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Detection and characterization of *Escherichia coli* associated *Oreochromis niloticus* sold in the retail markets at Sohag Governorate, Egypt

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Abstract

Fish is considered a major vehicle for transmission several bacterial diseases to human. *Escherichia coli* (*E. coli*) is one of the main causes of food poisoning outbreaks occurring in Egypt due to consumption of contaminated fish and fish products. This study was performed to investigate prevalence of contamination of *Oreochromis niloticus* (*O. niloticus*) sold in the retail markets at Sohag Governorate, Egypt with *E. coli* and Shiga toxin-producing *E. coli* (STEC) and to determine the antimicrobial susceptibility of the isolates. Therefore, 110 apparently healthy *O. niloticus* samples were randomly collected from the fish retail markets at Sohag Governorate cities, Egypt and they were bacteriologically examined for presence of *E. coli* on the surface and in the gills and muscles. Susceptibility of *E. coli* isolates were tested to 10 different antibiotics and subsequently representative *E. coli* isolates (n=12) were serotyped and screened by PCR for presence of *stx1* and *stx2* genes. 22 *E. coli* isolates were isolated and identified from surface, gills and muscles of the examined *O. niloticus* with percentage of (8.2%), (10.0%) and (1.8%) respectively, these isolates were isolated from 12 fish only of the examined *O. niloticus* with total prevalence of 10.9% (12/110). Serotyping revealed that the investigated *E. coli* isolates belonging to 5 different O-serogroups comprising O_{55} (33.4%), O_{125} (25.0%), O_{26} (8.3%), O_{76} (8.3%) and O_{128} (8.3%) in addition to 2 non-typeable isolates (16.7%). Antimicrobial susceptibility testing for *E. coli* isolates revealed that they were sensitive to amikacin (100%), ciprofloxacin (95.5%), gentamicin (95.5%), chloramphenicol (90.9%), nalidixic acid (77.3%), tetracycline (72.7%) and sulfamethoxazole-trimethobrim (54.5%) while they were resistant to ampicillin (95.5%), cefotaxime (86.4%) and amoxicillin/clavulanic acid (68.2%). Screening 12 *E. coli* isolates by PCR for presence of *stx1* and *stx2* genes revealed that (33.4%) and (25.0%) of them harbor *stx1* gene and *stx2* gene respectively and that *stx1* and *stx2* genes present together in (16.7%) of them.

Keywords: Characterization, *Escherichia coli*, *Oreochromis niloticus*, Sohag Governorate.

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Introduction

Fish had long been regarded as a desirable and nutritional source of high-quality protein and generous supply of minerals and vitamins (Hastein et al., 2006). In the recent years, fish has been recommended as alternative source for animal protein in the developing countries and great attention was directed to fish industry in Egypt to meet the increasing demands for animal protein (Shokr et al., 2018). Egypt is the seventh-largest aquaculture producer in the world and the largest in Africa with approximately one million tons per year (Feidi, 2018 and Shaalan et al., 2018). *O. niloticus* represents the main cultured fish species in Egypt and contributes about 65.15% of Egyptian fish production (GAFRD, 2017). *O. niloticus* is highly consumed in Egypt due to its economical price, high nutritive value and palatability (Elsaidy et al., 2015).

Fish is considered a major vehicle for transmission several bacterial diseases to human (Saad et al., 2018) where it carries some zoonotic bacteria on the skin, gills or in the intestine (Salem and El-Newishy, 2010) which can infect both the consumers and handlers either through ingestion of fish or the direct contact with abraded skin during handling (Mohamed et al., 2011). Fish-transmitted infections to human are quite common but many of them go unreported and they depend on the season, individual contact with fish and related environment, dietary habits and the immune status of the exposed individual (Haile and Getahun, 2018). In addition to the infections, foodborne antibiotics resistant bacteria pose risk to consumers' health and support transfer of these phenotypes to human through the food chain (Rocha et al., 2014).

In Egypt, *E. coli* is widely recognized as one of the principles causes of food poisoning outbreaks occurring due to consumption of contaminated fish and fish products (El-Olemy et al., 2014). STEC have been emerged as pathogens can cause food-borne infections and severe potentially fatal diseases in human; hemorrhagic colitis and hemolytic uremic syndrome (Kargar and Homayoon, 2015). On the other hand, the pathogenic strains of *E. coli* may be one of fish spoilage causes (Haile and Getahun, 2018).

Fish can be contaminated by the pathogenic microorganisms from the aquatic environment contaminated with sewage or from the contaminated hands of fish handlers, or the contaminated utensils and equipment during harvesting, processing, preservation transportation, distribution (National Academy of Science, 1985) and in the retail markets (Kumar et al., 2005). *E. coli* is a member of the normal intestinal microflora of human, most animals and birds, so its presence in Nile tilapia indicates to fecal pollution which may occur due to contamination of aquaculture water by excreta of human and animals or using contaminated water in preparation of fish (James et al., 2001).

Microbiological quality and food safety have gained great attention among the present-day consumers, food processors and regulatory agencies and this continues to increase day by day (Jeyasanta et al., 2012). For our knowledge, there are no studies on contamination of *O. niloticus* sold in the retail markets at Sohag Governorate, Egypt with *E. coli* and STEC and their antibiotic resistance. Therefore, this study was designed to investigate prevalence of contamination of *O. niloticus* sold in the retail markets at Sohag Governorate, Egypt with *E. coli* and STEC and to determine the antimicrobial susceptibility of the isolates.

Material and Methods

1- Sampling:

One hundred and ten samples of apparently healthy *O. niloticus* were randomly collected from the fish retail

markets at Sohag Governorate cities, Egypt. The collected samples were separately packed in sterile plastic bags, kept in icebox containing ice bags and transported to the laboratory immediately.

2- Bacterial isolation and biochemical identification:

Under aseptic conditions, swabs were taken from surface, gills and muscles of the examined fish by sterilized cotton swabs. They were inoculated into tryptone soya broth (TSB) (Oxoid, England) and incubated at 37°C for 24 hours, then a loopful from the bacterial growth was streaked onto eosin methylene blue (EMB) agar (Oxoid, England) and incubated at 37°C for 24 hours. The suspected isolates were preserved in TSB supplemented with 15% glycerol at -20°C till their further identification. They were identified through their morphological characteristics, Gram-staining and some biochemical tests including oxidase, catalase, indole, methyl red, Vogas-Proskauer, citrate utilization and growth on Triple sugar iron agar (TSI) using the laboratory methods described by Holt et al. (1994) and identified as *E. coli* according to criteria of Mahon and Lehman (2019).

3- Serotyping of *E. coli* isolates:

Representative *E. coli* isolates (n=12; *E. coli* isolate from each contaminated fish) were serologically identified according to Edwards and Ewing (1972) by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) according to manufacturer's instructions.

4- Antimicrobial susceptibility testing:

The antimicrobial susceptibility for all *E. coli* isolates were determined by Kirby-Bauer disc diffusion method using the antibiotics disks illustrated in Table 1 (Bioanalyse, Turkey). Each *E. coli* isolate was streaked onto Mueller-Hinton agar (Oxoid, UK), antibiotic disks were dispensed on the inoculated plate and incubated at 37°C for 24 hours. Later, inhibition zones diameters were measured, and the results were interpreted according to the Clinical and Laboratory Standards Institute (2006).

Table 1. Antibiotics used in antimicrobial susceptibility testing of *E. coli* isolates.

Antibiotic	Disc concentration	Antibiotic	Disc concentration
Ampicillin (AMP)	30 mcg	**Amoxicillin/clavulanic acid (AMC)**	30 mcg
Cefotaxime (CTX)	30 mcg	**Gentamicin (CN)**	10 mcg
Amikacin (AK)	30 mcg	**Ciprofloxacin (CIP)**	5 mcg
Tetracycline (T)	30 mcg	**Chloramphenicol (C)**	30 mcg
Sulfamethoxazole-trimethoprim (SXT)	25 mcg	**Nalidixic acid (NA)**	30 mcg

5- Detection of *stx1* and *stx2* genes in *E. coli* isolates by PCR:

Representative *E. coli* isolates (n=12; *E. coli* isolate from each contaminated fish) were screened by PCR for presence of *stx1* and *stx2* genes.

5.1- DNA extraction:

Bacterial DNA was extracted from an overnight subculture for *E. coli* isolates on TSB using QIAamp DNA Mini Kit (QIAGEN Inc., USA) according to the manufacturer's instructions. Concentration of the extracted DNA from each isolate was measured by Nanodrop™ spectrometer

(NanoDrop Technologies, Inc., USA) and it was preserved at -20°C till be used.

5.2-PCR amplification:

PCR was conducted to amplify shiga toxin genes (*stx1* and *stx2*) by using the oligonucleotide primers illustrated in Table 2 (Dhanashree and Mallya, 2008) and Emerald Amp Max PCR Master Mix (Takara, Japan). PCR reaction mixture was prepared in a total volume of 25µl containing Master Mix (12.5µl), forward and reverse primers (1µl from each), extracted DNA (5µl) and nuclease-free water (5.5µl). PCR was conducted in a thermocycler (Applied biosystem 2720, USA) under PCR conditions previously described by Dhanashree and Mallya (2008); initial denaturation at 95°C for 5 minutes followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 58°C for 90 seconds and extension at 72°C for 90 seconds followed by final extension step at 72°C for 5 minutes.

Table 2. The target genes and oligonucleotide primers used in the study.

Target gene	Primers sequences (5`- 3`)		Product size (bp)
Stx1	F	ACACTGGATGATCTCAGTGG	614
	R	CTGAATCCCCCTCCATTATG	
Stx2	F	CCATGACAACGGACAGCAGTT	779
	R	CCTGTCAACTGAGCAGCACTTTG	

5.3- Analysis of PCR products:

Products of PCR were separated by electrophoresis on 1% agarose gel (Applichem GmbH, Germany) in 1x TBE buffer at room temperature using gradients of 5V/cm. Generuler 100 bp DNA ladder (Thermo scientific, Germany) was used to determine the fragments sizes. Gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

Results

1- Prevalence of *E. coli* among the examined fish samples:

Based on the morphological and biochemical characteristics of the isolates, 22 *E. coli* isolates were isolated and identified from surface, gills and muscles of the examined *O. niloticus* with percentage of 8.2%, 10.0% and 1.8% respectively (Table 3). On the other hand, these 22 isolates of *E. coli* were isolated from 12 fish only of the examined *O. niloticus* with total prevalence of 10.9% (12/110) as illustrated in Table 4.

On EMB media, *E. coli* isolates produced their characteristic dark colonies with green metallic sheen. Gram's staining and microscopical examination revealed gram-negative short rods. *E. coli* isolates were negative in oxidase, Voges-Proskauer and citrate utilization tests while they were positive in catalase, indole and methyl red tests. Moreover, they produced yellow acidic butt and slant on TSI without H_2S production.

Table 3. *E. coli* isolates isolated from surface, gills and muscles of the examined *O. niloticus*.

	Site of isolation						No. of total isolates
	Surface of fish		Gills		Muscles		
	No.	%*	No.	%	No.	%	
E. coli isolates	9	8.2	11	10	2	1.8	22

%* was calculated based on the total number of examined *O. niloticus* samples (n=110).

Table 4. Prevalence of *E. coli* among the examined *O. niloticus*.

Positive samples for *E. coli*	Number	%*
From surface, gills and muscles of the fish together	2	1.8
From surface and gills of the fish only	6	5.5
From gills of the fish only	3	2.7
From surface of the fish only	1	0.9
Total	12	10.9

* % was calculated based on the total number of examined *O. niloticus* samples (n=110).

2- *E. coli* isolates serotyping:

Serotyping revealed that the investigated *E. coli* isolates belonging to 5 different O-serogroups comprising O_{55}, O_{125}, O_{26}, O_{76} and O_{128} in addition to 2 non-typeable isolates as illustrated in Table 5.

3- Antimicrobial susceptibility testing:

E. coli isolates were sensitive to amikacin (100%), ciprofloxacin (95.5%), gentamicin (95.5%), chloramphenicol (90.9%), nalidixic acid (77.3%), tetracycline (72.7%) and sulfamethoxazole-trimethobrim (54.5%) while they showed highest resistance to ampicillin (95.5%) followed by cefotaxime (86.4%) and amoxicillin/clavulanic acid (68.2%) (Table 6).

Table 5. O-Serogroups of the investigated *E. coli* isolates.

O-Serogroup	*E. coli* isolates belonging to this O-Serogroup		O-Serogroup	*E. coli* isolates belonging to this O-Serogroup	
	No.	%		No.	%
O_{55}	4	33.4%	O_{125}	3	25.0%
O_{26}	1	8.3%	O_{76}	1	8.3%
O_{128}	1	8.3%	Non-typeable	2	16.7%

Table 6. Antimicrobial resistance of *E. coli* isolates.

Antibiotic	Resistant isolates		Antibiotic	Resistant isolates	
	No.	%		No.	%
Ampicillin (AMP)	21	95.5	**Amoxicillin/clavulanic acid (AMC)**	15	68.2
Cefotaxime (CTX)	19	86.4	**Gentamicin (CN)**	1	4.5
Amikacin (AK)	0	0	**Ciprofloxacin (CIP)**	1	4.5
Tetracycline (T)	6	27.3	**Chloramphenicol (C)**	2	9.1
Sulfamethoxazole-trimethoprim (SXT)	10	45.5	**Nalidixic acid (NA)**	5	22.7

4- Isolates investigation for shiga toxin genes by PCR:

Screening 12 *E. coli* isolates by PCR for presence of *stx1* and *stx2* genes revealed that (33.4%) and (25.0%) of them harbor *stx1* gene and *stx2* gene respectively and that *stx1* and *stx2* genes present together in (16.7%) of them. PCR results were summarized in Table 7 and illustrated in Fig. 1.

Discussion

Fish and fish products have been reported as vehicles for food-borne bacterial infections in human (Hastein et al., 2006). In the present study, *E. coli* was isolated from the examined *O. niloticus* with prevalence of 10.9%.

Table 7. Prevalence of *stx1* and *stx2* genes among *E. coli* isolates.

O-Serogroup	PCR results		*E. coli* isolates which have these PCR results	
	stx1	*stx2*	No.	%
O_{55}	+	+	2	16.7
O_{55}	-	-	2	16.7
O_{125}	+	-	1	8.3
O_{125}	-	-	2	16.7
O_{26}	-	+	1	8.3
O_{76}	-	-	1	8.3
O_{128}	+	-	1	8.3
Non-typeable	-	-	2	16.7
Total	4 (33.4%)	3 (25.0%)	12	100

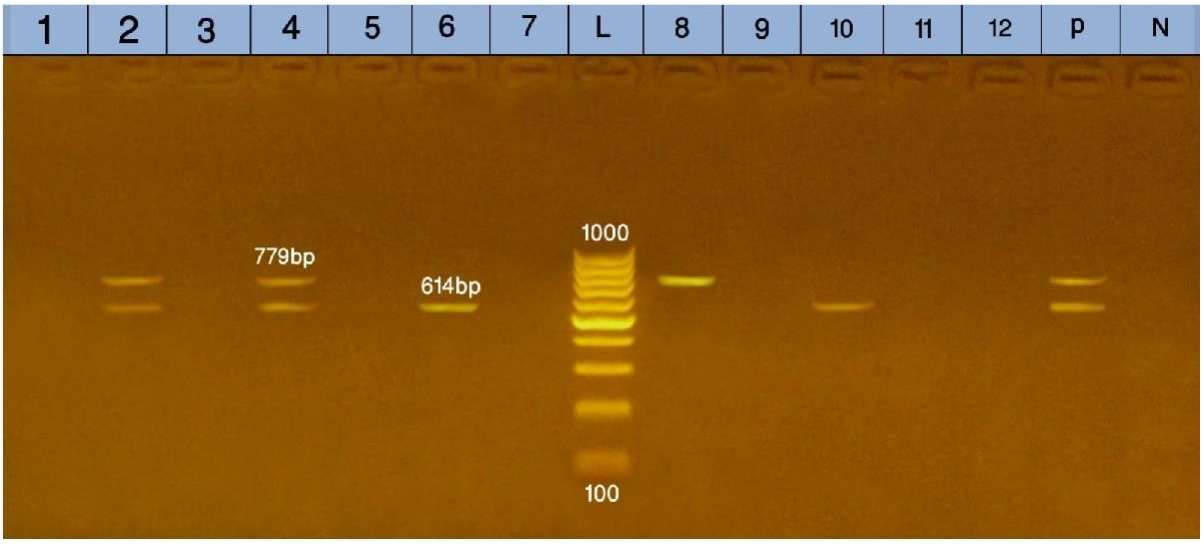

Fig. 1. Agar gel electrophoresis for PCR products using specific primers targeting *stx1* and *stx2* genes in *E. coli* isolates. Lane L: molecular weight marker 100 base pair, lane P: positive control, lane N: negative control and lanes 1-12: DNA extracted from *E. coli* isolates (O55, O125, O26, O76, O128 and non-typeable isolates respectively).

Our results agreed with findings of Salem and El-Newishy (2010), Elsherief et al. (2014) and Shokr et al. (2018) who isolated *E. coli* from *O. niloticus* sold in the retail markets at Qalyoubia, Kafr El Shiekh and Gharbia Governorates, Egypt with prevalence of 14.3%, 12% and 14.6% respectively while disagreed with findings of El-Olemy et al. (2014) and Saad et al. (2020) who isolated *E. coli* from *O. niloticus* sold in the retail markets at Qalyoubia and Gharbia Governorates, Egypt with prevalence of 1% and 70% respectively. These differences in prevalence of *E. coli* among the examined fish may be attributed to the differences in water quality of aquaculture and level of its pollution, management and sanitary conditions in aquaculture, sanitary conditions during fish handling, storage, transportation and display procedures (Saqr et al., 2016) and to the difference in sampling season where temperature affects *E. coli* population and favors bacterial growth (Akande and Onyedibe, 2019). On the other hand, as illustrated in Table (3), *E. coli* was isolated from surface, gills and muscles of the examined *O. niloticus* with percentages of (8.2%), (10%) and (1.8%) respectively. Isolation of *E. coli* with the highest percentage from the gills may be attributed to their direct contact with water, especially in plankton feeders as Nile tilapia (Rocha et al., 2014). Also, isolation of *E.*

coli with higher percentage from fish surface than that from the muscles is owing to that fish surface is exposed to the external environment and the different sources of contamination in contrast to the muscles where presence of *E. coli* could be attributed to the contamination from fish intestinal contents or after injury of body surface (Musefiu et al., 2011 and Rocha et al., 2014). Isolation of *E. coli* from apparently healthy *O. niloticus* in this study emphasizes that they could be a potential source for human infection and gives an indication on the faecal contamination of aquaculture water and/or from the subsequent unsanitary handling during catching, storage, distribution and marketing of fish. Therefore, good hygienic measures should be taken during all stages of fish production till reaching the consumers, also fish should be properly washed and cooked before consumption.

As illustrated in Table (5), serotyping of 12 *E. coli* isolates revealed that they belonged to 5 different O-serogroups including O_{55} (33.4%), O_{125} (25.0%), O_{26} (8.3%), O_{76} (8.3%) and O_{128} (8.3%) in addition to 2 non-typeable isolates (16.7%). Our results agreed with findings of Salem and El-Newishy (2010) and Shokr et al., (2018) who found that *E. coli* O_{55} and O_{125} are the most predominant serotypes of *E. coli* among *O. niloticus* respectively and also with those of Saad et al. (2020) who isolated *E. coli* O_{26} and O_{128} from *O. niloticus* in Gharbia Governorate, Egypt. While in contrast to our results, Gupta et al. (2013) found that *E. coli* O_{96} is the most predominant serotype of *E. coli* among the raw fish in India and isolated serotypes of *E. coli* completely different from those isolated in this study including O_{11}, O_{13}, O_{17}, O_{28}, O_{41}, O_{44}, O_{58}, O_{168} and O_{170} and this could be attributed to difference in the prevailing strains of *E. coli* colonising human and animals and those found in the different environments (Akande and Onyedibe, 2019). Some serotypes of *E. coli* isolated in this study as O_{55} and O_{128} have been identified to cause gastroenteritis in human and implicated in food poisoning outbreaks (El-Olemy et al., 2014).

The indiscriminate use of antibiotics in aquaculture has been paralleled by a significant increase in number of the reports of resistant bacteria isolated from aquaculture stock (Tendencia and dela Pena, 2002). In the present study, antimicrobial susceptibility testing for *E. coli* isolates revealed that they were sensitive to amikacin (100%), ciprofloxacin (95.5%), gentamicin (95.5%), chloramphenicol (90.9%), nalidixic acid (77.3%), tetracycline (72.7%) and sulfamethoxazole-trimethobrim (54.5%) while they showed highest resistance to ampicillin (95.5%) followed by cefotaxime (86.4%) and amoxicillin/clavulanic acid (68.2%). These results were nearly similar to those reported by Atwa (2017) except in susceptibility of the isolates to chloramphenicol and gentamicin while they disagreed with those reported by Saqr et al. (2016) in respect to susceptibility of the isolates to cefotaxime, amoxicillin/clavulanic acid, gentamicin and tetracycline. These differences in antimicrobial susceptibility may be attributed to use of different antibiotics in different settings and purposes as well as by human and animals in addition to the different applied hygienic measures (Saqr et al., 2016). Antimicrobial resistance of *E. coli* that contaminates *O. niloticus* represents a major risk to public health where it can act as reservoirs for the resistance genes and disseminate such genes to even the commensal human bacteria (Akande and Onyedibe, 2019). Transmission of resistant plasmids of *E. coli* from sea foods to human commonly occurs (Jeyasanta et al., 2012).

Pathogenic strains of *E. coli* are distinguished from the other strains by their ability to cause serious diseases due to their genetic elements for production of toxins, adhesion and invasion of host cells,

interference with cell metabolism and destruction of tissues (Galal et al., 2013). STEC is defined by production of one or more type of shiga toxins (*stx1* and *stx2*) through possession of shiga toxin genes (*stx1* and *stx2*). *Stx2*-producing *E. coli* strains are linked to increased risk of human diseases especially hemorrhagic colitis and haemolytic-uremic syndrome than *stx1*-producing *E. coli* strains especially when the former is associated with the intimin gene; *eaeA* (Paton and Paton, 2002). In this study and as illustrated in Table (7) and Fig. (1), screening of 12 *E. coli* isolates by PCR for presence of *stx1* and *stx2* genes revealed that (33.4%) and (25.0%) of them harbor *stx1* gene and *stx2* gene respectively and that *stx1* and *stx2* genes present together in (16.7%) of them. Our results agreed with findings of Galal *et al*. (2013) who reported that (33.4%) of *E. coli* isolated from fresh water fishes harbor *stx1* gene while disagreed with findings of Shokr et al. (2018) who reported that (83.3%) and (66.6%) of *E. coli* isolated from *O. niloticus* at Gharbiya Governorate harbor *stx1* and *stx2* genes respectively. Our results showed that prevalence of STEC among *O. niloticus* has potential health hazard on human.

Conclusion

The current study revealed that *O. niloticus* sold in the retail markets at Sohag Governorate cities, Egypt were contaminated with *E. coli* and STEC posing a potential public health threat to fish consumers and handlers. Therefore, adequate and efficient hygienic measures should be taken during all stages of fish production till reaching the consumers, also fish should be properly washed and cooked before consumption. Moreover, strict governing must be taken to the misuse of antibiotics not only in aquaculture but also in the other livestock production systems.

Conflicts of interest

The author declares that there is no conflict of interest.

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