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Microbiological Evaluation of Some Farmed Fish Species Marketed in Sharkia Governorate, Egypt

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Abstract

The present study was conducted to evaluate the microbiological status of some farmed fish species marketed in Sharkia Governorate then compared with Egyptian standard of chilled fish. keeping quality parameters also examined (pH, TVN, TMA and TBA) and compared with Egyptian standard of chilled fish. A total of 150 samples of farmed fish of Nile cages, concrete ponds and Earthen ponds 50 of each (25 *Tilapia nilotica* and 25 *Mugil cephalus*) respectively from the market in Sharkia governorate were examined during autumn 2021. For keeping quality parameters, all examined samples were within the acceptable limits according to ES (3494:2005). The obtained results of microbiological analysis revealed that the percentage of the exceeded permissible limits of aerobic plate count (APC) was 12% and 32%,20% and 16% ,12% and 20% for *Tilapia nilotica* and *Mugil cephalus* in Nile cages, concrete ponds and Earthen ponds respectively. For total coliform count, all positive examined samples exceed permissible limits of coliform count (2 log10 CFU/g) according to ES (3494:2005). The incidence of *Staphylococcus aureus* was 0% and 11%, 36%, and 32%, 16% and 12% for *Tilapia nilotica* and *Mugil cephalus* in Nile cages, concrete ponds and Earthen ponds respectively. *Listeria* species were detected in 20% and 8% ,8% and 16% , 20% and 0% for *Tilapia nilotica* and *Mugil cephalus* in Nile cages ,concrete ponds and Earthen ponds, respectively. *Escherichia coli* was 24% and 20% ,8% and 16%, 16% and 20% for *Tilapia nilotica* and *Mugil cephalus* in Nile cages, concrete ponds and Earthen ponds respectively. *Salmonella* species were 4% and 12% for both from Nile cages and from concrete ponds and 4% and 12% in earthen pond *Tilapia nilotica* and *Mugil cephalus* respectively. Serological identification of *E. coli*, *Salmonella* and *Listeria* in these samples was showed in this search. So hygienic and proper practices performed during transportation and handling of fish are needed before consumption of this fish.

KEYWORDS
Farm fish, Keeping quality parameters, Microbiological analysis.

INTRODUCTION

Egypt owns the major aquaculture industry in Africa that provides more than seventy percent of the country's fish production (GAFRD, 2013). There are various methods of farming fish all over the world, such as intensive commercial operations and extensive small-scale or subsistence systems. Possible food safety hazards related to aquaculture differ according to the system used including biological contamination, such as foodborne pathogens or even chemical contamination. The causes of health hazards are varied, starting from unfortunate aquacultural practices, ecological contamination and cultural habits of food preparation and consumption (Reilly and Kdferstein, 1997). Semi-intensive aquaculture in clay ponds was considered the main fish farming system in Egypt, meanwhile intensive systems in tanks and cages have noticed fast progress during latest years (Kleih *et al.*, 2012). Fish is a very perishable food that requires cautious storage and handling. Fish quality to be kept, preserve it fresh as possible until cooking and consumption. So farm fish is a better choice for fresh, non-preserved fish, and higher nutritional content (Bremner, 2003). Detection of pH of fish muscle can point to physical changes happening in fish muscle throughout the storage time (Izumi, 2012). Volatile amines (total volatile nitrogen and trimethyl-amine) are molecules that cause fishy aroma and taste in fish which last for some days following catching and considered as measurement for evaluating the quality of fish (Etienne, 2005). According to data from (CDC) fish cause about twenty four percent of food related outbreaks of illness and six percent of all food poisoning (CDC, 2013). Food poisoning is caused by handling diseased fish or coming into touch with polluted water or other aquatic environment components (Gauthier, 2015). Also can caused by eating raw or undercooked fish, that could be infected with pathogens such as *Salmonella* spp., *E. coli* and *S. aureus* from water sources, or during the different processing stages (Pal *et al*., 2014).

Listeria spp. other than *L. monocytogenes* are widespread in hot environments (Fuchs and Reilly, 1992). *L. monocytogenes* are widespread all over the world, The incidence ratio in seafood can vary from zero to more than fifty percent (Jinneman *et al*., 1999). According to Food Net US, listeriosis mortality rate reach to 16.9 % with a percent 30% of foodborne deaths from 1996 to 2005 (Barton *et al*., 2011). *Salmonella* and *E. coli* in fish or fish products are indicators of fecal and environmental pollution of the fish environment (Wogu and Maduakol, 2010).

Escherichia coli are facultative anaerobes found in human and animal GIT so their presence in foods indicates fecal contamina-

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tion (Fadel *et al*., 2017). *Salmonella* was the main cause of bacterial food-borne disease in urban countries.

S. aureus responsible for production of Staphylococcal enterotoxins and various disease syndromes. Staphylococcal intoxication mainly characterized by emesis. Although enterotoxigenic strains mainly come from food handlers with hand infections or with a cold or a sore throat, *Staphylococci* could be also isolated from newly caught fish, especially in warm waters (Gram and Huss, 2000).

The aim of the study was to examine the keeping quality parameters (pH, TVN, TMA and TBA) and assess the bacteriological quality of farm fish in Sharkia market.

MATERIALS AND METHODS

Collection and preparation of fish samples

A total of 150 samples of farmed fish of Nile cages, concrete ponds, and Earthen ponds 50 of each (25 *Tilapia nilotica* and 25 *Mugil cephalus*) respectively were collected from the market in Sharkia governorate during autumn 2021. The samples were kept in a separated sterile plastic bag, labeled, and conserved in an ice box then transferred to the laboratory. The samples were prepared according to ISO 6887-3:2017. 10 grams of each sample taken under aseptic conditions, transferred into a sterile polyethylene bag to which 90 ml of 0.1% Sterile peptone water (Oxoid CM9) was aseptically added to provide a dilution 1/10, the content was blended for not more than 2.5 minutes using blender at high speed not less than (2000 r.p.m). Then the mixture was allowed to stand for15 minutes at room temperature. The contents of the jar were mixed by shaking before applying the following technique.

One ml from the original suspension (10^{-1}) was transferred aseptically with a sterile test tube containing 9 ml of sterile peptone water 0.1 % to obtain a dilution of (10^{-2}) from which further 10 fold decimal dilution were prepared up to suitable countable dilution.

Keeping quality tests

Detection of pH (Pearson, 2006)

In a blender, approximately 10 g of the sample were blended in 10ml of neutralized distilled water. The homogenate was left at room temperature for 10 minutes with continuous shaking. The pH value was determined by using an electrical pH meter (Bye model 6020, USA).

Detection of total volatile nitrogen (TVN)

The technique applied for determination of total volatile nitrogen (TVN) was recommended by Food and Agriculture Organization (FAO,1980)

Detection of trimethylamine (TMA)

The method adopted by FAO (1980) was applied using Conway test.

Detection of thiobarbituric acid (TBA)

The method adopted for estimation of TBA by Pikul *et al*. (1989) was applied.

Bacteriological evaluation

Aerobic Plate Count was done according to ISO 4833-1:2013. Briefly, dilutions were mixed thoroughly, 0.1 ml was pipetted of each dilution onto surfaces of solid media of plate count agar in pre-labeled petri dishes. Inoculum was spread over the entire surface with a bent glass rod that was first sterilized by dipping in 95% ethanol and quickly flamed to remove the ethanol. After absorption of the medium by the inoculum, the plates were inverted and incubated for 48 h at 35±2°C. All colonies in plates that contained 25-250 colonies were counted, and results were recorded per dilution counted. Average the colony counts obtained and multiply the average by 10 and then by the appropriate dilution factor (10^{1}-10^{-6}). Results were reported as APC/g sample.

Total coliform count (ICMSF, 1996)

The technique recommended by ICMSF (1996) using the surface plating method and Violet Red Bile agar medium.

Staphylococcus aureus (FDA, 2001)

One ml of the prepared dilution was poured into plates contained Baird Parker media with Egg Yolk-Tellurite emulsion incubated at 35ºC and observed after 48 hours. Characteristic black colonies surrounded by a narrow white margin with a zone of clearance was counted to obtain the total *Staphylococcus aureus* counts per g. Confirmation of *Staphylococcus aureus* was carried out by using the following biochemical tests: Gram staining, catalase test, coagulase test and detection of hemolysis.

Determination of *Listeria* species

Samples were analyzed according to Food and Drug Administration, Bacteriological Analytical Manual Standard (FAD-BAM) for the detection of *Listeria* (Hitchins, 2003).

A Sample of 25 g was added to 225 ml of half strength Fraser broth which was used as primary enrichment media, to obtain a 1:10 sample dilution. All samples were homogenized 30- 60 seconds and incubated at 30°C for 24 h. From this primary enrichment, 0.1 ml was then inoculated into 10 mL of Fraser Broth which had been used as a secondary enrichment medium and incubated for 48 h at 37°C. A loopful of the Fraser Broth enrichment culture was streaked on the surface of OXFORD agar with supplement then incubated for up to 48 h at 37°C. Selective agars were observed for suspected colonies at 24 to 48 h of incubation. Suspected colonies were those that appeared greyish colonies surrounded by black halos and sunken centre with possible greenish sheen on Oxford agar.

Listeria species was identified using *Listeria* Latex Agglutination Kit. Done in the Faculty of Vet. Med. Benha univ. The Oxoid *Listeria* Test Kit (Oxoid, Basingstoke, Hampshire, England) is a rapid latex agglutination test for the presumptive identification of *Listeria* species in selective and/or enrichment cultures.

Escherichia coli

Isolation and identification (Acumedia, 2011)

After samples were inoculated into buffered peptone water, the homogenate was incubated at 37±1°C for 18 h. By using sterile loop, the pre-enrichment broth culture was cultivated into Eosin methylene blue agar plates. The inoculated plates were incubated at 37°C for 24 h. Convincing evidence for the presence of

Escherichia coli was given by the appearance of dark blue–black colonies with metallic green sheen on Eosin methylene blue agar Serodiagnosis of *E. coli* (Kok *et al*., 1996)

The isolates were serologically identified in the Faculty of Vet. Med., Benha Univ. The isolates were serologically identified according to Kok *et al*. (1996) by using rapid diagnostic *E. coli* antisera sets (Denka Seiken Co., Japan) for diagnosis of the Enteropathogenic types.

Determination for *Salmonella*e

Using the selective solid media, Xylose Lysine Deoxycholate ager (XLD agar), *Salmonella* colonies have a black center and lightly transparent zone of reddish color.

Serological identification of *Salmonella*e was carried out according to Kauffman-White scheme (Kauffman, 1974) for the determination of Somatic (O) and flagellar (H) antigens using *Salmonella* antiserum (DENKA SEIKEN Co., Japan). Done in the Faculty of Vet. Med. Benha Univ.

RESULTS AND DISCUSSION

Chemical indices as pH, TVN, TMA and TBA are the most used parameters to evaluate fish quality and freshness. In this research, two types of farmed fish (*T. niloticus* and *M. cephalus*) were examined to determine their keeping quality and safety for human consumption. pH values are suitable index for freshness assessment and useful guideline for quality of fish (Ruiz-Capillas and Moral, 2001). Egyptian Organization for Standardization (ES.3439:2005) had reported the critical limits of pH in chilled fish not to be more than 6.5. Accurately 100% of samples were accepted in *T. niloticus* and *M. cephalus* as recorded in Table 1. These results were nearly closed to those of (Ibrahim, 2017). TVN levels are affected by the method of catch, postmortem treatment, and storage temperature. It differs according to fish species (Nazemroaya *et al*., 2011). According to ES 3439:2005 TVN permissible limit is 30 mg % so all the examined samples were accepted.

TMA is a good index of quality for many fish species (Baixas-Nogueras *et al*., 2002). TMA changes according to species and it is affected by time of storage and catching time. According to ES 3439:2005 the permissible limit of TMA is 10mg%. So, the examined samples were accepted. TBA factor is responsible for a rancid flavor, off odor, colors and texture deterioration. The permissible limit of TBA is 4.5 mg/kg as recorded in ES 3439:2005. All the examined samples were within the acceptable limits.

Foodborne microbes such as *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes* contaminate the food production systems or products from these systems (Nurudeen *et al*., 2014; Ibrahim *et al*., 2014).

Aerobic plate count (APC) of fish generally related to food safety hazards also can be useful to indicate quality and shelf life. Total aerobic plate count shouldn't exceed 6 (log_{10} CFU/g) according to ES 3439:2005.The data recorded in Table 2 declared that the mean values of APC were 5.27±0.15 and 5.98±0.05, 5.72±0.08 and 5.66±0.09, 5.58±0.07 and 5.71±0.09 for *Tilapia nilotica* and *Mugil cephalus* in Nile cages ,concrete ponds and Earthen ponds respectively and the percentage of the exceeded permissible limits of (APC) was 12% and 32%, 20% and 16%, 12% and 20% for *Tilapia nilotica* and *Mugil cephalus* in Nile cages ,concrete ponds and Earthen ponds respectively. APC in fish

Table 1. keeping quality parameters of examined farm fish samples.

Keeping quality indices		Nile cages		Concrete ponds		Earthen ponds	
		Tilapia	Mugil	Tilapia	Mugil	Tilapia	Mugil
pH	Min	5.59	5.49	6.01	5.16	6.01	5.16
	Max	6.36	6.23	6.27	6.19	6.27	6.19
	Mean ±SE	6.12±0.03	6.05 ±0.03	6.14±0.01	6.01±0.04	6.14±0.01	6.01±0.04
TVN (mg %)	Min	2.09	2.41	2.91	2.81	2.91	2.81
	Max	8.18	6.75	8.38	5.65	8.38	5.65
	Mean ±SE	5.17±0.3	4.33±0.23	5.51±0.32	4.01±0.18	5.50±0.32	4.02±0.18
TMA (mg %)	Min	0.73	0.72	0.89	0.72	0.89	0.72
	Max	3.68	2.96	4	2.81	4	2.81
	Mean ±SE	2.13±0.15	1.40±0.10	2.18±0.18	1.52±0.10	2.18±0.18	1.53± 0.10
TBA (mg/K)	Min	0.59	0.4	0.61	0.33	0.61	0.33
	Max	2.41	1.83	2.41	1.5	2.41	1.5
	Mean ±SE	1.41±0.14	1.06±0.06	1.53±0.10	0.97±0.06	1.54±0.10	0.96±0.07

All examined samples are within the permissible limits according to ES:3494 (2005)

Table 2. Total aerobic plate count (log_{10} CFU/g) in examined farm fish samples (25 for each).

Sample		MIN.	MAX.	Mean ± SE	Samples exceed P.L.	
					No.	%
Nile Cages	Tilapia	3.4	6.4	5.27 ± 0.15	3	12
	Mugil	5.56	6.54	5.98 ± 0.05	8	32
Concrete ponds	Tilapia	5	6.36	5.72±0.08	5	20
	Mugil	4.7	6.6	5.66±0.09	4	16
Earthen ponds	Tilapia	5.04	6.25	5.58±0.07	3	12
	Mugil	4.48	6.4	5.71± 0.09	5	20

Total aerobic plate count shouldn't exceed 6 (log_{10}CFU/g) according to ES:3494:2005

from farm type (concrete and earthen ponds) had significantly higher counts than in natural channels type (Nile cages) fish. This may be due to that fish farmers use organic fertilizers to increase the fertility of pond and production of natural food for fish and use agriculture run off water that both factors can increase the bacterial load of fish (Ampofo and Clerk, 2002). *Mugil cephalus* had higher bacterial count. This is due to its omnivorous bottom feeder feeding habit where the fish greases the pond bottom detritus and organic matter which are usually of high bacterial count (Lartseva *et al*.,1996; Abdelhamid *et al*., 2007).

Eltholth *et al*. (2018) recorded that the mean APC for more than 85% of samples was within the permissible limits as in ES (3494:2005). The contamination of tilapia may occur pre and post harvesting and during handling the fish (Eltholth *et al*., 2015). Methods of harvesting and handling of tilapia from the farm to market could contribute to the bacterial load of tilapia as well as the lack of a prober cold chain. Fish could be contaminated post-capture from fishing tools or contaminated water and contaminated ice beside soiled surfaces and boxes, as well as by poor hygienic handling practices (Mhango *et al*., 2010).

In Table 3, total coliform count of all positive examined samples exceeds permissible limits of coliform count (2 $\log_{10}$ CFU/g) according to ES (3494:2005) as the mean value of total coliform count in positive fish was 3.60±0.09 and 3.73±0.07, 4.09±0.10 and 3.92±0.07, 3.61±0.06 and 3.80±0.07 of *Tilapia nilotica* and *Mugil cephalus* in Nile cages, concrete ponds and Earthen ponds respectively. Lower results recorded by Budiati *et al*. (2015) as the coliform count ranged from 1.6 to 4.04 log MPN gr^{-1} for tilapia. Fecal coliform bacteria are used as the fecal indicator in guidelines for wastewater reuse in irrigation and aquaculture. Falcao *et al*. (2002) explained that this is because of the ice used to refrigerating seafood may be contaminated with coliform bacteria which cause human infection, as they discovered the presence of high numbers of coliforms and pathogenic strains in ice used for chilling fish; therefore, some of the contamination detected in this study could be due to the ice used for chilling purposes.

Staphylococcus aureus are known as enteriotoxic producing microorganism which is poisonous. The incidence of *Staphylococcus aureus* in Table 4, was 0% and11%, 36% and 32% , 16% and 12% for *Tilapia nilotica* and *Mugil cephalus* in Nile cages ,concrete ponds and Earthen ponds respectively. These results are higher than results reported by Hardi *et al*. (2018) which were 24.32% and lower than results reported by Maysoon (2014) as the percentage was 40%. All positive examined samples exceed permissible limits of *Staphylococcus aureus* count (3 $\log_{10}$ CFU/g) according to ES, 3439:2005. This may be due to hand contamination of fish handlers during catching, sorting and selling which in turn contaminates fish and the water and ice used for their preparation for selling (Saad *et al*., 2015; Edris *et al*., 2017). There are four common pathogens have recorded as being significant

Table 3. Total coliform count ($\log_{10}$ CFU/g) in positive examined farm fish samples (25 for each).

Sample		Positive samples		MIN.	MAX.	Mean ± SE
		No.	%			
Nile Cages	Tilapia	21	84	3	4.48	3.60 ± 0.0.09
	Mugil	17	68	3	4.23	3.73 ±0.07
Concrete ponds	Tilapia	21	84	3.3	4.97	4.09±0.10
	Mugil	22	88	3.3	4.39	3.92±0.07
Earthen ponds	Tilapia	17	68	3	4.15	3.61±0.06
	Mugil	14	56	3	4.2	3.80±0.07

All positive examined samples exceed permissible limits of coliform count (2 log10 CFU/g) according to ES:3494(2005)

Table 4. *Staphylococcus aureus* count (log10 CFU/g) in positive examined farm fish samples (25 for each).

Sample		Positive samples		MIN.	MAX.	Mean ± SE
		No.	%			
Nile Cages	Tilapia	0	0	---	-	-
	Mugil	11	44	3.04	3.95	3.53± 0.06
Concrete ponds	Tilapia	9	36	3.04	4.36	3.53±0.13
	Mugil	8	32	3.6	3.95	3.78±0.05
Earthen ponds	Tilapia	4	16	3.04	4.3	3.61±0.29
	Mugil	3	12	3.04	3.48	3.16± 0.14

All positive examined samples exceed permissible limits of *Staphylococcus aureus* count (3 log10 CFU/g) according to ES:3494(2005)

Table 5. Incidence ratio of food-borne pathogens in the examined farm fish samples (n=25 for each)

Sample types	Bacterial types	*S. aureus*		*Listeria monocytogenes*		*E. coli*		*Salmonella* Serotypes	
		No.	%	No.	%	No.	%	No.	%
Nile cages	Tilapia	0	0	5	20	6	24	1	4
	Mugil	11	44	2	8	5	20	1	4
Concrete ponds	Tilapia	9	36	2	8	2	8	3	12
	Mugil	8	32	4	16	4	16	3	12
Earthen ponds	Tilapia	4	16	5	20	4	16	1	4
	Mugil	3	12	0	0	5	20	3	12

Accepted fish meat should be free from *Salmonella* spp. and *Listeria monocytogenes* according to ES:3494(2005)

in terms of human health and disease. These include *Listeria monocytogenes*, Vibrio parahaemolyticus, *Staphylococcus aureus*, and *Salmonella* spp. (Feldhusen, 2000).

Table 5 shows incidence ratio of some food-borne pathogens in the examined farm fish as following: *Listeria monocytogenes* were detected in 20% and 8%, 8% and 16%, 20% and 0% for *Tilapia nilotica* and *Mugil cephalus* in Nile cages, concrete ponds, and Earthen ponds respectively. Lower findings were 7.5% of *Listeria* spp. and 1.9% *L. monocytogenes* from fresh fish samples by Ebrahim *et al*. (2012). But higher results were reported by Soultos *et al*. (2007) who could isolate 44.5% *L. monocytogenes* from fresh samples. Edris *et al*. (2017) couldn't detect *L. monocytogenes* in their study while *L. monocytogenes* contamination rate in fresh fish was 4.1% by Wang *et al*. (2011). According to ES,3494 :2005 fish meat should free from *L. monocytogenes*.

L. monocytogenes has been isolated from fish and seafood products all over the world. Contamination with *L. monocytogenes* depends on factors as cleaning and processing procedures, working habits, and the existence of surface persistent *L. monocytogenes* types in processing facilities. Also, raw materials contaminated with *Listeria* may be a reason for the contamination of the final product (Miettinen and Wirtanen, 2005). *L. monocytogenes*is most associated with disease in both animals and humans. Pregnant women, neonates, elderly, or immune-compromised people are the most susceptible to *Listeria* manifestations as abortion, stillbirth, septicemia, meningitis, and meningoencephalitis (WHO, 2004). Serological identification of *Listeria* spp. isolated from examined farm fish samples present in Table 6. *L. innocua*, *L. grayi*, *L. welshimeri*, *L. seeligeri*and *L. ivanovii* also present in the examined samples.

Escherichia coli was 24% and 20%, 8% and 16%, 16% and 20% for *Tilapia nilotica* and *Mugil cephalus* in Nile cages, concrete ponds and Earthen ponds respectively, as in Table 5. Razavilar *et al*. (2013) and Mamdouh *et al*. (2022) recorded the incidence of

Table 6. Serological identification of *Listeria* spp. isolated from examined farm fish samples.

Listeria serotypes	Nile Cages (n=8)		Concrete ponds (n=5)		Earthen ponds (n=2)		Latex Agglutinating Kit
	No.	%	No.	%	No.	%	
L. monocytogenes	2	8	1	4	-	-	+ve agglutination clumps
L. innocua	2	8	2	8	3	12	+ve agglutination clumps
L. grayi	-	-	-	-	1	4	+ve agglutination clumps
L. welshimeri	2	8	2	8	-	-	+ve agglutination clumps
L. seeligeri	-	-	-	-	-	-	+ve agglutination clumps
L. ivanovii	-	-	1	4	1	4	+ve agglutination clumps
Total	6	24	6	24	5	20	

Table 7. Serological identification of *E. coli* isolated from examined farm fish samples.

E. coli strains	Nile cages (n=11)		Concrete ponds (n=6)		Earthen ponds (n=9)		Strain Characteristics
	No.	%	No.	%	No.	%	
O127:H6	4	16	-	-	-	-	ETEC
O91:H21	1	4	1	4	2	8	EHEC
O26: H11	1	4	1	4	2	8	EHEC
O15	2	8	1	4	-	-	EPEC
O163:H2	1	4	-	-	1	4	EPEC
O44:H18	2	8	-	-	1	4	EPEC
O103:H2	-	-	2	8	2	8	EHEC
O124	-	-	1	4	-	-	EIEC
O153:H2	-	-	–	–	1	4	EPEC
Total	11	44	6	24	9	36	

Table 8. Serological identification of *Salmonella*e isolated from farm fish samples.

Salmonella serotypes	Nile cages (n=2)		Concrete ponds (n=6)		Earthen ponds (n=4)		Group	Antigenic Structure	
	No.	%	No.	%	No.	%		O	H
S. Typhimurium	1	4	3	12	-	-	B	1,4,5,12	i: 1,2
S. Enteritidis	-	-	2	8	1	4	D	1,9,12	g, m: -
S. Tsevie	1	4	-	-	-	-	B	1,4,5,12	i: 1,2
S. Heidelberg	-	-	-	-	1	4	B	4,5,12	r: 1,2
S. Infantis	-	-	-	-	1	4			
S. Takorodi	--	-	-	-	1	4			
S. Inganda		-	1	4	-	-			
Total	2	8	6	24	4	16			

E. coli was 36.6% and 26.6% in Tilapia and Mugil. Lower results obtained by Egbere *et al.* (2010).

E. coli isolates from the examined fish samples in Table 7 were16% O127, 4% for O91, O26 and O163 and 8% O44 and O15 in Nile cages reared fish. *E. coli* isolates from concrete pond reared fish were one isolate for O91, O26, O15 and O124 and two isolates for O103. While in earthen pond reared fish the isolates were 8% for O91, O26 and O103 and 4% for O136, O44 and O153, nearly similar serotypes O121:H7, O44:H18 and O158:H2 obtained from fish by Barbosa *et al.* (2014).

Koo *et al.* (2012) reported having isolated ETEC strain from farm fish sold in South Korea. *Escherichia coli* has been involved for several gastroenteric diseases such as diarrhea (traveler's disease), vomiting, dysentery, fever, colitis, hemolytic uremic syndrome with renal failure (Egbere *et al*., 2010).

Salmonella species were 4% and 12% for both from Nile cages and from concrete ponds and 4% and 12% in earthen pond *Tilapia nilotica* and *Mugil cephalus* respectively as in Table 5. *Salmonella* spp. was detected in 10% of seafood samples collected from markets in Alexandria by Bakr *et al.* (2011), While Hassan *et al.* (2021) recorded 33.3% in *T. niloticus* and 16.6% in *Mugil cephalus*. The presence of *Salmonella* spp. indicates fecal contamination of water from which the fishes were harvested. *Salmonella* spp. were reported in raw retail frozen imported fresh-water tilapia to eastern province of Saudi Arabia 64% (16/25) from Thailand, and 28% (14/50) from India (Ribeiro *et al*., 2016). Similar results to this study were revealed with fresh tilapia fish in Sokoto, Nigeria. The detected rate of *Salmonella* in fish meal was 8%. *Salmonella* in naturally contaminated feeds ranged from 3 to 21 *Salmonella*/100 g by Starkey (2013).

The use of wastewater for fish culture or the practice of fertilizing ponds with animal manure may result in products that harbor pathogenic bacteria. Aquatic birds are known to harbour pathogenic strains of *V. cholera* and *Salmonella* and are a possible source of these organisms in fish farms, that are very difficult to control (Ogg *et al*., 1989 and Fenlon, 1983).

The results of the microbiological evaluation in this study were better than that from previous studies by Emire and Gebremariam (2010) and Hamed *et al.* (2013).

Table 8 showed the serotyping of *Salmonella* which showed *S.* Typhimurium group B in 4%, 12% and zero in Nile cages, concrete ponds, and earthen pond reared fish respectively. *S.* Enteritidis group D present in 0 %, 8% and 4% in Nile cages, concrete ponds and earthen pond reared fish respectively.

CONCLUSION

Hygienic and proper practices should be performed during transportation and handling of fish. Adequate cleaning and sanitization of utensils, effective training for workers on hygiene and safety, application of hygienic measures during handling of fish are required to control the growth of microorganisms.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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