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Bacteriological evaluation of raw Catfish (*Clarias gariepinus*)

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ABSTRACT

Fish is a worldwide inexpensive source of protein especially in the developing countries like Egypt. This study aimed to bacteriologically evaluate fifty random samples of catfish collected from retail and supermarkets in Benha city, Qalubiya governorate, Egypt. Results revealed that the mean values of aerobic plate count (APC), total enterobacteriaceae, and total coliforms counts were 9.74×10^5, 3.25×10^3 and 2.35×10^3CFU/g, respectively. *Escherichia coli*, Salmonellae, and *Yarsenia enterocolitica* were detected in 18, 6, and 10% of examined samples, respectively, which went in further serological identification. This study indicated that the hygienic status of raw catfish is strongly related to source of collection, conditions of storage and handling, and recommended following proper hygienic properties of rearing, transportation, storage and handling

1. INTRODUCTION

African catfish (*C. gariepinus*) is generally considered to be one of the most important tropical catfish species for aquaculture in Africa (Clay, 1979), it is widely distributed throughout Africa, inhabiting tropical swamps, lakes, and rivers, some of which are subjected to seasonal drying (Olufemi *et al.*, 1991).

Fish is a highly perishable food, which needs proper handling and preservation if it is to have a long shelf life and retain a desirable quality and nutritional value. The most obvious method for preserving the quality of fish is to keep them alive until they are ready for cooking and eating. So, farm fish is better way to have fresh, healthy, non-preserved fish, and higher nutritional value (Bremner, 2003). On the other hand, retailed fish must be handled very carefully so they can be delivered to the next part of the marketing chain in a fresh and undamaged condition, and to allow consumer to have all the nutritional value of fish (Ananou *et al.*, 2007).

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 harvesting by workers, utensils and equipment during transportation, distribution and food preparation (National Academy of Science, 1985; El-Leboudi, 2002).

Although fish flesh is generally thought to be sterile immediately after catching (Kasing *et al.*, 1999), the hygienic quality of fish is often more difficult to control due to variations in species, sex, age, habitats and action of autolytic enzymes as well hydrolytic enzymes of microorganisms on the fish muscle (Venugopal, 2002), which may become contaminated with different microorganisms during subsequent handling (Sumner and Rose, 2002) as these microorganisms can penetrate from skin and the gut to the flesh (Samaha *et al.*, 2004).

Contamination of fish with organisms of public health significant remains primarily a problem of handling and processing (WHO, 1999). *Enterobacteriaceae* group has an epidemiological importance as some of its members are pathogenic and may cause serious infections and food poisoning to human. High prevalence of *Enterobacteriaceae* indicates unsatisfactory hygienic measures during catching and distribution of the fish (Pogorelova *et al.*, 1993; Valdivia *et al.*, 1997).

Foodborne diseases are still one of the major public health problems worldwide and account for considerably high cases of illness. Many reports indicated that *Salmonella* species and pathogenic *E. coli* were considered the most frequent pathogens (White *et al.*, 2002). In addition, Aziz and Dapgh (2005), and Pao *et al.* (2008) considered *Salmonellae spp.* and *E. coli* as Enterobacteriaceae members of a potential public health hazard; where it causes food poisoning associated with severe diarrhea and gastroenteritis in infants and adults as well.

Therefore, the aim of the present study was investigation and evaluation to the hygienic status of raw catfish sold in Benha city.

2. MATERIAL AND METHODS

2.1. Collection of samples

A total of 50 samples of African catfish were collected from different retails and markets located in Benha city, Qalubiya governorate, Egypt. Samples were transported to the laboratory under complete aseptic conditions in an ice box within one hour and examined for bacteriological detection of hygienic quality.

*2.2. Preparation of sample a*ccording to APHA (2013)

Twenty-five grams of muscle sample were mixed with 225 ml sterile 0.1% peptone water. The contents were

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homogenized at Stomacher (M A 106402, France, 450 to 640 strokes per minute) for 2 minutes and 1 ml of the mixture was transferred into separate tube each containing 9 ml sterile 0.1% peptone water, from which tenth-fold serial dilutions were prepared. The prepared samples were subjected to the following bacteriological examination:

2.3. Determination of Aerobic plate count (APC) according to ISO 4833-1: 2013.

2.4. Identification and enumeration of Enterobacteriaceae

Identification of family *Enterobacteriaceae* was conducted according to Cowan and Steel (1974) performed by Gram's stain, Biochemical tests, and motility tests.

For enumeration to the *Enterobacteriaceae* 0.1 ml from each of the previously prepared dilution was spread on Violet Red Bile Glucose agar (VRBG) and incubated at 37 °C for 24 hours. All purple colonies were then counted, and the average number of colonies was determined (ICMSF, 1996).

2.5. Determination of coliform count

1 ml from each of the previously prepared dilution was cultured in Violet Red Bile agar (VRBA) by pour-plate technique and incubated at 37 °C for 24 hours. All purple colonies were then counted, and the average number of colonies was determined (ISO 4832:2006).

2.6. Isolation and identification of E. coli:

2.6.1. Isolation of E. coli.

1 ml from each of the previously prepared dilution was cultured in Tryptone-Bile-Glucuronic Agar (TBX) by pour-plate technique and incubated at 44 °C for 24 hours. All bluish-green colonies were then counted, and the average number of colonies was determined (ISO 16649-2:2001).

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2.6.2. Identification of E. coli.

Gram's Stain according to (Cruickshank *et al.*, 1975), and Biochemical tests (MacFaddin, 2000).

2.6.3. Serological Identification of E. coli according to (Kok *et al.*, 1996).

2.7. Isolation and identification of Salmonellae

2.7.1. Isolation of salmonellae: According to (ISO 6579:2017), Pre-enrichment in non-selective buffered peptone water broth, which then incubated at 37±1 °C for 18 ± 2 hours.

Enrichment in Rappaport Vassilidis broth (RV broth), then the tube was incubated at 43°C for 24 hours.

Selective Plating on Xylose lysine Desoxy choclate (XLD) agar and Brilliant Green agar. The plates were incubated at 37 °C for 24 hours. Plates were examined for suspected Salmonella colonies which appeared as red with black centers on XLD agar and pink on Brilliant Green agar.

2.7.2. Identification of salmonellae

Gram's Stain according to Cruickshank et al. (1975), biochemical identification Krieg and Holt (1984), and Serological identification (Confirmatory test) according to Kauffman (1974).

2.8. Detection of Y. enterocolitica according to (ISO 10273:2017).

2.9. Statistical analysis

The obtained results were statistically analyzed according to Feldman *et al.* (2003).

3. RESULTS AND DISCUSSION

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

Results demonstrated in table (1) showed the incidences and counts of APC, *Enterobacteriaceae*, and coliform which were 100, 96, and 94%; with mean counts 9.74×10^5, 3.25×10^3, and 2.35×10^3 CFU/g, respectively, which indicating a high prevalence of *Enterobacteriaceae* and coliforms in the examined samples. The obtained results somewhat agreed with those reported by Mahmoud (2001), El-Shabasy (2009) and Mhango *et al.* (2010), who recorded that the counts of TEC, APC, and TCC were $2.3x10^3$, $1.4x10^5$, and $4.2x10^3$ cfu/g, respectively. While, results were lower than those recorded by Mahmoud (2001), who recorded that the mean APC was $3.5x10^6$, and El-Shabasy, (2009), who recorded that the mean TEC, and TCC in her examined samples were $1.48x10^4$, and $6.4x10^4$ cfu/g, respectively. Moreover, results were higher than those recorded by Danba *et al.* (2014), and Budiati *et al.* (2015) ($2.24x10^3$, and $2.8x10^2$ CFU/g for APC and TCC, respectively).

Table 1 Aerobic plate counts cfu/g in the examined samples of Catfish (n=50).

Hygienic parameter	Positive samples		Min.	Max.	Mean ±S.E.
	No.	%			
APC	50	100.0	4.9×10^5	1.86×10^6	9.74×10^5 ±0.42 $\times10^5$
Enterobacteriaceae	48	96.0	1.5×10^3	5.60×10^3	3.25×10^3±0.14$\times10^3$
Coliform	47	94.0	4.0×10^2	4.60×10^3	2.35×10^3±0.15×10

Bacteriological and biochemical identification of Enterobacteriaceae members (Table 2) revealed the detection of *Citerobacter diversus, Citrobacter freundii, Edwardsiella tarda, E. coli, Enterobacter aerogenes, Enterobacter cloacae, Klebsiella pneumonia, Klebsiella oxytoca, Proteus mirabilis, Proteus vulgaris, Salmonella* spp., *Y. enterocolitica* with incidences of 4, 4, 2, 18, 4, 6, 4, 8, 2, 2, 6, and 10%, respectively. Generally, 35 strains were isolated with total incidence of 70% from all examined samples. The results agreed with those recorded by Morshdy (1992), Mhango *et al.* (2010), and Hassan (2013), who detected *Citrobacter diversus, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Klebsiella pneumoniae, Proteus mirabilis,* and *Proteus vulgaris* in raw catfish samples.

The incidence of bacteriological detection of some pathogenic food poisoning bacteria and the incidence of hygienic acceptance of the examined samples were summarized in table (3). *E. coli*, *Salmonellae,* and *Yarsenia enterocolitica* were detected in 18, 6, and 10%, respectively.

In addition, 41(82%), 47(94%), and 45(90%) of the examined samples were accepted in relation to the number and incidences of microbial detection following EEC, 2005 specifications. These results partially agreed with those recorded by Hefnawy *et al.* (1989), Mekhael (2003) and Papadopoulou *et al.* (2007), who detected *Y. enterocolitica, Salmonella,* and *E. coli* in their examined samples at the incidence of 10.0, 6.2, and 14.0%, respectively. While, they were higher than those recorded by El-Adamy, (2002), who detected *E. coli*, *Y. enterocolitica,* in the examined samples by the incidence of 11.3, and 2.0%, respectively. While, Mahmoud (2001) could not detect *Salmonella* in the examined catfish samples. In addition, the present results were lower than those recorded by Ali (2017), who detected *E. coli*, *Salmonella,* and *Y. enterocolitica* at rate of 48.0, 32.0, and 16.0%, respectively, while they are totally disagreed with the results that recorded by Kasing *et al.* (1999), who could not detect any bacterial isolates in examined musculature samples.

Table 2 Prevalence of Enterobacteriaceae species in examined samples of Catfish (n=50).

Isolates	Incidences	
	No.	%
Citerobacter diversus	2	4.0
Citrobacter freundii	2	4.0
Edwardsiella tarda	1	2.0
E. coli	9	18.0
Enterobacter aerogenes	2	4.0
Enterobacter cloacae	3	6.0
Klebsiella pneumoniae	2	4.0
Klebsiella oxytoca	4	8.0
Proteus mirabilis	1	2.0
Proteus vulgaris	1	2.0
Salmonella spp.	3	6.0
Yersenia enterocolitica	5	10.0
Total	35	70.0

Table 3 Incidence and acceptability of some food poisoning bacteria in examined samples of catfish (n=50).

microorganism	Positive		Accepted samples**	
	No.	%*	No.	%*
E. coli	9	18.0	41	82.0
Salmonellae	3	6.0	47	94.0
Yarsenia enterocolitica	5	10.0	45	90.0

* Percentage was recorded according to total number of samples (50). **Accepted samples according to (EEC, 2005).

Serological identification of *E. coli,* and *Salmonella* isolates was presented in tables (4 and 5). The serological classification of isolated *E.coli* strains revealed detection of O_{27}:H_2, O_{63}:H_2, O_{158}:H_4, and O_{159}:H_7 strains with incidences of 8, 2, 6, and 2%, respectively. In addition, *Salmonella* isolates were serologically classified to *S. essen*, *S. saint paul*, and *S. enteritidis*. Results of the serological identification of isolated *E. coli* and *Salmonellae* are in line with the results that recorded by Hassan (2013) and Ibrahim, (2018), who identified *E.* coli and *Salmonella* as O_{153}, O_1, O_{125}, O_{78}; *S. enteritidis, S. typhimurium* and *S. haifa*, respectively. Variations between authors may be attributed to the differences in collection area, hygienic practices performed during catching, transportation, storage and handling; presence of such entero-pathogens proved that bacteria can migrate from the skin and the gut and infect musculature which renders it even of inferior quality or loss its safety for human consumption (Kasing *et al.*, 1999).

4. CONCULSION

The results of the present study revealed high contamination levels of fresh catfish can be considered as risky factors which may affect human health especially due to detection of coliforms, *E. coli*, *Salmonellae*, and *Yersinia enterocolitica.* Also, it could be concluded that hygienic and proper practices should be performed during transportation and handling of fish.

Table 4 Serotyping of *E. coli* isolated from examined samples of Catfish (n=50).

E. coli strains	Incidences		Strain characteristic
	No.	%*	
O27:H2	4	8.0	EPEC
O63:H2	1	2.0	ETEC
O158:H4	3	6.0	ETEC
O159:H7	1	2.0	EHEC
Total	9	18.0	-

* Percentage in relation to total number of each sample (50). EPEC: Enteropathogenic *E. coli*. ETEC: Enterotoxigenic *E. coli*. EHEC: Enterohaemorrhagic *E. coli*

Table 5 Serotyping of *Salmonellae* isolated from examined samples of Catfish (n=50).

Salmonella strains	Group	Antigenic structure	
		O	H
S. Essen	B	4,12	g, m:-
S. Saint Paul	B	1,4,5,12	e,h:1,2
S. Enteritidis	D_1	1,9,12	g, m:-

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CONFLICT OF INTEREST

The content of this report solely reflects the opinions of the authors, and we report no conflicts of interest

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