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"DETECTION OF STX1GENE, STX2 GENE AND FLICH7 GENE SHIGA TOXIN OF *ESCHERICHIA COLI* ISOLATED FROM HUMAN AND CHICKENS IN KARBALA CITY"

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ABSTRACT

Aims: This study was conducted to isolate and identify by PCR analysis for presence stx1gene**,** stx2 gene and fliCh7 gene of Shiga toxin *Escherichia coli* from broiler chickens and humans from live chicken markets in different areas of Karbala.

Material and methods: A total of 200 fecal samples were collected from chicken broiler (n=150) and human (n=50) . *E. coli* were isolated and identified by cultural, biochemical, Api-20E, VITEK 2 system and PCR.

Results: 13 Human and chicken positive samples include 10% , 5.3% and were derived from human and broiler chickens respectively that diagnosis by using PCR analysis for presence stx1gene**,** stx2 gene and fliCh7 gene. Shiga toxin–producing *E. coli* results negative stx1gene and positive stx2 gene .

Conclusion: Considering the findings of this study demonstrate that the chicken's digestive track hosts a bacterial pathogen, indicating that interferences are required to reduce Shiga-toxin-producing Escherichia coli(STEC) transmission.

Keywords**:** *E.coli***,** stx1gene **,** stx2 gene **,** Shiga toxin

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INTRODUCTION

For most of history, Escherichia coli has been one of the most studied bacteria, and it has served as the foundation for many important biochemical and genetic principles [1]. E. coli is a broad classification of bacteria strains. Some of them can be discovered in the gut microbiota of humans and other animals[2]. E. coli bacteria are found in intestines of (animals and humans) as commensals, although pathogenic forms reason sickness in chickens [3]. Other domestic and wild animals, such as birds, feral pigs, chickens, and insects, shiga toxin-producing *Escherichia coli* (STEC) strains maybe carried. STEC (verocytotoxin-producing *E. coli*) is a type of the bacteria that produces verocytotoxin (VTEC) [5]. STEC is a zoonotic pathotype foodborne pathogen that has been linked to occasional instances to major outbreaks over the world. The death and morbidity linked with several recently STEC Outbreaks have

been highlighted the public health hazard
this pathogen poses [6,7] , and gastrointestinal disorders in humans that can lead to serious consequences [8]. STEC infects people of all ages and genders, but young and elderly people account for the majority of reported cases [9]. E. coli strains produce two kinds of Shiga toxins: (Stx1&Stx2) , Stx1which have three strains a, b, and c while Stx2 have seven strains (a to f). The most prevalent toxigenic profile is Stx2linked to serious human disorders, and it may have two clinically significant variants, Stx2c and Stx2d. Enterohaemorrhagic E. coli (EHEC) is a name that has been used to refer to a subgroup of highly aggressive E. coli bacteria [10]. STEC strains, on the other hand, can induce Heamolytic uremic syndrome (HUS) with hemorrhagic colitis (HC). The 'big six' serogroups (O121,O111,O103 O45,O26 and O145) are the most widely seen non-O157STEC strains. [11].

MATERIALS AND METHODS

- **Samples Collection:** 200 samples were gathered from poultry coops in the holy city of Karbala, with 50 human stool samples and 150 fecal swabs from broiler chickens. All samples were taken with sterile swabs, placed in sterile tubes with 5 ml of Buffer Peptone Water, and carried to the lab in a refrigerated box.
- **E. coli Isolation and Identification*:*** all the samples were placed in(5) ml of Buffer Peptone Water (BPW) and incubated overnight at 37°C before being subculture.
- **Eosin methylene blue agar (EMB):** It was prepared and sanitized according to the manufacture company (Himedia (India)), poured onto petri dishes beneath a sterile hood, and refrigerated at 4°C for E. coli identification.
- **Microscopic study by Gram's staining method:** Following the isolation of bacteria on Eosin methylene blue agar, single colonies were picked up, gram stain was used to stain the bacteria &studied under a microscope to assess (size, shape, and arrangement) of bacteria [12].
- **Biochemical tests:** E.coli was identified using biochemical assays that included the IMViC test (indol, methyl red, Voges proskauer, citrate utilization) and conservative glucose and lactose fermentation using the Triple suger iron test (TSI) and urease. The confirmation was carried out using API 20 E System, kit (BioMerieux, France) [13]. Furthermore, the VITEK 2 system was used to identify bacterial strains using a gram negative (GN ID) card, as per the manufacturer's instructions BioMerieux, France [14].
- **PCR detection:** DNA was extracted from bacterial growth using the Wizpio solution Extraction, kit procedure. (Wizpio, south Korea).The conventional PCR was performed to recognize STEC on stx1 gene using primers F (ATAAATC GCCATTCGTTGACTAC), R (AGAACGCCCACTGAGATCATC) and stx2 gene using primers F(GGCACTGTCTGAAACTGCTCC), R (TCGCCAGTTATCTGA CATTCTG) and fliCh7 gene using primers F(GCGCTGTCGAGTTCTATCGAGC), R (CAACGGTGACTTTATCG CCATTCC).
  - **Reaction Setup and Thermal Cycling Protocol:** There was a total volume of 25µl used with 12.5 µl of PCR, 1 µl of each forward primer and reverse primer. 3 µl of template DNA, Nuclease Free Water up to 25 µl .
- **Statistical analysis :** The results were analyzed at the level of significant by Chi square statistic when p- value < 0.05. The statistical study was carried out with the help of the SPSS program [15].

RESULTS

- **Sample collection :** A total of 200 fecal samples were obtained from chicken broiler and human with diarrhea they were isolated and identified . In the current study , found most ages were positive

for a human in ages (15 - 20) years with non-significant (P>0.05), while the ages were in broiler chickens which more susceptible to infection (20- 30) days with significant difference (P<0.05) .

- **Result of isolation** the results the *E. coli* growth on Eosin methylene blue (EMB) agar color appearance (green metallic sheen) , (EMB) used to identification of *E. coli* was established on colonizer morphology and color. The result of of *E .coli* according to (culture method , Api-20E system , Biochemical tests and demonstrated by a vitike2 compact system assay . It is consider non-Significant (P<0.05). *STEC* confirmed through results biochemical test by use Api-20E system where the results of Api-20E test detected Numerical definition file (7144572) which gave diagnosis (99.8 %) , demonstrated by a vitike2 compact system assay diagnosis (99 %) (table 1).

Table (1): The results of *STEC* isolate's by using EMB , VITK and PCR

Isolation results	EMB +ve	VITK 2 +ve	PCR/STEC +ve No.	PCR/ STEC - ve No.	X2 value
Workers	28 (56%)	20 (40%)	5 (10%)	45 (90%)	Calculated X2=1.344 Tabulated X2 =3.84 df= 1 non-significant (P>0.05)
Broiler	110 (73%)	98 (65%)	8 (5%)	142 (94%)	
Total	138	118	13	187	

- **Result of PCR**: Electrophoresis on a 1% agarose gel was used to detect the results, which revealed the DNA as compact bands when exposed to UV light. PCR amplification result of 255 bp fragments of (Stx2) genes was seen on an agarose gel electrophoresis (Figure 1) . While in chicken showed amplification product of 255 bp fragments of (Stx2 and fliCh7) genes by PCR (Figure 2). There were 13 positive samples from humans and chickens, with 10% and 5.3 percent from humans and broilers, respectively that diagnosis by using PCR analysis for presence stx1gene**,** stx2 gene and fliCh7 gene. Shiga toxin–producing *E. coli* results negative stx1gene and positive stx2 gene .

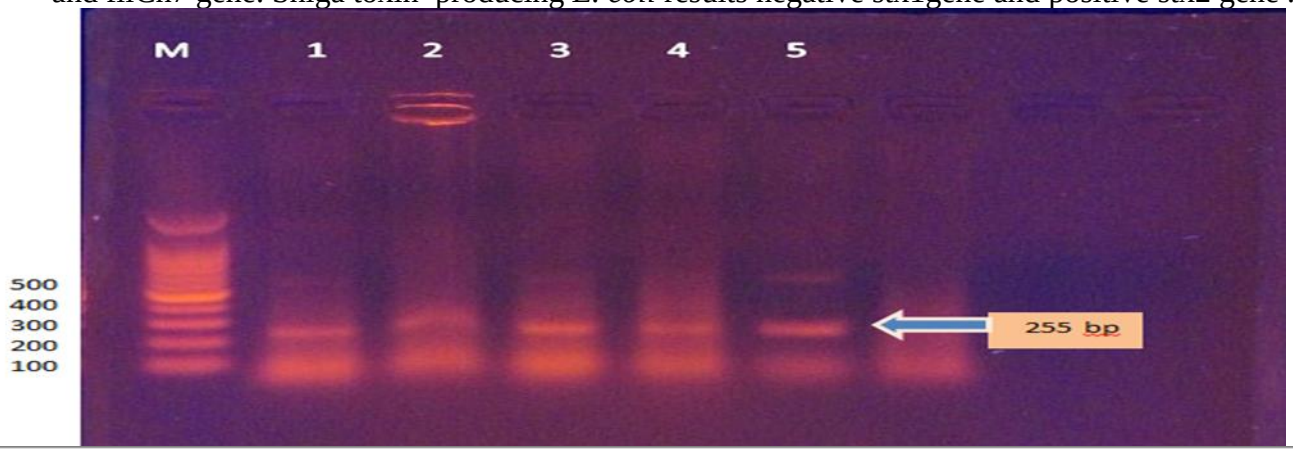

Figure (1): PCR amplification product of 255 bp fragments of (Stx2) genes on agarose gel electrophoresis. A 2% agarose gel was used (5 volt\ cm2\ 1.5 hours) Marker ladder (Lane M) (100-500 bp) Lanes 1 to 5 show the human PCR product.

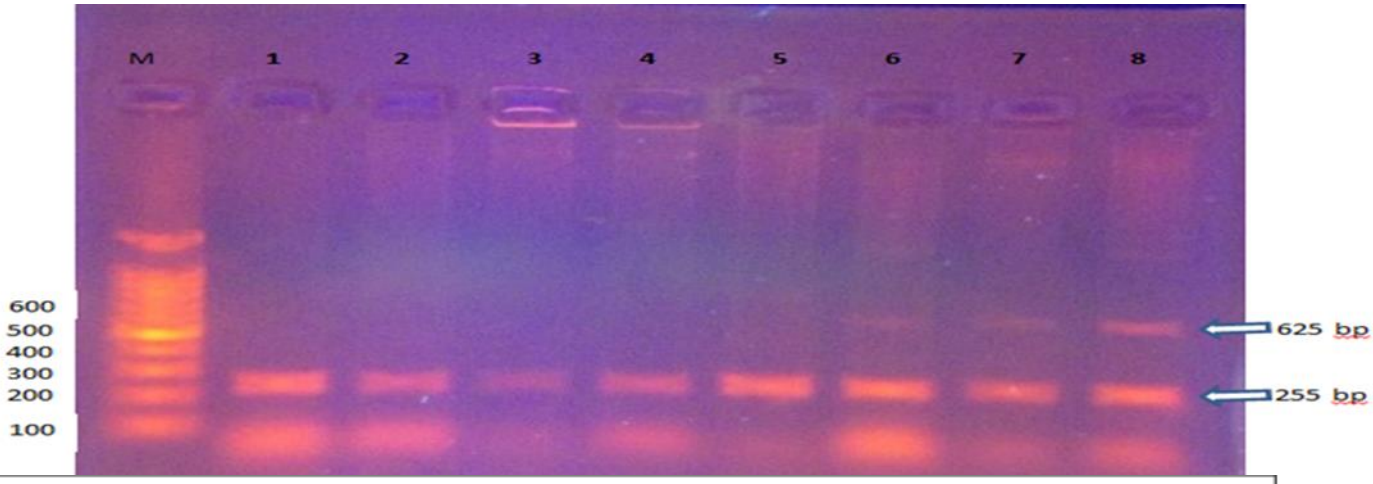

Figure (2): PCR amplification product of 255 bp fragments of (Stx2 and fliCh7) genes on agarose gel electrophoresis. A 2% agarose gel was used (5 volt\ cm2\ 1.5 hours) Marker ladder (Lane M) (100-500 bp) The product PCR of chickens is shown in Lanes 1 to 8.

DISCUSSION

In the current study was found most ages were positive for a human in ages (15 - 20) years with non-significant (P>0.05), in Nigeria have been recordedshowed the highest number *E. coli* infection O157: H7 among adults 10-29 years [16]. It was observed during the study that positive ratios chicken broiler was more infected than laying hens that agreement with several researchers which found the proportions infection of Broiler *E. coli* levels were substantially greater than in the laying-hen population. [17] . This may be due to the high immunity of laying hens, in laying hens was in ages (28-58) weeks with significant difference (P<0.05), Joseph Fuh, (2018) referred that most infected ages 18-72 weeks old on laying hens [18]. while the ages were in broiler chickens which more susceptible to infection (20- 30) days with significant difference (P<0.05), the study agreed with a study in Ethiopia that discovered that the frequency of *E. coli* O157: H7 is disease in poultry more frequent in young ages than in older adults [19]. A total of 5(10%) positive sample of *E.coli* were isolated from 50 stool specimen selected from human (workers in chicken fields). The results Our findings were agreement with Lagos, Nigeria, researchers show *E. coli* from 6% of diarrhea sufferers [20].On the other hand, our study did disagreement up with other researchers, Klaif *et al.*, 2019 found in his search a high incidence of infection [21] . As for others, they recorded lower incidence such as O157 : H7 prevalence in patients with 0.4% diarrhea was recorded by Bolukaoto et al., 2019 [22] , On the other hand, our study disagreement with that of other researchers, as Cloacal E. coli O157: H7 was isolated in 13.4% of specimen selected from chicken farmhouses [19] . And another study found 13% and 14% of serotype O157: H7 from poultry farms in (Lagos and Ibadan) correspondingly, this confirms the existence of *E. coli* O157: H7 strains in poultry dropping and their prevalence in a high classification [23] . This variation could be attributable to sample methods, time, a lack of stringent sanitary standards between fields, or mutual pollution. Based on 16SrRNA gene analysis, and the match was made utilizing the National Center for Biotechnology Information's basic local search Tool (BLAST) (NCBI) , sepsi T test and EzTaxaon. And all isolates After DNA sequencing, *E. coli* serotype O157:H7 was obtained, which was 100% identical to one from a broiler origin. (Sample one MN689684.1) is the accession number, except for two isolations, one from human origin (sample four MT370824.1) is accession number, one of layer origin (sample two MT370810.1) is accession number sequencing correspond 99.93% . Analysis sequencing has been used in a number of studies to identify E. coli serotype O157:H7 [24] . Also, the results of the current research show up the E. coli prevalence O157: H7 for six months with non-significant association (P>0.05) survey of broiler chickens

feces, laying hen feces taken from cloacal swabs and human stool showed that the *E. coli* O157: H7 serotype was not noted in November and February. While *E. coli* O157: H7 was found in equal levels in the other months, In Turkey, near the geographical location of our study, where results showed agreement our study on chicken broiler, the most important event of *E. coli* O157: H7 was detected into both September , October, while bacteria were not detected between November and February. Among the same months, they showed in their study that the warm climate contributed to the infected may be that because the changing climatic conditions that occur in Iraq from time to time. In other study incidence of *E. coli* O157:H7 isolation come about the warm periods of spring and summer [25]. This disagreement due to the fact that the sample collection in our study was not the summer season.

CONCLUSION

Considering The findings of this study demonstrate that the chicken's digestive track harbors a bacterial pathogen, implying that interferences are required to reduce (STEC) transmission , so there must be protocols in place. Public health education and stringent hygiene practices in live chicken markets during chicken handling are critical to reducing the danger of cross contamination and infection spread among live chicken market workers.

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