Prevalence of Escherichia Coli in Fish Obtained from Retail Fish Markets in Gharbia Governorate, Egypt

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A B S T R A C T

A total of 100 random samples of fresh and marine fishes represented by Tilapia niloticus, Mugil cephalus, Sardine and Barboni (25 0f each) were collected from the different fish markets located in Gharbia governorate. E. coli was identified by standard microbiological, biochemical tests, and further confirmed by multiplex PCR. The faecal coliform loads in fish samples were assessed. The bacterium was detected in 8(32%), 6(24%), 4(16%) and 3(12%) of the examined samples of Oreochromis niloticus, Mugil cephalus, Sardine and Barboni respectively. The accepted fish samples were 17 (68 %), 19 (76 %), 21 (86 %) and 22 (88 %) of the examined samples of O. niloticus, Mugil cephalus, Sardine and Barboni respectively while the unaccepted samples were 8 (32 %), 6 (24 %), 4 (16 %) and 3 (12 %) of the examined samples of O. niloticus, Mugilcephalus, Sardine and Barboni respectively according to Egyptian standards (E.S 3494/2005). the results cleared that, the main isolates include O26: H11; O44: H18, O86: H; O91: H21; O103: H2; O119: H6; O121: H7; O124: H; O128: H2; O146: H21 and it isolated mainly from O. niloticus, Mugil cephalus, then Sardine and Barboni. The work was aimed to study prevalence of Escherichia coli contamination in fish sold in different fish markets located in Gharbia governorate.

Keywords: Escherichia coli, Oreochromis niloticus, Mugil cephalus, Sardine, Barboni.

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1. INTRODUCTION:

Fish is a food of excellent nutritional value, providing high-quality protein rich in essential amino acids, and a wide variety of minerals, including phosphorus, magnesium, iron, zinc, and iodine in marine fish *(Ariño et al., 2013)*. Fish are generally considered as major vehicles for several bacterial disease transmissions. Escherichia coli has been generally recognized as an indicator organism for faecal contamination of water and seafood *(Geldrich 1997)*.

The poor unhygienic conditions of the landing centers, storage and domestic retail markets exacerbate the problem of poor hygiene and consumer safety of fish *(Kumar et al.,2005)*. Most strains of E. coli or faecal coliform are harmless, but some may cause diarrhea. Reed (1994) classified *E. coli (EEC*) strains to several major subgroups:

Enterohemorrhagic *E. coli (EHEC)* causes hemorrhagic colitis and hemolytic uremic syndrome. Six verotoxins have been identified within this group, but only stx-1 and stx-2 seem to be important in human infections. *E. coli* O157:H7 is the principle serotype of this group Enteroinvasive *E. coli* (EIEC) causes a diarrheal illness similar to shigellosis. Enterotoxigenic *E. coli* (ETEC) is a major cause of travelers' diarrhea and infant diarrhea in developing countries. These strains product a heat-labile toxin (LT) and/or a heat-stable toxin (ST). Enteropathogenic *E. coli* (EPEC) is an important cause of infant diarrhea.

The objectives of our study were to assess the prevalence of *E. coli* in fish sold at retail fish markets in Gharbia government and to enumerate the faecal coliform loads in fish.

2. MATERIALS AND METHODS:

2.1. Collection of samples:

A total of 100 random samples of Nile and marine fishes represented by Tilapia niloticus, Mugil cephalus, Sardine and Barboni (25 of each) were collected from the different fish markets located in Gharbia governorate. Each sample was kept in a separated sterile plastic bag and preserved in an ice box then transferred to the laboratory under complete aseptic conditions without undue delay and examined as quickly as possible. The collected samples were subjected to the bacteriological examination to evaluate their quality.

Preparation of samples (ICMSF, 1996):

To 5 grams of the sample, 45 ml of sterile peptone water 0.1% were added and thoroughly mixed using sterile blender for 1 - 1.5 minutes, from which tenth fold serial dilutions were prepared. The prepared samples were subjected to the following examinations

Isolation and identification of Enteropathogenic Escherichia coli (ICMSF, 1996). Serodiagnosis of *E. coli (Kok et al. 1996*).

Statistical Analysis:

The evaluation and interpretation of obtained results were carried out using of Analysis of Variance (ANOVA) test according to *Feldman et al.* (*2003*).

3. RESULTS:

Our results cleared in table (1) indicated that, there is a significant difference of the incidences of Enteropathogenic *E. coli* isolates among examined fish samples at ($P < 0.01$).

The serotypes of *E. coli* organisms were O26: H11 in 2 (8 %) 1 (4 %), 1 (4 %) and 0 (0 %). O44:H18 1 (4 %), 0 (0 %), 0 (0 %) and 1 (4 %). O86: H 0(0 %), 0(0 %), 1 (4 %) and 0 (0 %). O91:H21 0 (0 %), 1 (4 %), 0 (0 %) and 1 (4 %). O103: H2 1(4 %), 0 (0 %), 0(0 %) and 0 (0 %). O119: H6 0 (0 %), 2 (8 %), 0 (0 %) and 0(0 %). O121: H7 1(4 %), 0 (0 %), 2 (8 %) and 0(0 %). O124: H 1 (4 %), 0 (0 %), 0 (0 %) and 0 (0 %). O128: H2 2 (8 %), 1 (4 %), 0 (0 %) and 1 (4 %). O146: H21 0 (0 %), 1 (4 %), 0 (0 %) and 0 (0 %) of the examined samples of O. niloticus, Mugil cephalus, Sardine and Barboni respectively.

Our results cleared in table (2) indicated that, there is a significant difference ($P < 0.01$) of the acceptance of fish samples based on contamination with E. coli.

The accepted O. niloticus fish were 17 (68 %) and unaccepted samples 8 (32 %). The Mugil cephalus accepted samples were 19 (76 %), while the unaccepted samples were 6 (24 %). While, the Sardine accepted samples were 21 (86 %0, while, the unaccepted samples were 4 (16 %) and in Barboni the accepted samples were 22 (88 %0 and the unaccepted samples were 3 (12 %).

Our results on Table (3), indicated that, the incidence of virulence genes of Enteropathogenic E. coli isolated from the examined samples of fresh and marine fish cleared that, the Shiga-toxin 1 gene (*stx1*), observed in the E. coli isolates of O26: H11, O44: H18, O91: H21, O103: H2, O119: H6and O128: H2.

While, the Shiga-toxin 2 gene (*stx2*), observed in the E. coli isolates of O26: H11, O86, O91: H21, O103: H2, O119: H6 and O121: H7 and O146: H21.While, the Intimin gene (*eaeA*), observed only in *E. coli* isolates of O26: H11.

The results observed in Photograh (1), indicated that, the unaccepted samples are +ve for the *E. coli* isolates as the agrose gel electrophoresis of multiple PCR gave +ve results of stx1 (614 bp), *stx2* (779 bp) and *eaeA* (890 bp) genes for characterization of Enteropathogenic E. coli.

Table (1): Incidence of Enteropathogenic *E. coli* isolated from examined samples of fresh and marine fish (n=25).

Fish Spp.	*Oreochromis niloticus*		*Mugil cephalus*		Sardine		Barboni		Strain characteristics
E. coli strains	No.	%	No.	%	No.	%	No.	%	
O26: H11	2	8	1	4	1	4	0	0	EHEC
O44: H18	1	4	0	0	0	0	1	4	EPEC
O86	0	0	0	0	1	4	0	0	EPEC
O91: H21	0	0	1	4	0	0	1	4	EHEC
O103: H2	1	4	0	0	0	0	0	0	EHEC
O119: H6	0	0	2	8	0	0	0	0	EPEC
O121: H7	1	4	0	0	2	8	0	0	EHEC
O124	1	4	0	0	0	0	0	0	EIEC
O128: H2	2	8	1	4	0	0	1	4	ETEC
O146: H21	0	0	1	4	0	0	0	0	EPEC
Total	8	32	6	24	4	16	3	12	

Chi2 = 22.455** ** = Significant at (P < 0.01).

N.B. The isolation % was calculated according to the number of examined samples

EPEC=Enteropathogenic*E. coli* EIEC =Enteroinvasive *E. coli*

ETEC=Enterotoxigenic *E. coli* EHEC =Enterohaemorrhagic *E. coli*

Table (2): Acceptability of the examined samples of fresh and marine fish based on their contamination with *E. coli* (n=25).

Fish species	*E. coli* /g*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Oreochromisniloticus	Free	17	68	8	32
Mugilcephalus	Free	19	76	6	24
Sardine	Free	21	86	4	16
Barboni	Free	22	88	3	12

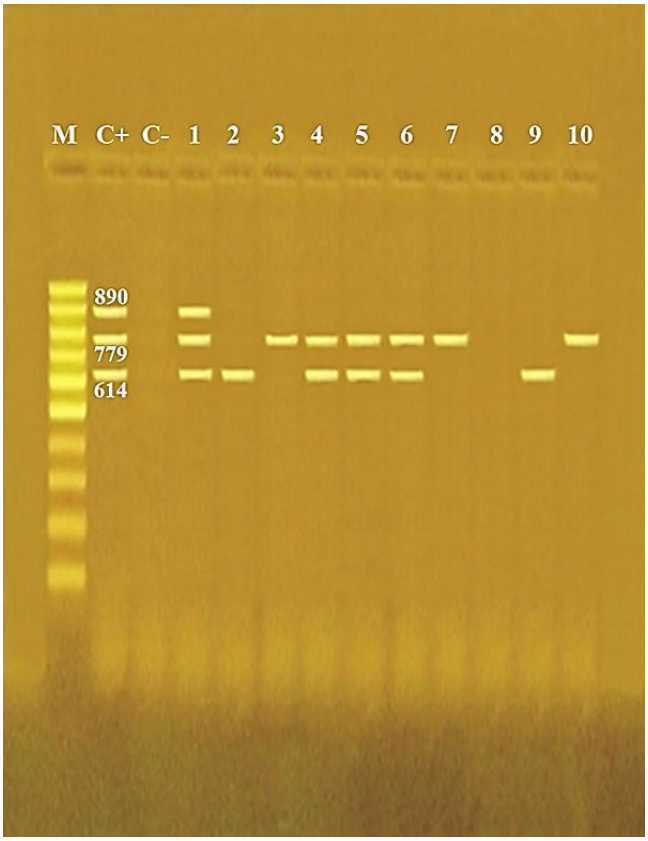
Chi2 = 9.55** ** = Significant at (P < 0.01).

*Egyptian Organization for Standardization "EOS" (2005).
ES 3494-2005 for chilled fish

Table (3): Incidence of virulence genes of Enteropathogenic *E. coli* isolated from the examined samples of fresh and marine fish.

E. coli Serovars	*stx1*	*stx2*	*eaeA*
O26: H11	+	+	+
O44: H18	+	-	-
O86	-	+	-
O91: H21	+	+	-
O103: H2	+	+	-
O119: H6	+	+	-
O121: H7	-	+	-
O124	-	-	-
O128: H2	+	-	-
O146: H21	-	+	-

Stx1: Shiga- toxin 1 gene
Stx2: Shiga- toxin 2 gene
EaeA: intimin gene



Photograph (1): Agarose gel electrophoresis of multiplex PCR
Of *stx1* (614 bp), *stx2* (779 bp) and *eae*A (890 bp) genes for characterization of *Enteropathogenic E. coli*.
Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive *E. coli* for *stx1*, *stx2* and *eae*A genes.
Lane C-: Control negative *E. coli* for stx1, stx2 and eaeA genes.
Lane 1 (O26): Positive *E. coli* strain for *stx1*, *stx2* and *eaeA* genes.
Lanes 2 (O44) & 9 (O128): Positive *E. coli* strains for *stx1* gene.
Lanes 3 (O86), 7 (O121) & 10 (O146): Positive *E. coli* for *stx2* gene.
Lanes 4 (O91), 5 (O103) & 6 (O119): Positive *E. coli* for *stx1* and *stx2* gene.
Lane 8 (O124): Negative *E. coli* strain for *stx1*, *stx2* and *eae*A genes.

4. DISCUSSION:

Quality of sea foods depends on the quality of waters from where the fishes are captured and the sanitary conditions of the landing centers. Proper Sanitation facilities at the retail markets play an important role in the overall quality of the fish. Even if the seafood samples collected from fish catch is landed in prime condition, contamination at poor landing sites and cross contamination may cause faecal contamination (*Sugumar 2002*). However, *E. coli* does not thrive in the marine environment for long period of time and so this organism cannot be expected to harbor in fish harvested from the sea. The detection of E. coli in some fishes might represent post-harvest cross contamination (*Martinez-manzaar 1992*).

Our results in table (1) agreed with those of (*Samaha and Hendawy, 2017*), where they observed that, the most important isolates of E. coli from imported fish were Serotyping of *E. coli* isolated from the examined samples of imported fishes stated 5 strains of *E. coli* isolated from Basa,

Barboniand Mackerel, as O86:K61 (B7), O111:K58 (B9), O124:K72 (B17), O26:K60(B6), and O128:K67(B12). Furthermore, 4 strains were serologically isolated from Denise as O86:K61 (B7), O124:K72 (B17), O26:K60 (B6), and O128:K67 (B12).

Also, our results agreed with those of (Edris et al., 2014), where the observed that, the higher bacterial count (E. coli) was found in Basa and gently went down in Mackerel, Barboni and Denise, respectively. Moreover, results show that the higher bacterial count (Shigella) was found in Mackerel and gently went down in, Basa, Barboni and Denise, respectively.

The current result of isolation of E. coli from the examined samples of imported fish was higher than those obtained by Singh & Kulshrestha (1994), who could isolate 17 strains from all examined samples.

As well as Ahmed (2006) who examined 15 samples of imported fish and stated that it is accepted for (E. S889/2009). Serotyping of enteropathogenic E. coli isolated from the examined samples of imported fish.

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