Coliform count	2.1±1.5	2.5±1.3	$<10^2$
	Anaerobic count	2±1.6	2.3±1.5	**
	Staph.aureus count	2.5±1.4	2.5±1.7	$<10^3$

*=Egyptian Organization for Standardization and Quality Control, **= not mentioned

Table 2: Number of isolated organisms in the examined fresh fish samples.

Isolated organisms	*Tilapia nilotica*				*Mugil cephalus*				*
	rural areas		urban areas		rural areas		urban areas		
	No	%	No	%	No	%	No	%	
Anaerobes	3	6	4	8	2	4	2	4	**
Staph.aureus	2	4	3	6	0	0	1	2	$<10^3$
E. coli	5	10	8	16	3	6	4	8	**
Salmonellae	1	2	2	4	0	0	1	2	**
L. monocytogenes	9	18	13	26	6	12	8	16	**

* = Egyptian Organization number of examined positive samples from rural areas or urban areas.

Table 3: Serological identification of isolated *E.coli* in the examined positive fresh fish samples.

Serotype	*Tilapia nilotica*			*Mugil cephalus*	
	No	rural areas	urban areas	rural areas	urban areas
O44:H18	3	-	-	3	-
O111:H4	5	3	-	-	2
O125:H21	2	2	-	-	-
O114:H21	5	-	5	-	-
O127:H6	5	-	3	-	2

Table4: Serological identification of isolated *Salmonella serovars* in the examined positive fresh fish samples.

Identified strains	Antigenic structure		*Tilapia nilotica*				*Mugil cephalus*		*
			rural areas		urban areas		urban areas		
	O	H	No	%	No	%	No	%	
Salmonella enteritidis	1,9 ,12	g,m: 1,7	-	-	-	-	1	2	**
Salmonella anatum	3,10 ,15	e,h: 1,6	1	2	1	2	-	-	**
Salmonella typhimurium	1,4, 5,12	I:1,2	-	-	1	2	-	-	**

DISCUSSION

Contamination of hands and surfaces during catching, cleaning and evisceration of fish is the common route of pathogen infection to other food (Buras, 1993) hence, Fish not only transmit diseases to man but received many diseases and capable of transmitting some of the established food borne microbial infections and intoxications (FAO/WHO, 1974).

The obtained results were calculated and analysed statistically as shown in Table (1) where the APC were 4.5±3,4.8±3.5,3.9±2 and 4.4± 3.2 $\log_{10}$cfu/g for *Tilapia nilotica* from (rural and urban areas) and *Mugil cephalus* from (rural and urban areas) respectively, these results were nearly in accordance with Mahmoud (1999) who recorded that the APC were 3±0.12 $x10^3$ and 1.86±0.10 $x10^3$ cfu/g for *Tilapia nilotica* and *Mugil cephalus*, Vieira, *et al*. (2000) found that the APC were $0.3x10^4$cfu/g for frozen tilapia, Samaha *et al.* (2011) who reported that the APC were $4.38x10^4$±3.2x 10^3 and $6.6x10^3$± $4.0x10^2$cfu /g for *Tilapia nilotica* and *Mugil cephalus* musculature. Meanwhile, higher results were obtained by Abd El-Aziz, (2010) who recorded that the APC were $1.8x10^7$±$4.0x10^7$cfu/g for *Tilapia nilotica*, Wang

et al. (2011) found that the APC were 4.96-6.53 $\log_{10}$ cfu/g with 15.8% which were unacceptable. As the recorded limit was (APC>7 log cfu/g) for seafood, Hafez and Megahed (2011) found that the APC were $4.2X10^6$ $\pm0.2x10^3$cfu/g for *Tilapia nilotica* and Budiati *et al.* (2015) who found that 5.77-9.12 $\log_{10}$ cfu /g for Tilapia, the APC in rural areas were lower than in urban areas with significant difference (P<0.05) in the examined samples of *Tilapia nilotica* and *Mugil cephalus*. These results were in accordance with the Egyptian Organization for Standardization and Quality Control (EOS) (2007) for APC ($<10^6$cfu/g).

The aforementioned results in Table (1) declared that all the examined samples were positive for Coliforms with variant mean counts 2.5±1.3, 2.7±1.7, 2.1±1.5 and 2.5±1.3$\log_{10}$cfu/g for *Tilapia nilotica* and *Mugil cephalus* from (rural and urban areas) respectively, these results were nearlyin accordance with Abd ELShahid *et al.* (2009) who found that Coliform count were$1.02x10^3$ and2.1×10^2cfu/g.for *O.niloticus* and *Mugil cephalus*, Abd El-Aziz (2010) found Coliform count were $4.3x10^2\pm8.4x10^2$cfu/g. for *Tilapia nilotica*, Hafez and Megahed (2011) showed that Coliform count were $2.8x10^2 \pm 0.1x10^2$cfu/gfor *Tilapia nilotica*, El-Hakem *et al.* (2013) couldisolate Coliform by 2.4 x 10^2 ±1.4 $x10^2$cfu/g from Tilapia musculaturealso, Eissa *et al.* (2014) found coliform count $9.5x10^2\pm4.2x10^2$ and $9.3x10^2\pm4.3x10^2$cfu/g in raw *Tilapia nilotica* and *Mugil cephalus*, Junior, *et al.* (2014) obtained counts of coliforms were 3-1100 cfu/g in fish and Budiati *et al.* (2015) isolate 1.6 - 4.04 $\log_{10}$cfu coliforms /g. for tilapia, while Samaha *et al*. (2011) recorded higher results in *Tilapia nilotica* and *Mugil cephalus* $1.69x10^3\pm0.15x10^3$ and $1.98x10^3\pm0.32x10^3$cfu/g which may be due to water pollution with sewage, improper handling during catching, storage and distribution in the markets. Coliforms in rural areas were lower than in urban areas with significant difference (P<0.05) in the examined samples of *Tilapia nilotica* and *Mugil cephalus.* The results which were higher than the EOS (2007) for Coliforms were unacceptable.

The anaerobic countresults in tables(1&2)were 2±1.8, 2.3±2, 2±1.6 and 2.3±1.5 $\log_{10}$ cfu/g the counts were higher in urban areas than rural areas, with incidence percent 6%,8%,4% and 4% for *Tilapia nilotica* from (rural and urban areas) and *Mugil cephalu*s from (rural and urban areas) respectively. Meanwhile the vegetative form of Clostridium perfringens was detected in 2%, 4% and 2% of examined samples from *Tilapia nilotica* collected from (rural and urban areas) and *Mugil cephalus* from (urban areas) and the spore form of Clostridium perfringens were present in 4%, 4% 2% and 2% of examined samples from *Tilapia nilotica* collected from (rural and urban areas) and *Mugil cephalus* from (rural and urban areas). There was a significant difference (P<0.05) in anaerobic count between rural and urban areas in the examined samples of *Tilapia nilotica* and *Mugil cephalus*, these results were relatively in accordance with Voidarou *et al.* (2011) who isolate the vegetative and spore forms of *Clostridium perfringens* from 6% and 35% of the examined *Mugil cephalus* while, Novotny *et al.* (2004) found *Cl. Botulinum* in fresh water fish. The positive results were not in accordance with the EOS (2007) for anaerobic counts.

The obtained results of *Staph.aureus* in Tables (1&2) were 2.6±1.4, 2.7±1.8, 2.5±1.4 and 2.5±1.7 $\log_{10}$cfu/g with incidence percent 4%,6%,0% and 2% for *Tilapia nilotica* from (rural and urban areas) and *Mugil cephalus* from (rural and urban areas) respectively. There was a significant difference (P<0.05) in *Staph. aureus* count between rural and urban areas which were lower in rural areas than urban areas of the examined samples. These results were in accordance with Vieira *et al.* (2000) who found that the counts were 10.0 and $10.6x10^2$cfu/g in frozen Tilapia, Pacheco *et al.* (2000), Novotny *et al.* (2004) found coagulase-positive *Staph. aureus* counts ranged from $10\text{-}21x10^3$cfu/g.in frozen Tilapia and Voidarou *et al.* (2011) found 8% of *Mugil cephalus* contain *Staph. aureus*, Samaha *et al.* (2011) could isolate *Staph. Aureus* by $3.84x10^2\pm0.46x10^2$ and $3.56x10^2$ ±0.41 $x10^2$cfu/g from *Tilapia niloticus* and *Mugil cephalus,* Hafez and Megahed (2011) could isolate $5.1x10^1\pm0.114$ cfu/g *Staph. Aureus* from 12% of the examined *Tilapia nilotica*, El-Hakem *et al.* (2013) detect $6.8x10^2\pm2.9x10^2$cfu/g *Staph. Aureus* in *Tilapia nilotica* musculature and Eissa *et al.* (2014) could isolate 7.5X10±1.2 and 5.0x10±1.2cfu/g from raw *Tilapia nilotica* and *Mugil cephilus* fish. Meanwhile, higher results were obtained by Junior *et al.* (2014) who examined skin and muscles of Tilapia for Coagulase positive *Staph. Aureus* which were 1.0×10^2–1.2×10^6cfu/g the results were in accordance with the EOS (2007) for *Staph. Aureus* ($<10^3$cfu/g), while coagulase positive *Staph. Aureus* were negative.

The results of *E.coli* incidence in (tables 2&3) were 10%,16%,6% and 8% in the examined *Tilapia nilotica* and *Mugil cephalus* samples collected from rural and urban areas respectively, with three serotypes of *E. coli* O111:H4 and two serotypes of *E. coli* O125:H21isolated from *Tilapia nilotica* samples collected from rural areas, five serotypes of *E. coli* O114:H21 and three serotypes of *E. coli* O127:H6 in Tilapia nilotica samples collected from urban areas, three serotypes of *E. coli*O44:H18 in *Mugil cephalus* collected from rural areas and two serotypes of *E. coli* O111:H4 and two serotypes of *E. coli* O127:H6 in *Mugil cephalus* collected from urban areas.

These results were nearly similar with those achieved by Mahmoud (1999), Novotny *et al.* (2004), Abd EL-Shahid *et al.* (2009) who found *E. coli* in 20% and 8% for *Oreochromis niloticus* and *Mugil cephalus*

samples and so Voidarou *et al.* (2011) (6%) for *Mugil cephalus* and *Tilapia nilotica* respectively. Wang *et al.* (2011) could isolate *E.coli* by 9.4% from seafood, Elsherief *et al.* (2014) found *E.coli* in 12% and 4% of examined *Tilapia nilotica* and *Mugilcephalus*. Meanwhile, higher results were recorded by Hassan *et al.* (2012) 27% and 42.8% for *Oreochromus niloticus* and *Mugilcapito* respectively and Amr *et al.* (2012) 57.10% and 91.40% from Tilapia and *Mugil cephalus.* The presence of high counts and incidence of *E. coli* serotypes as shown in Table (3) in some samples indicates sewage pollution of fish inducing food poisoning and hemorrhagic enterocolitis in human due to eating improperly processed fish meals Galal, (2013). The positive results were not in accordance with the EOS (2007).

The results in (Table2) declared that the incidence of *Salmonellae spp.* were 2%,4%,0% and 2% in the examined *Tilapia nilotica* and *Mugil cephalus* samples collected from rural and urban areas respectively, Nearly similar or slightly higher results were obtained by Vieira *et al.* (2000) isolate 8.3% *Salmonellae spp.* from *Tilapia nilotica*, Abd EL-Shahid *et al.* (2009) detect *Salmonellae spp.* In 8% and 4% of examined *Oreochromis niloticus* and *Mugil cephalus*, Shinkafi and Ukwaja (2010) found *Salmonellae spp.* In 3.2% of examined *Tilapia nilotica*, Voidarou *et al.* (2011) isolate *Salmonellae spp.* from 2% of examined *Mugil cephalus* samples, Elsherief *et al.* (2014) detect *Salmonellae spp.* In *Tilapia nilotica* and *Mugil cephalus* by 8% and 16% and so Mahmoud (1999), Novotny *et al.* (2004). In contrary Pacheco *et al.* (2000) found higher results from *Salmonellae spp.* in the examined Tilapia (60%) and Wang *et al.* (2011) isolate *Salmonellae spp.* from 17.5% of examined seafood, Hassan *et al.* (2012) could isolate *Salmonella arizonae* from *O. niloticus* and *Mugil capito* which were 21.6% and 14.2%. Amr *et al.* (2012) could isolate *Salmonellae spp.* by 57.10% and 17.1% from Tilapia and *Mugil cephalus,* while Hafez and Megahed (2011), EL-Hakem *et al.* (2013) and Eissa *et al.* (2014) failed to detect *Salmonellae spp.* from the examined raw *Tilapia nilotica* and *Mugil cephalus*. The positive results were unacceptable and not in accordance with the EOS (2007).

The data reported in (Table4) revealed that serotypes of *Salmonellae spp.* isolated from *Tilapia nilotica* were represented by *Salmonella typhimurium* and *Salmonella anatu* m in rural and urban areas While, in *Mugil cephalus Salmonella enteritidis* could be isolated. Some members of *Salmonellae* are pathogenic and may cause infection and food poisoning to human and unacceptable according to the EOS (2007). *Salmonella typhimuium* represents about 50 - 60 % of food poisoning and commonly isolated from cases of food poisoning. Meanwhile, presence of *Salmonellae* in fish reflect unsatisfactory hygienic conditions during catching, handling and marketing of fish WHO (1997).

The obtained results in Table (2) declared that *L. monocytogenes* could be isolated from *Tilapia nilotica* and *Mugil cephalus* collected from rural and urban areas by18%, 26%, 12% and 16% respectively, these results were in agreement with Novotny *et al*. (2004) and Abd El-Aziz, (2010) who could isolate *L. monocytogenes* from *Tilapia nilotica* by 26.7% while lower results were recorded by Wang *et al.* (2011) 4.1% and Shinkafi and Ukwaja (2010) 9.67 %. This may be attributed to fish species, methods of catching, handling sanitation level during transportation, distribution and storage conditions as well as marketing Wang *et al.* (1994) and the positive results were unacceptable according to the EOS (2007).

CONCLUSION AND RECOMMENDATIONS

From the obtained results, it could be concluded that raw fish had a high bacterial load. Some samples were free from Salmonella, *E.coli* and *L. monocytogenes* this may be attributed to the condition of fish itself or method of fish manipulation after catching. *Staph. aureus* and Coliform count which present in raw fish indicate pollution which must be taken in consideration, hence Fish should be chilled as quickly as possible to the lowest temperature from the harvesting point up to consumption. Containers used for fish transportation should be cleaned and disinfected periodically. Education of the fishermen and fish handlers about the hygienic methods for fish preservation, transportation and distribution.

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السلامة البكتيرولوجية للاسماك الطازجة بالمناطق الحضرية والريفية المباعة فى مدينة المنصورة

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اجريت هذه الدراسة على عدد ٢٠٠ سمكة (١٠٠ سمكة من كل من اسماك البلطى النيلى واسماك البورى الطازجة) والمجمعة من اسواق بيع الاسماك من اماكن مختلفة نصفها من المناطق الحضرية والنصف الاخر من المناطق الريفية حيث تم فحص جميع العينات بكتيريولوجيا لمعرفة العد الكلى للبكتيريا الهوائية وعد الميكروبات القولونية وعد البكتيريا اللاهوائية وعزل وتصنيف ميكروب المكورالعنقودى الذهبى والسالمونيلا والايشيريشيا كولاى واللستيريا مونوسيتوجين حيث وجد ان الحمل البكتيرى فى الاسماك المجمعة من المناطق الحضرية اقل من الاسماك المجمعة من المناطق الريفية وان العد البكتيرى غير مطابق للمواصفات المصرية فى بعض العينات نتيجة لتلوث المياه واختلاطها بمياه الصرف الصحى بالاضافة الى عدم وجود والوعى الصحى اللازم عن تداول الاسماك وطرق حفظها لدى الصيادين وبائعى الاسماك لذا يجب توعية الصيادين وبائعى الاسماك عن طرق الحفظ السليمة وضرورة حفظ الاسماك بالتبريد مباشرة بعد صيدها وتطهير صناديق نقل الاسماك بصورة متكررة لتقليل الحمل البكتيرى للاسماك الطازجة.