Phenotypic and Genotypic Characterization of *E. Coli* Isolated From Fish and Human

Ashraf A. AbdEl-Tawab, [1]Fatma I. El-Hofy, [1]Adel M.El-Gamal[2]andHeba O. Ibrahim[3]

[1]*Bacteriology, Immunology and Mycology Department Faculty of Veterinary Medicine, Benha University, Egypt.*

[2]*BacteriologyDept, Animal Health Research Institute, Kafr El-Sheikh branch. Egypt.*

[3]*Veterinary Medicine Directorate, Kafr El-Sheikh branch. Egypt*

A B S T R A C T

The present work aimed to isolate and characterize *E.* coli bacteria that transmitted from fish to fish handlers in markets and farm workers, for this 200 apparently healthy Oreochromis niloticus were collected randomly from different markets and farms and 50 human skin swabs from sellers at markets and workers at farms were obtained at Kafr El-sheikh governorate, Egypt. These samples were cultured and biochemical characters was studied. Serological identification, antibiogram activity and molecular characterization of some virulence factors were detected. Results, the prevalence of *Escherichia* coli was 17.5% in Tilapia while its prevalence in human was 24%. *Escherichia* coli serotypes from fish were O_{153} :H_2, O_1 :H_7, O_{125} :H_{21} and O_{78}. Only one from four isolates of *Escherichia* coli was positive to *eaeA* gene and the four isolates were negative to *hly* gene.

Key words: *E. coli*, biochemical, in vitro sensitivity, virulence genes, transmission, fish, human

http://www.bvmj.bu.edu.eg) **(BVMJ-34(2): 41-50, 2018)**

1. INTRODUCTION

The most popular fresh water fish in Egypt are Oreochromis niloticus. The fish flesh, which is the main edible part, is generally sterile immediately after catching; however, it may become contaminated with different microorganisms during subsequent handling as these microorganisms can penetrate from skin and the gut to the flesh (El-olemy *et al*., 2014). Zoonotic diseases occurred during handling the affected fish (Magdy *et al*., 2014).

Escherichia coli in fish is considered as an indicator of sewage pollution. Most of the *E.* coli is normal inhabitants in the small intestine and they are non-pathogenic, meaning they do not cause disease in the intestine. *E.* coli spreads outside the intestine cause disease. The pathogenic strains of *E.* coli may cause diarrhea by producing and releasing toxins and cause deaths in fish (Soliman *et al*., 2010).

Antibiotics used in both veterinary and human medicine include penicillins, cephalosporins, tetracyclines, chloramphenicols, amynoglycosides, spectinomycin, lincosamide, macrolides, nitrofurans, nitromidazoles, sulfonamides, trimethoprim, polymyxins, quinolones .However, evolution of bacteria towards resistance has been considerably accelerated by the selective pressure exerted by over prescription of drugs in clinical settings and their heavy use as growth promoters for farm animals such as fish.(Samuel *et al*., 2011)

Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on different reasons such as the season, patients' contact with fish, dietary habits and the immune system status of the exposed individual (Novotny *et al*., 2004).

With the increasing intensification of aquaculture production, diseases cause problems in the fish farming industry.Although vaccines are being developed and marketed but cannot be used as universal disease control measures in aquaculture. During the last decades, antibiotics used for fish diseases management, improvement of growth and efficiency of feed conversion (Denev *et al*., 2009).

So, The aim of this study is to detect, isolate and characterize *E. coli* transmitted from Oreochromis niloticus to fish handlers and farm workers.

2. MATERIALS AND METHODS

2.1.Sampling:-

2.1.1. Fish samples:

A total of 800 samples, 200 of each (gill, liver, spleen and kidney samples) from 200 apparently healthy 200 Tilapia niloticus were obtained randomly from some markets and farms in KafrEl-sheikh governorate (Egypt). 100 Tilapia fish were collected from markets and 100 from farms. Fish samples were placed in strong, clean and aseptic bags then packed in column and surrounded with ice bags and brought to laboratory on the day of collection to microbiological department, Animal health research institute, Kafrelsheikh Lab.

2.1.2. Human samples: A total of 50 human skin swabs (25 skin swabs from sellers at markets and 25 from workers at farms) were collected with 5 ml saline to avoid dryness of samples and transported to the bacteriological lab to Microbiological department, Animal health research institute, Kafrelsheikh Lab.

2.2.Bacteriological examination:-

2.2.1.Isolation of *E*. coli from fishes: (Soliman *et al*., 2010)

Apiece of liver, spleen, kidney and gills were cultured separately on to MacConkey broth for isolation of *E*. coli. The MacConkey broth samples were streaked onto Eosine Methylene Blue agar for isolation of *E*. coli (selective plating) and incubated overnight at 37 °C.

2.2.2. Isolation of *E*. coli from human skin swab samples: (El-olemy *et al.*, 2014)

Wetted human skin swab was inoculated separately in 5ml MacConkey broth, for isolation *E*. coli, the inoculated tubes were incubated at incubator. The MacConkey broth samples were streaked on to Eosine Methylene Blue agar plates, and incubated overnight at 37 °C.

2.2.3.Biochemical identification:-

The pure colonies of isolates were identified biochemically according to (Koneman *et al*., 1983 and Quinn *et al*., 2002).

Indole, Methyl red, Vogues-Proskauer, Simmon citrate and Triple sugar iron tests were done.

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

- Two separate drops of saline were put on a glass slide and a portion of the colony from the suspected culture was emulsified with the saline solution to give a smooth fairly dense suspension.
- To one suspension, control, one loopful of saline was added and mixed. To the other suspension one loopful of undiluted antiserum was added and titled back and forward for one minute.
- Agglutination was observed using indirect lighting over a dark background. When a colony gave a strongly positive agglutination with one of the pools of polyvalent serum, a further portion of it was inoculated onto a nutrient agar slant and incubated at 37°C for 24 hours to grow as a culture for testing with mono-valent sera.
- A heavy suspension of bacteria from each slope culture was prepared in saline, and slide agglutination tests were performed with the diagnostic sera to identify the O-antigen.

2.4.In-Vitro antibiotic sensitivity of bacteria according to Srivani (2001).

As shown in Table(1, 6). Subcultures from the isolates were prepared and the test was applied as follows:

A smooth single colony was inoculated in 5 ml nutrient broth and incubated at 37°C for 18 hrs, then turbidity was adjusted to 0.5 McFarland contain (1.5×10^8) colony forming unit/ml, then few drops of the inoculated broth were flooded on to the surface of Muller-Hinton agar plates. Excess of cultural fluid was removed aseptically and the plates were allowed to stand for 15 minutes at 37°C for dryness. Then the inoculated plates were over laid with antibiotic discs using a sterile forceps, the discs were distributed in a manner where the distance among them was optimum and away from the edge of plate to avoid overlapping of inhibition zones and give more wide area for the zone of inhibition. The inoculated plates were incubated at 37°C for 24 hours. The Inhibition zones, in mm were measured and scored as sensitive, intermediate and resistant categories with the critical break points recommends by CLSI (CLSI, 2011).

2.5. Detection of virulence factors of *E*. coli:.

Extraction of DNA according to QIAamp DNA mini kit instructions, Preparation of PCR Master Mix According to Emerald Amp GT PCR mastermix (Takara) Code No. RR310Akit,Cycling conditions of the primers during cPCR (temperature and time conditions of the two primers during PCR according to specific authors and Emerald Amp GT PCR mastermix (Takara) kit) they have specific sequence and amplify a specific product as shown in Table (2), DNA Molecular weight marker the ladder 100bp was mixed gently by pipetting up and down. 6 µl of the required ladder were directly loaded and agarose gel electrophoreses (Sambrook *et al*., 1989).

3. RESULTS

The morphological characters of the colonies on Eosine Methylene Blue were brilliant green. A total of 47 bacterial isolates were obtained from examined samples, 35 bacterial isolates from fish and 12 bacterial isolates from human. *E*.coli isolated from Tilapia with an incidence (17.5%) as shown in (Table 3).

E. coli isolated from human with an incidence (20%) of isolates were from sellers at markets and (28%) from workers at farms. as shown in (Table 3).

E. coli give positive results to Indole, Methyl red and negative results to Vogues-Proskauer and simmons citrate. *E*. coli give yellow slant

and yellow bottom without H_2S production as shown in Table (4).

Ten *E*. coli isolates were serotyped, six from Tilapia and four from human, serological identification revealed that five isolates from Tilapia were belonging to (O_{153} :H_2, O1 :H_7, O_{125} :$H_{21}$and 2 O_{78}). Three isolates from human belonging to (O_{153} :H_2, O_{26} :H_{11} and O_{78}) as shown in (Table 5).

Antibiogram results revealed that *E*.coliO_{153}, O_1 isolates were resistant to Flumequine but *E*.coliO_{125} isolates were sensitive to Flumequine. *E*. coli O_{78}, O_{26} isolates were resistant to Doxycillin but *E*.coliO_{78} from human was resistant to Ciprofloxacin and Chloramphenicol. (Table 6).

Identification of *eaeA* and *hly* virulence genes of four *E*. coli isolates that were serotyped and the results revealed that only one isolate contain *eaeA* gene as shown in (Table 7) and(Figures 1,2).

Table (1): Effect of GSPE administration on serum glucose, urea and creatinine concentrations in streptozotocin induced diabetic nephropathy rats (mg/dl).

Data are presented as (Mean ± S.E). S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table (1): Antimicrobial discs, concentration or antibiogram profile of antibiotic susceptibility of their action on the isolated *E.coli*.

Susceptible (mm)	Intermediate (mm)	Resistant (mm)	Sensitivity disc content (µg)	Antimicrobial agent
14 or more	12-13	11 or less	10	Amoxicillin (AML)
19 or more	15-18	14 or less	30	Doxycyclin (DO)
18 or more	13-17	12 or less	30	Chloramphenicol (C)
20 or more	15-19	15 or less	5	Ciprofloxacin (CP)
23 or more	14-22	13 or less	15	Erythromycin (E)
15 or more	12-14	11 or less	30	Flumoquin (UB)
17 or more	13-16	12 or less	30	Neomycin (N)
29 or more	21-28	20 or less	10 IU	Penicillin (P)
16 or more	11-15	10 or less	25	Sulphamethoxazol (SXT)

Table (2): Oligonucleotide primers sequences Source: Metabion (Germany).

Target MO	Gene	Sequence 5-3	Amplified product	Reference
E. coli	$eaeA_R^F$	ATGCTTAGTGCTGGTTTAGG GCCTTCATCATTTCGCTTTC	248 bp	Bisi-Johnson *et al*., (2011)
	Hly_R^F	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	1177 bp	Piva *et al*., (2003)

Table (3): Incidence of *E. coli* isolated from Tilapia and human.

Number of collected fishes and skin swabs from human	Incidence of *E. coli*	
	%	*E. coli*
Total Tilapia n=200	17.5	35
Tilapia from farms n=100	15	15
Tilapia from markets n=100	20	20
Sellers from markets n=25	5	20
Workers from farms n=25	7	28

Table (4)Biochemical tests of *E*. coli:

Bacteria	Biochemical tests				
	Indole	Methyl red	Voccus prosqwer	Citrate utilization	TSI
E. coli	+	+	-	-	A/A -ve H_2S

Table (5): Results of serological identification of *E*.coli isolates.

Serial	Identified Bacterium	Serodiagnosis	Strain
1	*E*.coli(F)	O_{153} : H_2	EPEC
2	*E*.coli(F)	O_1 : H_7	EPEC
3	*E*.coli(F)	O_{125} : H_{21}	ETEC
4	*E*.coli(F)	O_{78}	EPEC
5	*E*.coli(F)	O_{78}	EPEC
6	*E*.coli(H)	O_{153} : H_2	EPEC
7	*E*.coli(H)	O_{26} : H_{11}	EHEC
8	*E*.coli(H)	O_{78}	EPEC

(F) *E*.coli isolated from fish (H) *E*. coli isolated from human

Table (6):Antimicrobials sensitivity results for *E.* coli isolates according to Srivani (2001).

Antimicrobial agents	Diffusion zone break point (mm)	*E*.coli O153(F)	*E*.coli O1(F)	*E*.coli O125(F)	*E*.coli O78(F)	*E*.coli O78(F)	*E*.coli O153(H)	*E*.coli O26(H)	*E*.coli O78(H)
Penicillin(P)	20≤	29(S)	31(S)	30(S)	21(I)	23(I)	28(S)	33(S)	30(S)
Amoxicillin(AML)	14≤	20(S)	19(S)	21(S)	19(S)	19(S)	21(S)	19(S)	22(S)
Ciprofloxacin(CP)	12≤	17(S)	5(R)	8(R)	15(I)	19(S)	16(I)	19(S)	11(R)
Chloramphenicol(C)	15≤	18(S)	10(R)	8(R)	16(I)	17(I)	15(I)	15(I)	4(R)
Erythromycin(E)	13≤	23(S)	24(S)	25(S)	11(R)	3(R)	5(R)	8(R)	22(S)
Doxacillin(DO)	16≤	17(I)	20(S)	17(I)	21(S)	20(S)	19(S)	17(I)	20(S)
Neomycin(N)	12≤	19(S)	18(S)	17(S)	31(I)	19(S)	18(S)	19(S)	19(S)
Flumquine(UB)	15≤	18(S)	13(I)	20(S)	6(R)	21(S)	22(S)	8(R)	17(S)
Sulphamethoxazol+trimethoprime(SXT)	10≤	8(R)	4(R)	16(S)	18(S)	12(I)	19(S)	20(S)	20(S)

(F)Bacteria isolated from fish (H) Bacteria isolated from human

Table (7): Results of molecular identification of *eaeA* and *Hly*gene of *E.* coli.

Results		Sample	Target MO
Hly	*eaeA*		
-ve	-ve	1	*E*. coli
-ve	+ve	2	
-ve	-ve	3	
-ve	-ve	4	

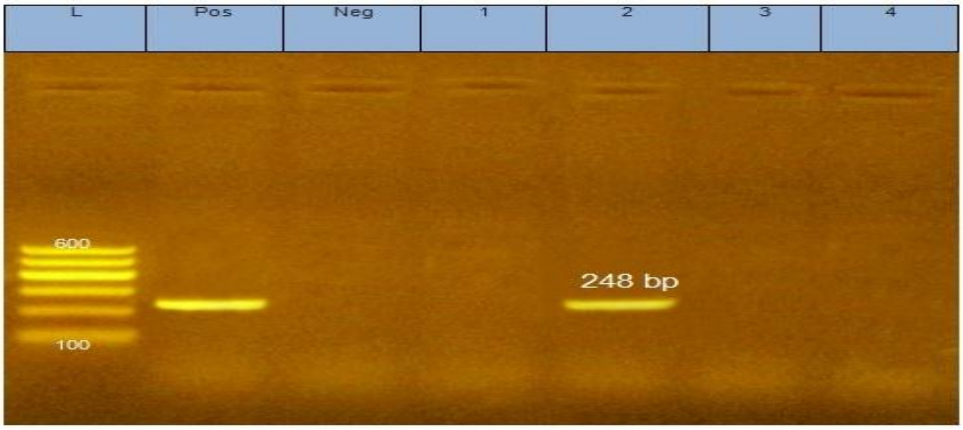

Figure (1): Agrose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Neg: Negative control, lane Pos: Positive control, lane 2: *eaeA* virulence gene of *E.* coli. The size in base pairs (248bP) of PCR product is indicated for the bands.

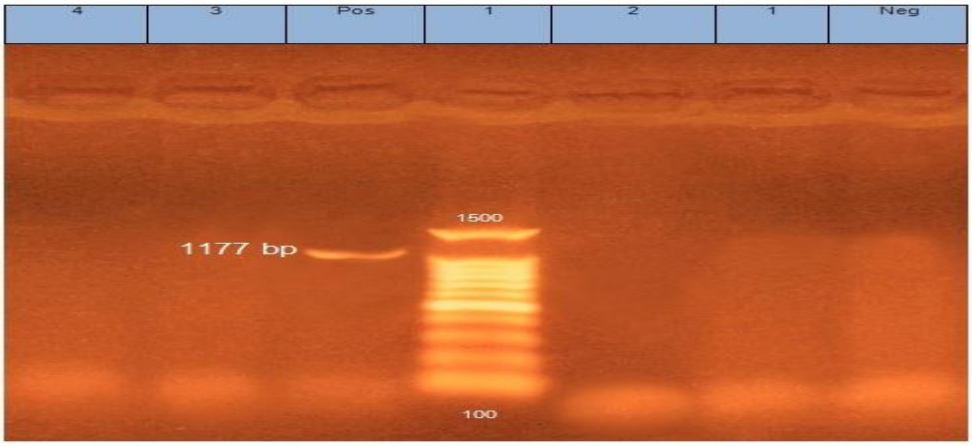

Figure (2): Agrose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Neg: Negative control, lane Pos: Positive control of *Hly* virulence gene of *E.*coli. The size in base pairs (1177bP) of PCR product is indicated for the bands.

4. DISCUSSION

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There are a variety of types of *E*. coli. *E*. coli is a bacterium that commonly lives in the intestine of people, animal and fish. There are many strains (types) of *E*. coli. Most of the *E*. coli are normal inhabitants in the small intestine and colon and they arenon-pathogenic, meaning they do not cause disease in the intestine. Soliman *et al*. (2010).

E. coli isolated from Oreochromis niloticus with an incidence of (17.5%) as shown in(Table 3).These results partial agree with Saqr et al. (2016) reporting incidences (18.3%). Higher incidences of *E*.coli were recovered by Amr et al. (2012), David et al. (2009), Galal et al. (2013)and Gupta et al. (2013) who reported incidences of *E*. coli 50%, 57.1%,29.34% and 36% respectively. But Atwa (2017) isolated *E*. coli from skin, muscle, intestine and liver with incidences 25, 22.5, 25 and 35% respectively.

E.coli isolated from human with incidence of (20%) from sellers in markets and (28%) from workers in farms as shown in (Table 3). The results partially similar with El-olemy et al. (2014) reporting incidences (20%) from fish handlers and (37.5%) from house wifes.

Ten *E*.coli isolates were serotyped (six from Tilapia and four from human). Serotyping revealed that five isolates from Tilapia were belonging to (O_{153} :H_2, O_1 :H_7, O_{125} :H_{21} and 2 O_{78}.) and three isolates from human belonging to (O_{153} :H_2,O_{26} :H_{11} and O_{78}) as shown in(Table 5). But Barbosa et al. (2014) by serological identification of 49 *E*. coli revealed that the most common serogroups were O_{125}:H_{21} ,O_{126}:H_{11} and O_{158}:H_2.

In this study as mentioned at (Table 6). *E*.coli O_{153} :H_2 strain isolated from fish and human, *E*. coli O_{125}:$H_{21}$and*E*.coli O_{78} isolated from fish and *E*. coli O_{26}:H_{11}were resistant to Doxicyclin. *E*. coli O_{78} strain isolated from human were resistant to Ciprofloxacin and Chloramphenicol. Soliman et al. (2010) reported that *E*.coli isolates were sensitive to Enrofloxacine, Oxanilic acid and Spectinomycine. Our results disagree with Samuel et al. (2011) who explained that there is no *E*. coli shows resistance to Norfloxacine, Sulphamethoxazol+ trimethoprim and Chloramphenicol.

Only one isolate of *E*.coli contain *eaeA* virulence gene of *E*.coli and none of the isolates contain *Hly* virulence gene of *E*. coli. Examination of *eaeA* virulence gene of *E*. coli giving PCR product of (248) bP size as shown in (Table 7). The prevalence of *eaeA* virulence gene of *E*. coli was 25%. And this agrees with Kargar and Hamayoon (2015) as they recorded only one isolate from seven isolates of *E*. coli O157:H7 contains *eaeA* virulence gene of *E*. coli but not has *hlyA* virulence gene of *E*. coli. But Aljanaby and Alfaham (2017) revealed that the lower prevalence of virulence genes in *E*.coli were (4%) of *eaeA* and *stx1* virulence gene of *E*.coli.

5. CONCLUSION

We can concluded that the most important *Escherichia* coli serotypes causing severe losses in fish are O_{153}:H_2, O_1: H_7, O_{125}: H_{21} and O_{78} these bacteria can transmitted to human and cause disease. *E*.coliO_{153}:H_2, O_1:H_7 isolates were resistant to Flumequine but *E*.coliO_{125}:H_{21} isolates were sensitive to Flumequine. *E*. coli O_{78}, O_{26}:H_{11} isolates were resistant to Doxycillin but *E*.coliO_{78} from

human was resistant to Ciprofloxacin and Chloramphenicol.

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