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Bacterial evaluation of the quality of farmed fish in Kafr El- Sheikh city in Egypt

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ABSTRACT

In the current study, a total 90 samples were collected random way from three different fish species (*Ttilapia niloticus, Mugil cephalus* and *Clarias)* 30 of each type collected from Kafr El-Sheikh city, Egypt. These samples were subjected for bacteriological examination to evaluate the quality and freshness in farmed fish. The mean value of *total bacterial count / g of Tilapia* niloticus, Mugil cephalus and *Clarias gariepinus* were $6.73\times10^4 \pm 2.03 \times10^4$, $8.33\times10^3\pm2.32\times 10^3$ and $4.1\times10^3 \pm 7.69\times10^2$ cfu/g, respectively. The mean values of coliforms count / g were $3.25\times10^2 \pm 8.04\times10$, $2.1\times10^2 \pm 6.74\times10$ and $1.06\times10^2 \pm 5.0\times10$ MPN/g, respectively. The mean values of staphylococcal count /g were $2.44\times10^5 \pm7.13\times10^4$, $8.67\times10^3 \pm2.23\times10^3$ and $4.74\times10^4 \pm 7.48\times10^3$ cfu/g respectively. The incidence of *S. aureus* was 33.3%, 30% and 46% respectively, *E. coli was* 36.6%,26.6% and 43.33%, salmonella was 33.3%,16.*6% and 36.3%, Aeromonas hydrophila was 80%,76.6%* and 90*% and Pseudomonas aeruginosa was 43.3%, 80% and 76.6%.*

1. INTRODUCTION

Egypt is the largest producer of farmed tilapia and the second in the world (FAO, 2019). Fish and its products are a very important source of protein and essential micronutrients for a good- balanced diet and perfect health (Arni, 2012). It possesses the biggest African aquaculture industry, which produced for 75.46% of the country's total fish production (GAFRD, 2013).

Clarias gariepinus is one of the famous extensively distributed fish species in Africa, and also it is a one of the most cultivated fish spp. This is because of its good-quality flesh, high level of water acceptance, production and high marketing value (Adeshina et al., 2016).

Fish is a highly sensitive food that needs careful storage and handling. The way to keep the quality of fish is to preserve it fresh until it's time to cook and eat it. Farm fish is therefore a better option for, nutritious, fresh, non-preserved fish, and higher nutritional content (Bremner, 2003) .

Staphylococcal enterotoxins are one of the most famous food poisonings all over the world. They considered 2nd or 3rd most prevalent microbiological food intoxication (Atnassova et al., 2001) .

When highly pathogenic organism as salmonella, E. coli and potentially pathogenic organisms such as citrobacter Spp. are isolated from fish or fish products, it indicates fecal and environmental pollution of the fish environmental pollution (Wogu. and Maduakol, 2010).

Salmonella is represented the most significant contaminant of the skin, muscle and gills (Joseph and George, 2010) .

Aeromonas hydrophila infection in fish causes a condition known as (motile Aeromonas septicemia) that causes death in farmed and wild fresh water and also fish species of marine water causing economic destroy to the aquaculture industry. Aeromonas spp can cause disease in humans and are mostly isolated from freshwater and healthy or diseased fish (Hidalgo and Figueras, 2013).

Pseudomonas species are commonly found in nature water sources and have been linked with septicemia in mainly aquatic animals. These bacteria are pathogenic, which cause disease when the host under stress (Soontornvit., 2002) .

Bacterial diseases of fish in fresh water are caused mainly by Aeromonas, pseudomonas and salmonella. Aeromonas hydrophila and Pseudomonas fluorescens are the highly widespread fish pathogens (Austin and Austin, 2012).

The high levels of fish diseases based on some factors as natural conditions, quality of pond water and number of pathogenic bacteria (Moore et al., 2014).

Foodborne diseases represented one of the most worldwide health problems and high cases of illness. High number of reports indicated that pathogenic E. coli and salmonella species were considered the most significant pathogens (White et al., 2002). As well as Pao et al. (2008) considered salmonellae species and E. coli leading to food poisoning with severe diarrhea and gastroenteritis in infants and adults as similar.

Staphylococcus aureus is living in nasal membranes and the skin with considerable pathogenic which cause a number of infections appeared in the environment and hospital (Oliveira et al., 2018) .

Escherichia coli are the most famous facultative anaerobic species found in human and animal gastrointestinal tracts and mainly found in the Enterobacteriaceae family, so the presence of such organisms in foods indicates fecal contamination (Mohamed., 2014) .

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Pathogenic E. coli divided into 2 main categories: extra intestinal pathogenic E. coli and diarrheagenic E. coli. There are currently six categories of diarrheagenic E. coli, including enteropathogenic E. coli (EPEC), Enterotoxigenic E. coli (ETEC), Entero-invasive E. coli (EIEC), Entero-aggregate E. coli (EAEC), E. coli (DAEC) and E. coli (EHEC) (Xiaodong, 2010).

So, this study aimed to investigate and evaluate the hygienic status of farmed fish bacteriologically in Kafr El sheikh city.

2. MATERIAL AND METHODS

2.1. Collection of samples:

About 90 samples of farm fish (Tilapia, Mugil and Clarias) represented by (30 of each) were collected randomly in Kafr-Elsheikh governorate.

2.2.Preparation of samples for microbiological examination (ISO, 2007):

Ten grams were taken aseptically from examined samples and put into a sterile homogenizer flask contain 90 ml of sterile peptone water (0.1%) and homogenized at 14000 r.p.m. for 2.5 minutes to provide a homogenate of 1/10 dilution. One ml of this homogenate was transferred by a sterile pipette to another test tube contain 9 ml of sterile peptone water (0.1%) to give 10^{-2}, from which tenth fold serial dilutions were prepared up to 10^{-6}.

2.3. Bacteriological examination:

2.3.1. Determination of Aerobic plate count (APHA, 2001).

2.3.2. Determination of Coliforms Count by Most Probable Number (MPN) (ISO, 2007):

2.3.3. Determination of Staphylococcal count (ISO, 2007):

2.3.4. Isolation and identification of Staphylococcus aureus according to (ISO, 2007):

2.3.5. Isolation of Escherichia coli (FDA, 1998):

A loopful from the positive tubes of MacConkey broth was streaked onto MacConkey agar plates and incubated at 35°C for 24 hrs. Following incubation, lactose - positive colonies (pink colonies) (3–5) were streaked onto eosin-methylene blue agar plates and incubated at 37^0 C for 24 hrs. Typical E. coli colonies on eosin-methylene blue agar (green shiny metallic with dark purple center) were picked up for further identification.

2.3.5.1. Serodiagnosis of E. coli:

The isolates of fish samples were serologically identified depending on Kok et al. (1996) by using rapid diagnostic E. coli antisera sets (DENKA SEIKEN Co., Japan) for Enteropathogenic type's diagnosis.

2.3.6. Isolation and identification of salmonellae: ISO: 6579(2002):

2.3.7. Isolation and identification of Aeromonas species:

From the prepared homogenate, 1 ml was transferred into a sterile test tube containing 9 ml of brain heart infusion broth (BHI) as an enrichment broth then incubated at 28°C for 24 hours. After incubation, a loopful from the enrichment broth was streaked onto Aeromonas Agar medium and incubated aerobically at 37°c for 18-24 hours according to Austin (1999). Suspected colonies (Translucent, pale green colonies 0.5-3.0 mm diameter) should be confirmed as presumptive Aeromonas species by performing the further identification.

2.3.8. Isolation and identification of pseudomonas species according to Mcfaddin (2000) as follows:

1 ml of original homogenate was inoculated onto tryptic soya broth incubating at 37 °C for 24 hours then steaked onto pseudomonas agar. Suspected colonies appeared as yellow colony.

3. RESULTS

Table 1 Analysis of variance (ANOVA) of total bacterial, staphylococcal and coliform count transformed data for different fish types

Variables	Source of Variance	Degree of freedom	Sum of squares	Mean square	F	P value
Total bacterial	Between groups	2	4.893	2.446	5.259	.007**
	Within groups	87	40.467	.465		
Staphylococcal	Between groups	2	45.111	22.555	25.799	.000**
	Within groups	87	76.061	.874		
Coliform	Between groups	2	7.100	3.550	6.357	.003**
	Within groups	87	48.581	.558		

** indicate P< 0.01 and significant at 1% level.

Table 2 Total bacterial count for different fish types

fish	Min.	Max.	Mean ± SE
Tilapia niloticus	7×10^2	4.6×10^5	6.73×10^4 a ± 2.03×10^4
Mugil cephalus	1×10^3	5.52×10^4	8.33×10^3 ab ± 2.32×10^3
Clarias gariepinus	1.55×10^2	1.33×10^4	4.1×10^3 b ± 7.69×10^2

The means of each factor designated by the same letter are not significantly different at the 5% level using Duncan's MRT

Table 3 Staphylococcal count for different fish types

Fish	Min.	Max.	Mean ± SE
Tilapia niloticus	2.78×10^3	1.46×10^6	2.44×10^5 a ± 7.13×10^4
Mugil cephalus	0.0	5.25×10^4	8.67×10^3 b ± 2.23×10^3
Clarias gariepinus	2.25×10^3	1.18×10^5	4.74×10^4 a ± 7.48×10^3

The means of each factor designated by the same letter are not significantly different at the 5% level using Duncan's MRT.

Table 4 Coliform count for different fish types

fish	Min.	Max.	Mean ± SE
Tilapia niloticus	3.6	1.1×10^3	3.25×10^2 a ± 8.04×10
Mugil cephalus	3.0	1.1×10^3	2.1×10^2 ab ± 6.74×10
Clarias gariepinus	3×10	1.1×10^3	1.06×10^2 b ± 5.00×10

The means of each factor designated by the same letter are not significantly different at the 5% level using Duncan's MRT

Table 5 Incidences of Staphylococcal aureus in examined fish samples (n=30)

Fish types	No of positive sample	%
Tilapia niloticus	10	33.3
Mugil cephalus	9	30
Clarias gariepinus	14	46

Table 6 Incidences of E. coli in examined fish samples (n=30)

	No of positive sample	%
Tilapia niloticus	11	36.6
Mugil cephalus	8	26.6
Clarias gariepinus	13	43.33

Table 7 Serotyping of isolated of E. coli in examined fish samples (n=30)

Fish types	Sero vars	NO	%	Strain characterization
Tilapia niloticus	086a	5	16.6	ELEC
	0125	4	13.3	EPEC
	Untyped	2	6.66	-
Mugil cephalus	0125	3	10	EPEC
	086a	2	6.6	ELEC
	0128	3	10	EPEC
Clarias gariepinus	0125	6	20	EPEC
	086a	3	10	ELEC
	055	1	3.3	EPEC
	untyped	1	3.3	-

Table 8 Incidences of salmonella in examined fish samples (n=30)

Fish types	No of positive sample	%
Tilapia niloticus	10	33.3
Mugil cephalus	5	16.6
Clarias gariepinus	11	36.3

The % according to n=30

Table 9 Serotyping of isolated of Salmonella in examined fish samples (n=30)

Salmonella strain	*T. niloticus*		*M. cephalus*		*C. gariepinus*		Antigenic structure	
	NO	%	NO	%	NO	%	O	H
*S.*Typhimurium group B	5	16.6	3	10	6	20	1,4,5.12	1:1,2
S. Bouake O:16 (I)	4	13.3	2	6.66	10	10	16	Z: Z_6
S. Enteritidis Group D1	1	3.33	-	-	2	6.66	1, 9, 12	9, m:1.7

Table 10 Incidences of Aeromonas hydrophila in examined fish samples (n=30)

Fish types	No of positive sample	%
Tilapia niloticus	24	80
Mugil cephalus	23	76.6
Clarias gariepinus	27	90

Table 11 Incidences of Pseudomonas aeruginosa in examined fish samples (n=30)

Fish types	No of positive sample	%
Tilapia niloticus	13	43.3
Mugil cephalus	24	80
Clarias gariepinus	23	76.6

Table 12 Serotyping of isolated of Pseudomonas aeruginosa in examined fish samples (n=30)

Pseudomonas strains	*T. niloticus*		*M. cephalus*		*C. gariepinus*	
	NO	%	NO	%	NO	%
P. polyvalent 111 Group (N)	7	23.3	10	3.33	9	30
P. polyvalent 111 Group (E)	4	13.3	9	30	9	30
P. polyvalent 111 Group (D)	2	6.6	5	16.6	5	16.6

4. DISCUSSION

The total bacterial count is used as an important index for the level of sanitation and hygienic quality of meat.

The bacteriological quality of fish meat is an indicator of the hygienic status of the rearing environment, handling and storage of caught fish. Gram and Huss (2000) reported that high coliforms count in the examined samples an indicative for massive contamination with deteriorative bacteria, which mostly lead to flavor deterioration in the fish.

The results demonstrated in Table (2) the mean value of Total bacterial count */ g of Tilapia, Mugil and Clarias* were $6.73\times10^4 \pm 2.03 \times10^4$, $8.33\times10^3\pm2.32\times 10^3$ and $4.1\times10^3 \pm 7.69\times10^2$ cfu/g, respectively. The mean values of staphylococci count / g were $2.44\times10^5 \pm7.13\times10^4$, $8.67\times10^3 \pm2.23\times10^3$ and $4.74\times10^4 \pm 7.48\times10^3$ cfu/g, respectively (Table, 3). Table (4) showed the mean values of coliforms count / g. They were $3.25\times10^2 \pm 8.04\times10$, $2.1\times10^2 \pm 6.74\times10$ and $1.06\times10^2 \pm 5.0\times10$ MPN/g, respectively. These results were higher than those recorded by Mahmoud (2001), who recorded that the mean aerobic plate count in (catfish) was $3.5x10^6$ and El-Shabasy, (2009), who recorded that the mean MPNn (coliforms count) of examined samples was $6.4x10^4$ cfu/g. In addition, the results were higher *S. aureus* than those recorded by Danba *et al.* (2014), and Budiati *et al.* (2015).

Staphylococcus species are serious bacteria in public health due to the severity of some infections they cause. Even when they were noticed at a very low frequency, their presence makes necessary to maintain microbiological quality investigation in tilapia culture and in general, in aquaculture (Allen *et al.,* 2004). It is a true food poisoning organism as it produces heat stable Staphylococcal enterotoxins (SEs) when allowed to grow in foods. Even the food is heated before eating; the poison in the food will cause illness although the heat has destroyed the bacterial cells (Soriano *et al.,* 2012). Staphylococcal food poisoning is associated with vomiting, nausea, diarrhea and abdominal cramps which are the most common symptoms appear 3- 8 hrs after ingestion (Pinchuk *et al.,* 2010).

The incidence of *S. aureus* was 33.3% in (*T. niloticus*) as shown in Table (5). This result was higher than those reported by Hardi *et al. (*2018) 24.32%. However, it was lower than (40%) as reported by Maysoon (2014). Staphylococcus aureus was isolated from 46% of the examined Catfish *(Clarias gariepinus)* samples. This result was higher than that reported by Danba *et a*l. (2014).

Escherichia coli has been involved for a number of gastroenteric diseases such as diarrhea (traveler's disease), vomiting, dysentery, fever, colitis, hemolytic uremic syndrome with renal failure *(Egbere et al., 2010).*

The incidence *E. coli* was 36.6%, 26.6% and 43.44% in *Tilapia, Mugil and Clarias,* respectively. The serotypes *of E. coli isolates from the examined fish samples were O86a, O55, and O128and O125 (*Tables, 6 & 7*).*

The result agreed with that reported by *Hassan et al. (2012); E. coli represented 33.3% of the examined Catfish (Clarias gariepinus) and lower than that of Egbebi et al,* (2016) and similar to Razavilar *et al. (*2013) in *(T. niloticus*).

Salmonella Species are the main cause of enteric diseases in animal and human with millions of sicknesses worldwide, whereas the non-typhoidal Salmonella species as a zoonotic agent is also principally associated with food borne diseases (Van *et al*., 2012). The main sources of *Salmonella* are soil, water, insects, animal feces, and surfaces of equipment, food factories and surfaces of utensil (Silva *et al.,* 2007). It causes salmonellosis which in humans could result in severe typhoid fever (enteric fever) or salmonella fever (Egbere et al., 2010). Table (8) showed the incidence of salmonella in fish types which showed 33.3% in Tilapia, 16.6% in Mugil and 36.3% in Clarias. While Table (9) showed serotyping of salmonella which showed S. *Typhimurium* group B in Tilapia 16.6%, 10% in Mugil and 20% in clarias, S. Bouke 0:16 (I) 133.3% in Tilapia,6.66% in Mugil and 10% in clarias while *S. Enteritidis* group (D1) 3.33% in Tilapia, 0% in Mugil and 6.66% in clarias.

Gastrointestinal tract infection is the mainly cause of Aeromonads followed with wound infections. In immune-suppressed persons or those with hepatobiliary disease, aeromonads can cause otitis media, meningitis, peritonitis, endocarditis, cholecystitis, hemolytic uremic syndrome, food poisoning and septicemia (Guerra *et al*., 2007). Aeromonas species was isolated from 80% of the examined *Tilapia* samples (Table, 10). Nearly similar result was reported by Escarpulli *et al., (*2003). Aeromonas species could be isolated from 90% of the examined catfish samples (Table10). Nearly similar to result was reported by Rahayu *et al.,* (2017) 95%. Lower result (43.8%) was reported by (Wamala *et al*. 2018).

Pseudomonas species is highly extent in natural sources of water and accompanying with septicemia in aquatic animals. These bacteria are opportunistic pathogens, causing disease when the host exposed to stress (Maysoon *et al.,* 2014). Pseudomonas species was isolated from 43.3% of the examined Tilapia samples (Table, 11). This result was lower than that reported by Maimona *et al.,* (2015) who observed 100% Pseudomonas spp. in all cultured samples. Higher incidence (30.83%) *Pseudomonas aeruginosa* was reported by Eissa *et al. (2010).* Pseudomonas spp. was isolated from 76.6% of the examined catfish samples (Table, 11). However, the current result was lower than that reported by Maimona et al. (2015) in all cultured samples (100%). Also, Kayıs *et al*. (2018) 99%. Lower incidence was recorded by (gbebi (2016). Contamination with enterotoxigenic pseudomonas has been testified from fish, food and drinking water resulting in diarrhea and skin infections in immune deficient persons (Wong *et al*., 2000).

5. CONCLUSION

The results in the present study revealed that high contamination levels of fresh catfish than other types can be

considered as risky factors which may affect human health especially due to detection of coliforms, *E. coli*, salmonellae, *Aeromonous hydrophila and Pseudomonas aeruginosa*. Also, it could be concluded that hygienic and proper safety, application of hygienic measures during handling of fish are required.

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practices should be performed during transportation and handling of fish. So, adequate cleaning and sanitization of utensils, effective training for workers on hygiene and

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