Antimicrobial-resistance and Virulence Genes of Shiga Toxin-producing *Escherichia coli* (STEC) Isolated from Tilapia and Mullet and its Public Health Significance

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Abstract

Shiga toxin-producing *Escherichia coli* (STEC) is responsible for several food-borne outbreaks worldwide. In this study, tissue samples of finfish (tilapia, n = 100) and (mullet, n = 100), and twenty human hand swabs from fish sellers and fishermen were tested bacteriologically for STEC presence. Isolates were tested for their antibiotic susceptibility and examined for the presence of the *eae*A, *stx*1, and *stx*2 genes. *E. coli* and STEC were identified from the tissues (36.5% and 12.5%) of the examined tilapia and mullet, respectively; however, *E. coli* and STEC from human hand swabs were as high as (60% and 40%), respectively. Of the recovered *E. coli* isolates, 25 presumptive STEC (17 from finfish and 8 from humans) yielded characteristic mauve-colored colonies on CHROMagar STEC medium. The highest prevalence of STEC was in mullet and tilapia from freshwater of Nile tributaries at 24% and 48%, followed by fish from freshwater fish farms at 16% and 12%, respectively. No STEC was isolated from fish from Suez Canal water and saltwater fish farms. Recovered STEC isolates from fish belonged to 14 serotypes belonging to (O121:H7, O113:H4, O119:H6, O128:H2, O153:H2, O91:H21, O26:H11, O44:H18, O146:H2, O55:H7, O124, O159, O78, and O117: H4). Isolates from human hand swabs belonged to (O26:H11, O91:H21, O15:H2, O121:H7, and O119:H6). One or two Shiga-toxin (*stx*1 and *stx*2) genes were confirmed in STEC isolates. STEC isolates from finfish and humans were multi-drug resistant (MDR). This study reported a high degree of contamination of freshwater finfish from the Ismailia governorate with STEC and highlighted the high level of antimicrobial resistance exhibited which is very hazardous to consumers.

Keywords: Fin fish, Zoonoses, *E. coli*, STEC, Virulence genes, Antimicrobial resistance.

Introduction

Egypt is regarded as one of the largest aquaculture-producing countries which considerably contributes to income as well as achieves food security all over the world. Although animal-sourced foods are noticeably linked to food borne illnesses, they are vital sources of energy **(*Eltholth et al., 2015; Randolph, 2013*)**. According to ***Jácome et al. (2019*)** freshwater fishes traced back to African origins are regarded as the main producers of animal protein all over the world, especially in developing countries.

Mullets and tilapia are among the most common aquaculture finfish species in Egypt. ***Ishak (1980*)** ascertains that more than 70 percent of the fish captured in the Nile River and nearby lakes are of the tilapia type. This type of fish is particularly more preferable by population than other types for a number of reasons. Namely, these are its palatable taste, high nutritional value, quick reproduction and growth, cheapness, and resistance to poor water quality. Tilapia is virtually classified as one of the highest aquaculture-produced finfish kinds, with a percentage amount of 10.2% of the total production coming from Asian countries, and China is ranked at top of the list **(*FAO, 2020*)**.

The isolation of *E. coli* is thought to be a significant indicator of the existence of water pollution in the surrounding environment of fish (***ELsaidy et al., 2015***). *E. coli* is thought to be one of the most dangerous diseases, causing losses at the economic level, an increase in the number of deaths, and damage to public health as well **(*Fatma et al., 2016*)**. Antimicrobial treatment is an essential means for minimizing the incidence and mortality caused by *E. coli* infection in fish, despite it is not the ideal approach due to the development of resistance **(*Schroeder et al., 2002*)**. Pollution taking place because of antibiotic-resistant bacteria leads to major difficulties in treatment options (***Al-Zarouni et al., 2008***).

STEC is regarded as a source of public health concern due to the severity of infections and the risk of a rise in mortality rates as a result of STEC poisoning. Fecal contamination, for example, participates in the transportation of STEC to animals and people through spreading to food crops and water supplies, as well as through instant contact. During the previously occurring epidemics, fish flesh was frequently regarded as the main component required in the population diets, which implies that these serious fish zoonotic outbreaks over the last few years shed light on the necessity to monitor diseases of fish-borne zoonotic origin. **(*Barrett et al., 2017*)**. Multiplex PCR was used in Egypt to identify virulence

genes (*stx*1, *stx*2, and *eae*A) in *E. coli* serotypes **(*Hussein et al., 2019*)**. Therefore, the current study was conducted to identify *E. coli* and STEC from finfish and human handlers in Egypt's Ismailia area, as well as to measure antibiotic sensitivity.

Material and methods

1. Ethics statement

The Ethics Committee of the Faculty of Veterinary Medicine at Suez Canal University, Egypt, has reviewed and approved the sample collection and laboratory procedures for the current study (No. 2017003).

2. Finfish samples

Finfish samples (100 Tilapia and 100 Mullet) were collected from: Suez Canal, fresh water of Nile tributaries, saltwater fish farms and freshwater fish farms at Ismailia governorate during the period from April 2021 to May 2022. Collected samples were transferred on ice promptly to the laboratory according to **(*Rocha et al., 2014*)**. Twenty hand swabs from sellers' hands and fishermen were also collected.

3. Isolation of *E. coli* and STEC Detection

Tissues of fish were collected by cutting a part after sterilization of the outer surface with 70% alcohol and a hot spatula according to the procedures previously described **(*Gupta et al., 2013*)**. Around twenty-five grams of each fish, were homogenized with 225 ml *Escherichia coli* broth (Biolife, Italiana) for 3 minutes in stomacher (Lab. Blender 400, Seward Lab, London). A tube containing 9 mL of sterile *E. coli* broth and 1 mL of the homogenized sample were used to incubate the samples for 24 hours at 37°C **(*Doyle et al., 2020*)**. Swabs were used to roll the palms and fingers of the sellers' hands and fishermen, which were then incubated in *E. coli* broth (Biolife, Italiana) for 24 hours at 37°C. *E. coli* broth loopfuls from each broth were subcultured for 24 hours at 37°C on EMB and MacConkey agar (Himedia, India).

In order to obtain pure colonies, typical *E. coli* colonies on EMB were repeatedly cultured ***(Doyle et al., 2020)***. Pure *E. coli* colonies were biochemically identified and then plated for 24 hours at 37°C on CHROMagar STEC agar medium (CHROMagar Microbiology, Paris, France) to identify STEC specifically **(*Meng et al., 2012*)**.

4. Serological identification of STEC

In the College of Veterinary Medicine at Benha University, the presumptive STEC isolates that were successfully grown on CHROMagar were identified serologically utilizing rapid diagnostic tests with Polyvalent *E. coli* antisera against the O and H antigens **(*Kok et al., 1996*)**.

5. Virulence of presumptive STEC from finfish and human

The virulence of 25 presumptive STEC (17 isolates from finfish and 8 isolates from human hand swabs)

was tested using PCR for three virulence genes (*stx*1, *stx*2, and *eae*A) (Table 1). According to the manufacturer's recommendations, bacterial DNA was extracted using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH). In a 50 µl reaction mixture including 25 µl of EmeraldAmp Master Mix (Takara, Japan), 6 µl of bacterial DNA, 1 µl of each primer (Metabion, Germany) (20 pmol concentration), 15 µl of molecular grade water, duplex PCR reactions for *stx*1 and *stx*2 were conducted. Furthermore, in a 25 µl reaction mixture, a uniplex PCR for the *eae*A gene was performed. The mixture consisted of 12.5 µl of EmeraldAmp Master Mix (Takara, Japan), 5 µl of bacterial DNA, 1 µl of each primer (Metabion, Germany) (20 pmol concentration), 5.5 µl of molecular grade water. All reactions were conducted in a thermal cycler (Applied biosystem 2720), followed by separation of PCR products (20 µl) by electrophoresis at room temperature using 100 bp molecular marker (Fermentas, Germany) using 1.5% agarose gel (Applichem, Germany, GmbH) and 1X Tris-borate-EDTA (TBE) buffer. After that, the gel was photographed with the Gel Documentation System (Alpha Innotech, Biometra).

6. Antimicrobial susceptibility of presumptive STEC from finfish and human

Identified presumptive STEC were evaluated for their susceptibility to 12 antibiotics from various antimicrobial classes on Muller Hinton agar (Oxoid, UK) using disc diffusion technique. The following antibiotics (Oxoid, UK) were used: piperacillin (PRL) 100 µg, ampicillin (AM) 10 µg, amoxycillin/clavulanic acid (AMC) 30 µg, ceftriaxone (CTR) 30 µg, ciprofloxacin (CIP) 5 µg, chloramphenicol (C) 30 µg, colistin (CT) 10 µg, azithromycin (AT) 15 µg , gentamycin (Gen) 10 µg, fosfomycin (FF) 50 µg, trimethoprim sulphamethoxazole (SXT) 25 µg and tetracycline (TE) 30 µg. Freshly grown pure STEC isolates were diluted to a density of 0.5 MacFarlane standard in 5 ml of Muller Hinton broth (Oxoid, UK). Sterile swabs were used to streak Mueller Hinton Agar (MHA) plates, which were then allowed to dry for 10 min. to dry. Antibiotic discs were firmly fixed on the plates using sterile forceps and incubated for 18 h at 37°C before measuring the inhibition zone suggested by the manufacturer as well as the Clinical and Laboratory Standard Institute **(*CLSI, 2020*)**.

Results

The frequency of *E. coli* in mullet and tilapia was highest among fish from the freshwater Nile tributaries (Table 2). Of the total *E. coli*, 25 presumptive STEC isolates exhibited characteristic mauve color on CHROMagar STEC media; those were recovered from fish from freshwater Nile tributaries and freshwater fish farms. No, STEC was not isolated from Suez Canal

water or saltwater fish farms (Table 2). Randomly selected 17 presumptive STEC isolates were found to belong to 14 serotypes (Table 3). PCR (polymerase chain reaction) was performed for the 17 presumptive STEC isolates for the detection of virulence genes (*eae*A, *stx*1, and *stx*2). Results revealed that out of 17 isolates from finfish samples, the detection rate of *eae*A, *stx*1, and *stx*2, genes was 41%, 70.5%, and 58.5% respectively (Table 4). The STEC isolates from finfish were multidrug-resistant, with complete resistance to amoxicillin/clavulanic acid, colistin, and fosfomycin, however, they were sensitive to gentamycin, azithromycin, and trimethoprim-sulphamethoxazole (Table 5).

Out of twenty hand swab samples taken from fishermen, sellers, and fish dealers, the prevalence of *E. coli* and STEC was as high as 60% and 40% respectively (Table 2). Serotyping revealed that five serotypes belonged to (O26:H11, O121:H7, O91:H21, O15:H2, and O119:H6) (Table 3). Results revealed that out of 8 presumptive STEC isolates, the detection rates of the *eae*A, *stx*1, and *stx*2 genes were 50%, 100%, and 87.5%, respectively. The human STEC isolates were multidrug-resistant in humans, with complete resistance to; piperacillin, chloramphenicol, colistin, amoxicillin/clavulanic acid, fosfomycin, and tetracycline, but they were susceptible to azithromycin (Table 6).

Table (1): *Primer sequences, product size, and cycling conditions of virulence genes from STEC.*

Target genes	Primer sequences 5'-3'	Product size (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				Den	Ann	Ext		
*eae*A	F: ATGCTTAGTGCTGGTTTAGG R: GCCTTCATCATTTCGCTTTC	248 bp	95˚C 3 min	95C 20 s	53C 30 s	72C 45 s	72˚C 7 min	*Bisi-Johnson et al., (2011)*
*stx*1	F: CTGGATTTAATGTCGCATAGTG R: AGAACGCCCACTGAGATCATC	150 bp	95˚C 3 min	95C 20 s	60C 30 s	72C 45 s	72˚C 7 min	*López-Saucedo et al., (2003)*
*stx*2	F: GGCACTGTCTGAAACTGCTCC R:TCGCCAGTTATCTGACATTCTG	255bp	95˚C 3 min	95C 20 s	60C 30 s	72C 45 s	72˚C 7 min	*López-Saucedo et al., (2003)*

Table (2): *Frequency of E. coli and STEC from finfish and human hand swabs.*

Type of sample			Total number of samples	Positive *E. coli*	Positive STEC from CHROMagar regarding the total samples examined
				No. (%)	No. (%)
finfish	Mullet	Suez canal water	25	3 (12)	0 (0.0)
		fresh water of Nile tributaries	25	18 (72)	6 (24)
		saltwater fish farms	25	9 (36)	4 (16)
		freshwater fish farms	25	9 (36)	4 (16)
	Tilapia	Suez canal water	25	2 (8)	0 (0.0)
		fresh water of Nile tributaries	25	17 (68)	12 (48)
		saltwater fish farms	25	7 (28)	0 (0.0)
		freshwater fish farms	25	8 (32)	3 (12)
Total			200	73 (36.5)	25 (12.5)
Human hand swabs			20	12 (60)	8 (40)

Table (3): *Serotypes of presumptive STEC from finfish and human hand swabs and their Virulence profile.*

Type of sample			No. of isolates	Serotype of presumptive STEC	Virulence genes from presumptive STEC		
					*eae*A	*stx*1	*stx*2
finfish	Mullet	Suez canal water	0	0	0	0	0
		fresh water of Nile tributaries	5	O121:H7	+	-	-
				O113:H4	-	-	+
				O128:H2	+	-	+
				O119:H6	+	+	-
				O146:H21	-	+	+
		saltwater fish farms	0	0	0	0	0
		freshwater fish farms	4	O153:H2	+	+	+
				O91:H21	-	+	-
				O91:H21	-	+	+
				O117:H4	-	-	-
	Tilapia	Suez canal water	0	0	0	0	0
		fresh water of Nile tributaries	5	O26:H11	+	+	-
				O44:H18	-	+	+
				O124	-	+	+
				O159	-	+	-
				O153:H2	-	+	+
		saltwater fish farms	0	0	0	0	0
		freshwater fish farms	3	O78	+	+	+
				O55:H7	+	-	+
				O128:H2	-	+	-
Total			17		7 (41.2)	12 (70.6)	10 (58.8)
Human hand swabs			8	O26:H11	+	+	+
				O121:H7	+	+	+
				O91:H21	-	+	+
				O15:H2	+	+	+
				O26:H11	-	+	+
				O91:H21	+	+	+
				O119:H6	-	+	+
				O15:H2	+	+	-
Total			8		5 (62.5)	8 (100)	7 (87.5)

Table (4): *Virulence genes from presumptive STEC serotypes from finfish and human hand swabs.*

Type of samples			No. of presumptive STEC serotypes	*eae*A gene		*stx*1 gene		*stx*2 gene	
				No.	(%)	No,	(%)	No.	(%)
Finfish	Mullet	freshwater of Nile tributaries	5	3	60	2	40	3	60
		freshwater fish farms	4	1	25	3	75	2	50
	Tilapia	freshwater of Nile tributaries	5	1	20	5	100	3	60
		freshwater fish farms	3	2	66.67	2	66.67	2	66.67
Human hand swabs			8	5	62.5	8	100	7	87.5
Total			25	11	41.18	20	80	17	58.82

Table (5): *Antimicrobial sensitivity testing of presumptive STEC obtained from finfish samples.*

Antimicrobial class	Antibiotic agent and concentration	Presumptive STEC (No. = 17)		
		Resistant No. (%)	Intermediate No. (%)	Susceptible No. (%)
Penicillin	Ampicillin	16 (94.12)	1(5.88)	0 (0.00)
	Piperacillin	15 (88.24)	1 (5.88)	1 (5.88)
B-lactams combination agents	Amoxcillin/clavulanic acid	17 (100)	0 (0.88)	0 (0.00)
Cephems	Ceftriaxone	3 (17.65)	2 (11.76)	12 (70.59)
Macrolides	Azithromycin	2 (11.76)	0 (0.00)	15 (88.24)
Phenicols	Chloramphenicol	5 (29.41)	3 (17.65)	9 (52.94)
Fluoroquinolones	Ciprofloxacin	3 (17.65)	10 (58.82)	4 (23.53)
Lipopeptides	Colistin	17 (100)	0 (0.00)	0 (0.00)
Fosfomycins	Fosfomycin	17 (100)	0 (0.00)	0 (0.00)
Aminogylcosides	Gentamycin	1 (5.88)	0 (0.00)	16 (94.12)
Folate pathway antagonists	Trimethoprim-Sulphamethoxazole	3 (17.65)	1(5.88)	13 (76.47)
Tetracyclines	Tetracycline	10 (58.82)	2 (11.76)	5 (29.41)

Table (6): *Antimicrobial sensitivity testing of presumptive STEC obtained from human hand swab samples.*

Antimicrobial class	Antibiotic agent and concentration	STEC (No. = 8)		
		Resistant No. (%)	Intermediate No. (%)	Susceptible No. (%)
Penicillin	Ampicillin	5 (62.5)	3	0 (0.00)
	Piperacillin	8 (100)	0 (0.00)	0 (0.00)
B-lactams combination agents	Amoxcillin/clavulanic acid	8 (100)	0 (0.00)	0 (0.00)
Cephems	Ceftriaxone	4 (50)	1 (12.5)	3 (37.5)
Macrolides	Azithromycin	1 (12.5)	0 (0.00)	7 (87.5)
Phenicols	Chloramphenicol	8 (100)	0 (0.00)	0 (0.00)
Fluoroquinolones	Ciprofloxacin	5 (62.5)	0 (0.00)	3 (37.5)
Lipopeptides	Colistin	8 (100)	0 (0.00)	0 (0.00)
Fosfomycins	Fosfomycin	8 (100)	0 (0.00)	0 (0.00)
Aminogylcosides	Gentamycin	3 (37.5)	1 (12.5)	4 (50)
Folate pathway antagonists	Trimethoprim-Sulphamethoxazole	1 (12.5)	2 (25)	5 (62.5)
Tetracyclines	Tetracycline	8 (100)	0 (0.00)	0 (0.00)

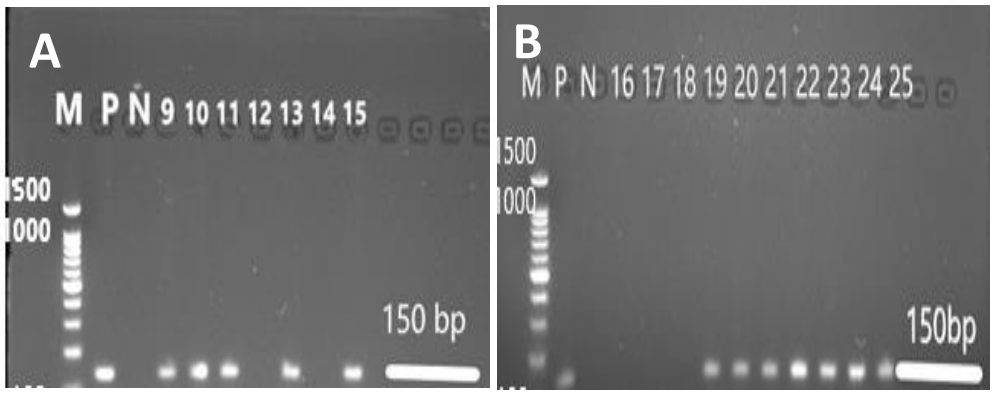

Photo (1): Agarose gel electrophoresis of s*tx*1 gene (150 bp) in STEC isolates from finfish. **A:** Lanes: M, molecular weight size DNA ladder (100-bp); P, *stx1*-positive control, N, *stx1*-negative control; 9–15, isolates from fresh water fish farms, (*stx1* positive strains; 9, 10, 11, 13, 15). **B:** Lanes: M, molecular weight size DNA ladder (100-bp); P, *stx*1-positive control, N, *stx*1-negative control; 16–25, isolates from fresh water of Nile tributaries, (*stx1* positive strains; 19, 20, 21, 22, 23, 24, 25).

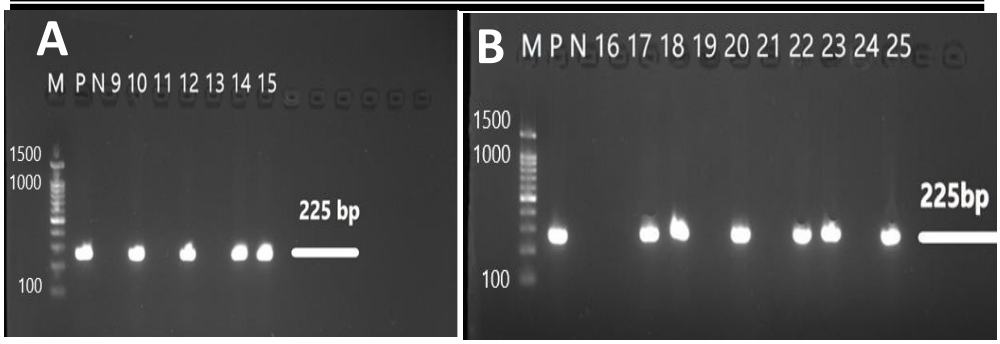

Photo (2): Agarose gel electrophoresis of s*tx*2 gene (225 bp) in STEC isolates from finfish. **A:** Lanes: M, molecular weight size DNA ladder (100-bp); P, *stx*2-positive control, N, *stx*2-negative control; 9–15, isolates from fresh water fish farms, (*stx2* positive strains; 10, 12, 14, 15). **B:** Lanes: M, molecular weight size DNA ladder (100-bp); P, *stx*2-positive control, N, *stx*2-negative control; 16–25, isolates from fresh water of Nile tributaries, (*stx2* positive strains; 17, 18, 20, 22, 23, 25).

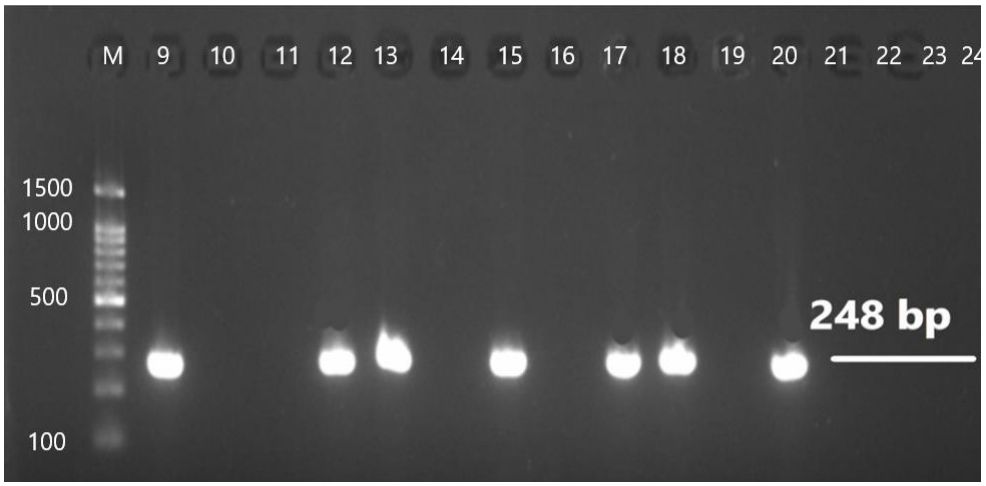

Photo (3): Agarose gel electrophoresis of *eae*A gene (248 bp) in STEC isolates from finfish. Lanes: M, molecular weight size DNA ladder (100-bp); 9–25, (*eaeA* positive strains; 9, 12, 13, 15, 17, 18, and 20).

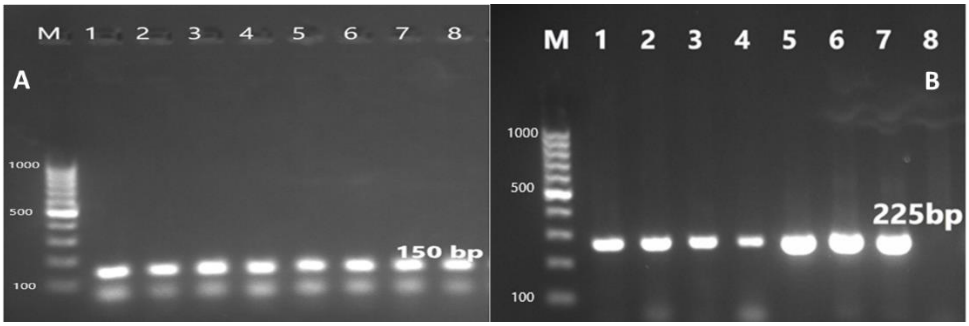

Photo (4): Agarose gel electrophoresis of s*tx*1 gene in STEC isolates from human hand swabs. Lanes: M, molecular weight size DNA ladder (100-bp); **A:** *stx1* positive strains; 1-8. **B:** *stx2* positive strains; 1-7.

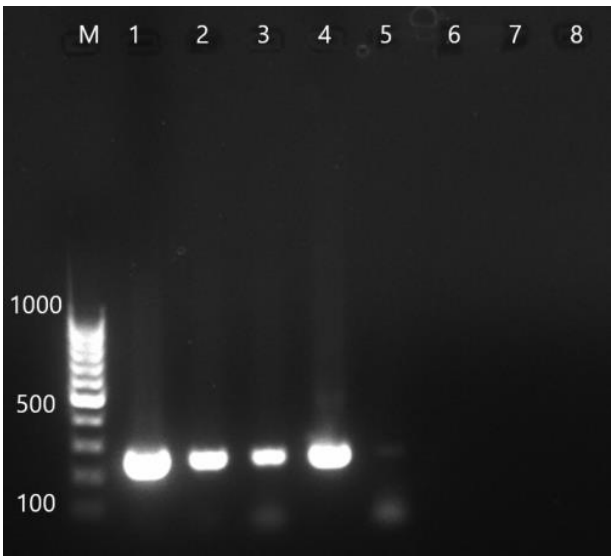

Photo (5): Agarose gel electrophoresis of *eae*A gene in STEC isolates from human hand swabs. Lanes: M, molecular weight size DNA ladder (100-bp), *eae*A positive strains; 1-5.

4. Discussion

Tilapia and mullet are the most common fish kinds used in aquaculture in Egypt. They are preferred by the population due to their palatable taste, high nutritional value, cheapness, quick growth and reproduction, and the ability to live in even unhygienic water **(*Mohamed, 2018*)**. In the present study, the occurrence rates of *E. coli* in mullet and tilapia of Suez Canal water, freshwater of Nile branches, salt water and freshwater fish farms were respectively 12% and 8%, 72% and 68%, 36% and 28%, and 36% and 32%. Such conclusions were almost similar to those reached in

studies conducted by ***Gaafar, 2007*** (8%), ***Amr et al. 2012*** (50%), ***Galal et al., 2013*** (36%), ***Hassan, 2013*** (57.1%), ***Ibrahim, 2014*** (8%), ***El-Sherief, 2015*** (12%), ***Saqr et al., 2016*** (18.3%), ***Atwa, 2017*** (25 %) and ***Rawash et al., 2019*** (13%); in India by ***Gupta et al., 2013*** (29.3%) and ***Dutta and Sengupta, 2016*** (65%), and in Ethiopia by ***Wendwesen et al., 2017*** (42.50%), ***Hiko et al., 2018*** (53.30%). While the percentages attained in this study were higher than those referred to in Ethiopia by ***Anwar et al., 2012*** (2.4%).

The prevalence rates of presumptive STEC for mullet and tilapia in fresh water of Nile tributaries were virtually 24% and 48%, whereas the percentage of their expansion in freshwater fish farms was 16% and 12%, nevertheless, STEC were not separated from finfish of Suez Canal water or saltwater fish farms. These results were more or less the same as those reached in a study carried out in India by ***Prakasan et al., 2018*** (16.6%).

The previous differences in prevalence rates of *E. coli* among the examined finfish might be the result of variations in aquaculture water quality and the level of contamination, management and sanitary matters of fish growing up, including hygiene and health conditions during fish handling, storage, transmission, and displaying techniques **(*Saqr et al., 2016*),** and also to the changing sampling season, where it is known that temperature affects the *E. coli* population and allows for the growth of bacteria **(*Akande and Onyedibe, 2019*)**. The occurrence rates for both *E. coli* and presumptive STEC separations from humans were respectively 60% and 40%. These findings were more or less the same as those ascertained by ***Onanuga et al. (2014)*** and ***Kumar et al. (2001)***.

At the overall, *E. coli* in fish are extremely dangerous for human health. As for, the health of fresh fish may generally be considered as a strong sign of bacterial infection, particularly the conditions related to unhygienic water and/or fish tank dirtiness, and above all *E. coli* as well **(*Jang et al., 2017*)**, since identifying the food sources of STEC and the ways of reserving it are of great importance.

In this study, 17 serotypes of presumptive STEC of finfish were obtained from Mullet (5 from fresh water of Nile tributaries and 4 from freshwater fish farms) and tilapia (5 from fresh water of Nile tributaries and 3 from freshwater fish farms). Aside from O157:H7, the four most regularly involved serotypes in human outbreaks of STEC in Europe are O26:H11, O103:H2, O145:H28, and O111:H8, which imply the 5 highly pathogenic serotypes **(*EFSA, 2013*)**.

The production of one or more kinds (*stx*1 and *stx*2) of Shiga toxins characterizes STEC **(*Paton and Paton 2002*)**. In this study, the detection rates of virulence genes (*eae*A, *stx*1, and *stx*2) using PCR

among the presumptive STEC isolates of finfish were respectively 41.2%, 70.6%, and 58.8%. Here, it might be worth denoting that the current study results were in accordance with ***Galal et al. (2013)*** who found the *stx2* gene in a few samples of Nile tilapia, however, they were all negative for *eaeA* gene. Furthermore, STEC was detected in fish and seafood samples in India by ***Kumar et al. (2001)*** and ***Kumar et al. (2004)***. In contrast, only *stx*2 (0.48%) was found out in some samples of Moroccan seafood, which were *eae*A negative **(*Bennani et al. 2011*)**. In human, the detection rates of *eae*A, *stx*1, and *stx*2 genes were respectively 62.5%, 100%, and 87.5%. ***Aljanaby and Alfaham (2017)*** revealed that the lowest expansion of virulence genes in *E. coli* was (4%) for *eae*A and *stx*1 virulence genes. Strains producing *stx*2, are combined with a higher level of hazard of human diseases, especially Hemorrhagic colitis (HC) as well as Haemolytic uremic syndrome (HUS) than *stx*1, particularly when the intimin gene; *eae*A, which exacerbates pathogenicity.

The distribution of multi-drug resistant (MDR) microorganisms constitutes a threat to public health all over the world. The fish handled for trade might be a vehicle for antibiotic-resistant microbes that are then transmitted to humans, causing great hazards to public health **(*Singh et al., 2020*)**. The selective demand resulted from overuse of medicine prescriptions in clinical settings, as well as their widespread use to prompt growth in the farms of animals and fish, has speeded up the evolution of bacteria toward resistance **(*Samuel et al., 2011*)**. In this study, isolates were entirely resistant to amoxcillin/clavulanic acid, colistin and fosfomycin, meanwhile, they were sensitive to gentamycin, azithromycin and trimethoprim- sulphamethoxazole. The majority of the finfish presumptive STEC isolates were MDR (see Table 5).

On the other hand, in this study, the antimicrobial sensitivity testing results of isolated presumptive STEC from human hand swabs revealed complete resistance to; piperacillins, chloramphenicol, amoxicillin/clavulanic acid, fosfomycin, colistin, and tetracycline, but they were susceptible to azithromycin. Most of the human STEC isolates were MDR. These results were nearly similar to that reported by ***Arias and Murray (2009); Schroeder et al. (2002)*** and ***Zhao et al. (2001)***, however, they were disagreed with that reported by ***Soliman et al. (2010)*** who found that *E. coli* isolates were shown to be susceptible to oxanilic acid, enrofloxacine, and spectinomycine. ***Samuel et al. (2011)*** noticed that there were no *E. coli* resistance to sulphamethoxazol+ trimethoprim, norfloxacine, or chloramphenicol. Differences in outcomes from previous reports might be attributed

to the usage of various antibiotics in different contexts, as well as, differing behavioral and sanitary environments. This study reported a high degree of contamination of freshwater finfish from the Ismailia governorate with STEC and highlighted the high level of antimicrobial resistance exhibited which is very hazardous to consumers.

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الملخص العربي

هدفت الدراسة الى تحديد مدى انتشار الإيشيريشيا كولاى مع التركيز على الإيشيريشيا كولاى المنتجة لسموم الشيقا في الأسماك ومخاطرها المحتملة على مستهلكي المأكولات البحرية في منطقة قناة السويس، مصر. تم جمع 200 عينة سمك من الأسماك الزعنفية اشتملت على (100 سمكة بوري و 100 سمكة بلطي) من مواقع واماكن مختلفة بمدينه الاسماعيلية وشاطئ قناه السويس وبحيره التمساح، بالاضافة الى 20 عينة مسحات ايدي من الانسان (الصيادين وبائعي الاسماك). تم فحص جكيع العينات ميكروبيولوجيا و تصنيفها سيرولوجيا للايشيريشيا كولاي المنتجه لسموم الشيقا وكذلك الكشف عن جينات الضراوة (*stx1* و *stx2* و *eae*A) باستخدام اختبار تفاعل عديد البمره المتسلسل وحساسيتها للمضادات الحيوية. كان معدل عزل الإيشيريشيا كولاي والإيشيريشيا كولاي الافتراضية المنتجه لسموم الشيقا من أنسجة الأسماك الزعنفية 36.5٪ و 12.5٪، و من مسحات اليد للانسان 60٪ و 40٪، على التوالي . تم تحديد 25 عزلة من الايشيريشيا كولاي الافتراضية المنتجه لسموم الشيقا من الأسماك الزعنفية (17) والانسان (8) والتي انتمت مصليًا إلى المجموعات التالية: O26:H11، O125:H6، O146:H21، O159، O44:H18، O128:H2، O117:H4، O121:H7، O113:H4، O119:H6، O153:H2، O91:H21، O124، O78، O15:H2 و O55:H7. فيما يتعلق بمعدلات الكشف عن جينات الضراوه *stx1* و *stx2* و *eae*A للأنماط المصلية للايشيريشيا كولاي الافتراضية المنتجه لسموم الشيقا للأسماك الزعنفية والانسان كانت70.6 ٪ و 58.8٪ و 41.2٪ ؛ و 100٪ و 87.5٪ و 62.5٪ على التوالي. أكدت النتائج ان هناك 23 عزلة من الايشيريشيا كولاي المنتجه لسموم الشيقا من الأسماك الزعنفية (15) والانسان (8) من بين الايشيريشيا كولاي الافتراضية المنتجه لسموم الشيقا المفحوصة. أظهرت جميع عزلات الايشيريشيا كولاي الافتراضية المنتجه لسموم الشيقا مقاومة كاملة للبنسلين، الأموكسيسيلين/حمض الكلافولانيك، الكوليستين، الفوسفوميسين، سيبروفلوكساسين، والتتراسيكلين، إلا أنها كانت حساسة للجنتامايسين والأزيثروميسين وتريميثوبريم-سلفاميثوكسازول. إن الانتشار المرتفع لعزلات الإيشيريشيا كولاي والإيشيريشيا كولاي الافتراضية المنتجه لسموم الشيقا من أنسجة الأسماك ومسحات الايدي من الإنسان ومقاومتها للعديد من المضادات الحيويه والكشف المرتفع نسبيًا عن جينات الضراوة يشير إلى وجود خطر محتمل للإصابة بعدوى التسمم الغذائي في محافظة الإسماعيلية اثر تناول لحوم هذه الاسماك اذا كانت غير مطهية جيدا او عن طريق تلوث الأغذية، وبالتالي، فإن الفحص الصحي المنتظم لمتداولي الطعام والنظافة الشخصية هي إجراءات أساسية لتقليل العدوى المنقولة بالغذاء للإيشيريشيا كولاي.