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Incidence of Enterobacteriaceae in some freshwater fishes

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ABSTRACT

It is evident that members of enterobacteriacae are one of the most incriminated bacterial food poisoning causes. Salmonella serovars and pathogenic *E. coli* may be responsible for more than 15% of reported annual food poisoning cases; and with increasing price of beef and poultry meats, and consumer's fish meat desire and demand. So, this study aimed to spot the light on the prevalence of enterobacteriacae members in some raw Nile fishes. 100 samples of *Oreochromis nilotica* (tilapia) and *Bagrus bayad* (Bayad), each of 50, were collected from different retail markets in Benha city, Qalubiya governorate, Egypt. Results revealed that 94% and 16% of examined samples were contaminated with enterobacteriacae and coliform bacteria, with mean counts 2.65×10^3 and 1.81×10^3 CFU/g for tilapia samples, and 2.07×10^3 and 1.74×10^3 CFU/g for bayad samples, respectively. Bacteriological classification of enterobacteriacae isolates revealed detection of *Citrobacter diversus, Citrobacter freundii, E. coli, Enterobacter aerogenes, Enterobacter cloacae, Klebsiella oxytoca, Proteus mirabilis, Proteus vulgaris, Yersinia enterocolitica* in variable percentages. 13% of examined samples were contaminated with *E. coli*, while 4% of examined tilapia samples only were contaminated with *Yersinia enterocolitica*. It is obvious that raw fishes may harbor bacterial contamination of significant health importance, so thorough cooking and processing hygienic practices are strongly recommended.

1. INTRODUCTION

Fish had long been regarded as a desirable and nutritional source of high-quality protein and generous supply of minerals and vitamins constituting the major part of human diet (Hastein *et al.*, 2006).

Tilapia nilotica and *Bagrus bayad* are two of the most widely distributed aquaculture commodities in Egypt (Sadek *et al.*, 2006). There has been a large increase in demand for tilapia and bayad, resulting in the increased production of both fishes through the method of high stocking density. However, the high stocking density method leads to rapid deterioration of water quality and increased fish stress (Sebastião *et al.*, 2015), resulting in bacterial invasion and proliferation in fish tissues.

Although, when a healthy fish is caught, the fish musculature is considered free of microbial contamination as its immune system prevents bacteria to proliferate easily whereas after death the immune system of fish collapses allowing easily penetration of microorganisms into the flesh (Kasing *et al.*, 1999). The penetration increase in case of fish caught from polluted area where there are high densities of bacteria (Sumner and Rose, 2002). Thus, many investigators convinced that fish from polluted environment may be passive carriers of pathogenic bacteria to man (Samaha *et al.*, 2004). In addition, contamination may be occurred during marketing at ground level or by hands (Sumner and Rose, 2002).

Enterobacteriaceae are a large, diverse heterogeneous group of rod-shaped Gram-negative bacilli that survive under aerobic conditions and normally inhabit the intestine of man and animals; some are motile while some others are not. The family includes many genera, some of which are part of the normal flora and incidentally cause diseases especially when given the opportunity. They are non-spore forming and some have capsules while others do not (Udeze *et al.*, 2012).

Enterobacteriaceae can be termed as common water-borne fish bacterial infections, which may normally in the tissues of apparently healthy fish, and the gastrointestinal tract of humans and animals (Newaj *et al.* 2008; Wogu and Maduakol, 2010), occurrence of fecal coliforms in fish was defined as a marker for the pollution level of the environment, they live in (CDC, 2013). Salmonella species and *E. coli* were the most repeatedly isolated (Sugumar *et al.*, 2008).

However, *Enterobacteriaceae* species including *Salmonella*, *E. coli* and *Yarsenia enterocolitica* are not typical of water or of aquatic products as reported by FAO (2003), Aziz and Dapgh (2005), Pao *et al.* (2008) exampled Salmonellae and

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E. coli as a microorganism act as a public health hazard if present where they cause food poisoning.
Therefore, the present study was conducted to investigate the presence of *Enterobacteriaceae* species in retailed *Tilapia nilotica* and *Bagrus bayad* fishes from Benha city markets.

2. MATERIAL AND METHODS

2.1. Collection of Samples:
A grand total of 100 random specimens of raw chilled freshwater fishes represented by *Tilapia nilotica* and *Bagrus bayad*, 50 of each, were collected randomly, along a year (March 2018 to February 2019), from retails and supermarkets in Benha city, Qalyubia governorate, Egypt. The collected samples were kept frozen and transported to the lab where subjected to bacteriological examinations to evaluate the incidence of enterobacteriacae contamination.

2.2. Preparation of samples according to (APHA, 2013)
Twenty-five grams of the sample were mixed with 225 ml sterile 0.1% peptone water. The contents were 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. Total Enterobacteriaceae count (ICMSF, 1996)
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 counted, and the average number of colonies was determined.
2.3.1. Identification of family Enterobacteriaceae
It was conducted according to Cowan and Steel (1974) performed by Gram's stain, Biochemical tests, and motility tests.

2.4. Determination of coliform count (ISO 4832, 2006).
One 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 counted, and the average number of colonies was determined.
2.5. Isolation and identification of E. coli
2.5.1. Isolation of E. coli according to (ISO 16649-2, 2001).
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 counted, and the average number of colonies was determined.
2.5.2. Identification of *E. coli.*
2.5.2.1. Gram's Stain according to (Cruickshank *et al.*, 1975).
2.5.2.2. Biochemical tests (MacFaddin, 2000).
2.5.2.3. Serological Identification according to Kok *et al.* (1996).
2.6. Isolation and identification of *Salmonellae*
2.6.1. Isolation of salmonellae according to (ISO 6579, 2017).
2.6.1.1. Pre-enrichment in non-selective buffered peptone water broth, which then incubated at 37±1 °C for 18±2 hours.
2.6.1.2. Enrichment in Rappaport Vassiliadis broth (RV broth), then the tube was incubated at 43°C for 24 hours.
2.6.1.3. 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.

2.6.2. Identification of salmonellae
2.6.2.1. Gram's Stain (Cruickshank *et al.*, 1975).
2.6.2.2. Biochemical identification (Krieg and Holt, 1984).
2.6.2.3. Serological identification (Confirmatory test) according to (Kauffmann, 1974).

2.7. *Statistical analysis*: of the obtained results was performed according to Feldman *et al.* (2003).

3. RESULTS

Results demonstrated in table (1) showed that *Enterobacteriaceae* were equally detected at rate of 94% of the examined tilapia and bayad samples, with mean values 2.65×10^3 and 2.07×10^3 cfu/g, respectively. The statistical results revealed that, there was a significant ($P \leq 0.05$) increase of *Enterobacteriaceae* counts of Nile tilapia when compared with Bayad samples.
Following results summarized in table (2) showed that coliform bacteria were detected in the examined tilapia and bayad samples with incidence of 14 and 18%, with mean values 1.81×10^3 and 1.74×10^3 cfu/g, respectively. The statistical results revealed that, there is no significant difference between tilapia and bayad samples.
Table (3) demonstrated a detailed bacteriological classification of *Enterobacteriaceae* isolates which revealed detection of *Citrobacter diversus, Citrobacter freundii, E. coli, Enterobacter aerogenes, Enterobacter cloacae, Klebsiella Oxytoca, Proteus mirabilis, Proteus vulgaris,* and *Yersinia enterocolitica* in different ratios. On the other hand, *Salmonella* sp. not detected in any of the examined tilapia and bayad samples.
Table (4) showed the serotyping of isolated *E. coli* strains revealed detection of O27:H2, O63:H2, and O158:H4 with incidences of 8, 2, 4% and 6, 2, 4% for tilapia and bayad samples, respectively.
Moreover, following (EEC, 2005) specifications, 14% and 4% of the examined tilapia samples were rejected due to detection of *E. coli* and *Y. enterocolitica*; while 12% of bayad samples were rejected due to detection of *E. coli* as shown in table (5).

Table 1 Total Enterobacteriaceae counts cfu/g in the examined samples of Nile fish (n=50).

Types of Samples	Positive		Min.	Max.	Mean ±S.E.
	No.	%			
Nile tilapia	47	94.0	3.0×10^2	4.90×10^3	$2.65\times10^3\pm0.16\times10^{3a}$
Bayad	47	94.0	3.0×10^2	4.50×10^3	$2.07\times10^3\pm0.01\times10^{3b}$
Total*	94	94.0			

[a, b] Mean value within a column followed by difference letter where significant difference ($P \leq 0.05$). Total*: number and percentage in relation to total number of samples (100).

Table 2 Total Coliform counts (cfu/g) in the examined samples of Nile fish (n=50).

Types of Samples	Negative		Positive		Min.	Max.	Mean ±S.E.
	No.	%	No.	%			
Nile tilapia	7	14.0	43	86.0	3.0×10^{2}	4.00×10^{3}	1.81×10^{3} $\pm0.13\times10^{3a}$
Bayad	9	18.0	41	82.0	1.0×10^{2}	2.90×10^{3}	1.74×10^{3} $\pm0.10\times13^{2a}$
Total*	16	16.0					

[a, b]: Mean value within a column followed by difference letter where significant difference ($p \leq 0.05$). Total*: number and percentage in relation to total number of samples (100).

Table 3 Prevalence of *Enterobacteriaceae* species in examined samples of Nile fish (n=50).

Bacterial isolates	Samples				Total
	Nile tilapia (*O. niloticus*)		Bayad (*B. bayad*)		
	No.	%*	No.	%*	No. & %**
Citrobacter diversus	2	4.0	1	2.0	3
Citrobacter freundii	1	2.0	1	2.0	2
Edwardsiella tarda	0	0.0	0	0.0	0
E. coli	7	14.0	6	12.0	13
Enterobacter aerogenes	1	2.0	1	2.0	2
Enterobacter cloacae	1	2.0	1	2.0	2
Kl. pneumoniae	0	0.0	0	0.0	0
Kl. oxytoca	3	6.0	3	6.0	6
Proteus mirabilis	1	2.0	1	2.0	2
Proteus vulgaris	1	2.0	1	2.0	2
Salmonella spp.	0	0.0	0	0.0	0
Y. enterocolitica	2	4.0	0	0.0	2
Total	19	38.0	15	30.0	24

%*: Percentage in relation to total number of each sample (50). %**: Percentage in relation to total number of samples (100).

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

E. coli strains	Samples					Strain characteristic
	Nile tilapia (*O. niloticus*)		Bayad (*B. bayad*)		Total	
	No.	%*	No.	%*	No. & %**	
O27:H2	4	8.0	3	6.0	7	EPEC
O63:H2	1	2.0	1	2.0	2	ETEC
O158:H4	2	4.0	2	4.0	4	ETEC
O159:H7	0	0.0	0	0.0	0	EHEC
Total	7	14.0	6	12.0	13	-

%*: Percentage in relation to total number of each sample (50). %**: Percentage in relation to total number of samples (100). EPEC: Enteropathogenic *E. coli.* ETEC: Enterotoxigenic *E. coli*. EHEC: Enterohaemorrhagic *E. coli*

Table 5 Incidence and acceptability of *E. coli* in the examined samples of Nile fish (n=50).

Microorganism	Fish samples							
	Nile tilapia (*O. niloticus*)				Bayad (*B. bayad*)			
	rejected		accepted		rejected		accepted	
	No.	%*	No.	%*	No.	%*	No.	%*
E. coli	7	14.0	43	86	6	12.0	44	88
Y. enterocolitica	2	4.0	48	96	0	0.0	50	100
Salmonella Sp.	0	0.0	50	100	0	0.0	50	100

%*: Percentage was recorded according to number of each sample (50).

4. DISCUSSION

Enterobacteriaceae especially food poisoning members represent a major and important group of microorganisms because of their frequent occurrence and activities that may have a negative impact on seafood quality. The presence of human pathogenic bacteria in fish and seafood may be attributed to contamination during processing. Several bacteria are, however, reported to cause infection and mortality in both fish and humans (Hastein *et al.*, 2006).

Results illustrated in table (1) showed the incidence and counts of *Enterobacteriaceae* in the examined samples which were nearly similar to those reported by Engelbrecht *et al.* (2008) and El-Shabasy (2009) ($3.8x10^3$ and $2.8x10^3$ cfu/g for tilapia and bayad samples, respectively). Lower results were recorded by Gaafar (2007) ($7.8x10^5$ and $6.24x10^5$ cfu\g for tilapia and bayad samples, respectively). On the other hand, the present results were higher than those recorded by El-Sherief (2015) ($7.64x10^2$ cfu\g in examined tilapia samples), and Hassan (2013) ($2.9x10^4$ cfu\g for bayad samples).

Following results of coliform tabulated in table (2), results were in agree with Engelbrecht *et al.* (2008) and El-Shabasy (2009) ($0.98x10^3$ and $2.4x10^3$ cfu\g for tilapia and bayad samples, respectively), while were lower than those recorded by Gaafar (2007) ($2.9x10^5$ and $1.1x10^5$ CFU\g for tilapia and bayad samples, respectively), also, they were higher than those recorded by Naser (1991) ($5.8x10^2$ cfu\g for tilapia samples).

Results of bacteriological classification of the isolated *Enterobacteriaceae* isolates as summarized in table (3) were in agreement with those recorded by Abo Samra (2001) who isolated *E. coli*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris* from examined freshwater fishes' samples).

The incidences of *E. coli* were nearly similar to those recorded by El-Sherief (2015) and Aki (2009) (12% and 16% for tilapia and bayad samples), while were lower than those recorded by Hassan (2013) (57.1 and 100% of examined tilapia and bayad samples, respectively), also, they were higher than those recorded by Ibrahim (2014) and Gaafar (2007) (8% for both tilapia and bayad samples).

The detected values of *Y. enterocolitica* detection in tilapia samples were nearly similar to these recorded by Aki (2009) (4%), while were lower than those recorded by Ammar (2016) (20%). The failure in detection of *Y. enterocolitica* from bayad samples is in agree with Aki (2009).

In addition, the failure of *Salmonella* detection in both tilapia and bayad samples is in agree with Papadopoulou (2007), while disagreed with Hassan (2013) and Ibrahim (2014) who detected *Salmonella* in examined samples at rate of 57.1 and 8%, respectively.

Variations between authors may be attributed to the differences in pollution levels of the environment of fish rearing, mishandling and improper storage practices, and the immunity status of the fish.

5. CONCULOSION

It could be concluded that the presence of *Enterobacteriaceae*, coliform and their members indicating several faults and improper practices of rearing, mishandling and improper storage properties during catching and marketing, that highlighted low hygienic knowledge and encourage authorities for more restrictions and observations, and recommend thorough heat treatment before consumption.

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CONFLICT OF INTREST

The content of this report solely reflects the opinions of the authors, and we report no conflicts of interest. FAPESB did not play a role in the research or writing of the paper.

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