Research Article

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Detection of Shiga Toxin Genes Presence in *Escherichia coli* Isolated from Chicken Fecal Samples

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Abstract

The current study was designed to isolate and identify *Escherichia coli* from chicken droppings and explore the presence of Shiga-toxin-producing *Escherichia coli* (STEC) in chickens in Basrah province, the period extending from October 18th, 2023, to 13 January 2024. Conventional microbiological techniques revealed that 109 (53.4%) of the 204 samples, 102 from backyard chickens, 48 from poultry fields, and 54 from chicken shops, tested positive for E. coli.The results of this technique indicated that 84 (77%) of them were *Escherichia coli* found in the examined samples. All isolates were undergone a polymerase chain reaction to detect Shiga-toxins genes (*stx1, stx2*). Neither *stx1* nor *stx2* were detected in any of the examined *E. coli* strains. These results indicated that the *E. coli* isolates from chicken were negative for Shiga toxin (*stx1, stx2*) genes.

Keywords: Chicken, *Escherichia coli,* Shiga toxin genes.

Introduction

The bacteria known as *Escherichia coli* are Gram-negative commensal bacteria that have a rod-like morphology and a flagellum, and they are members of the Enterobacteriaceae family (1). *E. coli* is a prevalent microbial flora of the human and poultry digestive tracts and other animals, though it can potentially become pathogenic for both. However, many *E. coli* isolates are not harmful. They are considered a sign of fecal contamination in food (2). It is the cause of several disorders in chickens, including coli granuloma, swollen head syndrome, cellulitis, omphalitis, and yolk sac infection (3). Extraintestinal pathogenic *Escherichia coli* (ExPEC) and intestinal pathogenic *Escherichia coli* (IPEC) are the two main categories into which pathogenic *E. coli* can be classified (4). The diverse collection of enteric pathogens known as

Shiga toxin-producing *E. coli* (STEC) is accountable for both widespread outbreaks and many sporadic infections across the globe (5). The basic virulence factors of these strains are Shiga toxins [Stx1 and Stx2] (6). *E. coli*, which produces Shiga toxins (STEC), is one of the most significant pathogens spread through food. These strains not only induce food poisoning but also serious illnesses such as hemolytic uremic syndrome, bleeding colitis, and diarrhea (7). STEC has been proven to be a zoonotic root with various groups of pathogenic E. coli in cattle from among ruminants, serving as the major reservoir for human diseases (8). An intensifying number of previous studies from different countries have listed low rates of prevalence of Shiga toxins in chicken (4%) in Northwest Iran (9). The rates of STEC in chicken carcasses, cloacae, chicken burgers, and giblets were confirmed to be 3.3%, 0.1%, 2.3%, and 10.3%, respectively, in Argentina (10), 1% in Turkey (11), and 0.5% in southern Vietnam (12). The current study aims to determine if *Escherichia coli* isolates from chicken fecal samples in the province of Basrah have Shiga toxin genes.

Materials And Methods

Samples collection: The samples used in this study were obtained from fresh droppings and cloacal swabs of chickens gathered from various parts of the Basrah region. There were 204 dropping and cloacal swabs from backyard chickens, poultry fields, and chicken shops from 18 October 2023 to 13 January 2024, (Table,1).

Microbiological methods: The samples were gathered by sterile swabs and transported directly to the Central Research Unit in the College of Veterinary Medicine by icebox. The lab cultivated the samples in peptone water for twenty-four hours at 37°C (13). After incubation, the samples were subcultured on MacConkey agar overnight at 37°C. From the pre-incubated samples, 3 pink colonies were chosen randomly from MacConkey agar and transferred to eosin methylene blue (EMB) agar overnight at 37◦C. The colonies were watched for metallic sheen appearance (14). The suspected *E. coli* colonies were submitted to various biochemical tests, including Gram stain (15), Simmons' Citrate (16), Indole production, Methyl red, and Voges–Proskauer (IMVIC) tests (17).

Molecular study: The suspected colonies were confirmed by being subjected to PCR to detect *the uid A* gene.

DNA Extraction: From nutrient agar, five E. coli colonies were inoculated into brain heart infusion (BHI) broth and incubated overnight at 37°C. The genomic bacterial DNA was extracted using the boiling method according to (18).

Molecular confirmation of *E. coli*: The *uidA* gene was amplified using the PCR technique to confirm the potential *E. coli* isolates discovered by conventional microbiological methods and

experimental conditions described by (19), using PCR technique, the amplicon size was 203 bp.

Detection of Shiga toxins genes (*stx1 and stx2*): Employing the primers listed in this work, the virulence genes *stx1 and stx2* were examined by the PCR technique to identify *E. coli* pathotypes. The primers were designed using the GenScript Tool, as shown in Table (2). The volumes of mixtures used for amplification of virulence genes *stx1* and *stx2* were prepared in a total of 25 µl PCR reaction, 7.5µL of nuclease-free water, 3µL of DNA template, 2µL of each primer, and 12.5 µL of the master mix (Promega, USA) were used.

Thermal cycling for *stx1* was conducted using the initial denaturation of 94 °C for 3 minutes, followed by 30 amplification cycles of 45 seconds at 94 °C, 45 seconds at 54 °C, and 45 seconds at 72 °C. This step was followed by a final extension step of 5min at 72 °C. As for *stx2*, the condition PCR consisted of 95 °C for 4 min, followed by 30 cycles of 95 °C for 45sec, 53 °C for 1min, and 72 °C for 1min, with a final extension step of 72 °C for 5min.

Table (1): Number and sources of samples used in this study

Source of sample	Type of sample		Total No.
	Cloacal swab	Droppings swab	
Backyard chicken	67	35	102
Poultry fields	48	0	48
Chicken shop (markets)	38	16	54
Total	153	51	204

Table (2): The primer sequences for detecting *Stx1 and Stx2.*

Primer	Primer sequences (5-3)	Length	Product size	GenBank accession no	Reference	Manufacturer
Stx 1	F:5′-CTGTGGCAAGAGCGATGTTA-3′ R:5′- CTCAACCTTCCCCAGTTCAA-3′	20bp	196 bp	NC_002695.2	This study	Promega / USA
Stx 2	F:5′-GTTCCGGAATGCAAATCAGT-3′ R:5′- CGGCGTCATCGTATACACAG-3′	20bp	206 bp	BA000007.3	This study	Promega / USA

Results

Identification of *E. coli* isolates

Of 204 dropping and cloacal swabs from chicken samples, 109 (53.4 %) samples were positive for *Escherichia coli* based on biochemical and morphological features. The isolates displayed short-rod Gram-negative bacteria, green metallic sheen colonies on EMB agar, pink colonies on MacConkey agar, and were positive for indole and methyl red: hence, the isolates were negative for Voges-Proskauer and Citrate utilization tests (Figure 1).

Molecular detection of *uidA gene*

The *uidA* gene was found using the PCR method, confirming the suspected isolates as *E. coli.* The product's size was 203 bp. Of 109 suspected isolates by using conventional biochemical tests, 84(77%) were confirmed as *E. coli* (Table 3), (Figures 2 and 3).

Molecular detection of *stx1 and stx2*

The results are shown in Figures (4 and 5) of the PCR analysis of E. coli isolates for the stx1 and stx2 genes, which are virulence genes in STEC. he results show that all isolated E. coli isolates lack the stx1 and stx2 genes, with a positive control for stx1 and stx2 provided by (20).

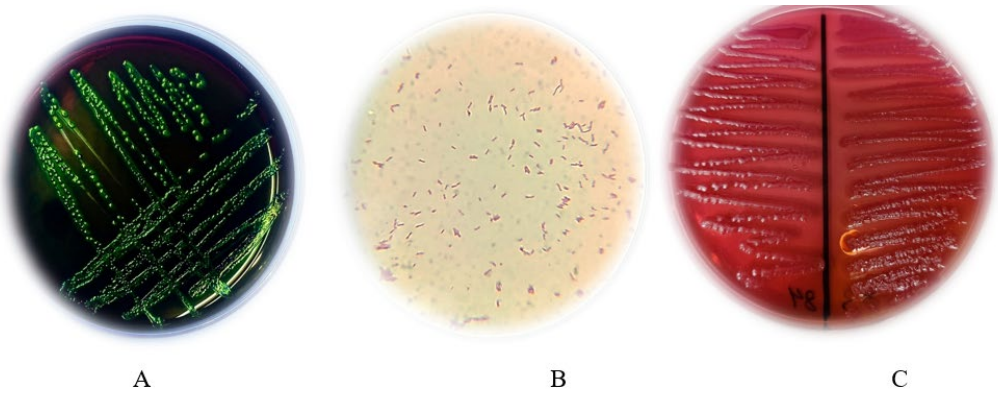

Figure (1): Morphological characteristics of isolates A. *E. coli* on EMB agar medium. B. Microscopical appearance of *E. coli*, short-rod Gram-negative bacteria. C. Growth on MacConkey agar.

Table (3): Results of *E. coli* identification using conventional microbiological techniques and PCR

Source	Total No.	conventional microbiological techniques		Detection by *uidA*	
		No.	%	No.	%
Backyard chicken	102	39	38.2	34	87.1
Poultry fields	48	44	91.6	36	81.8
Chicken shop (Market)	54	26	48.1	14	53.8
Total	204	109	53.4	84	77

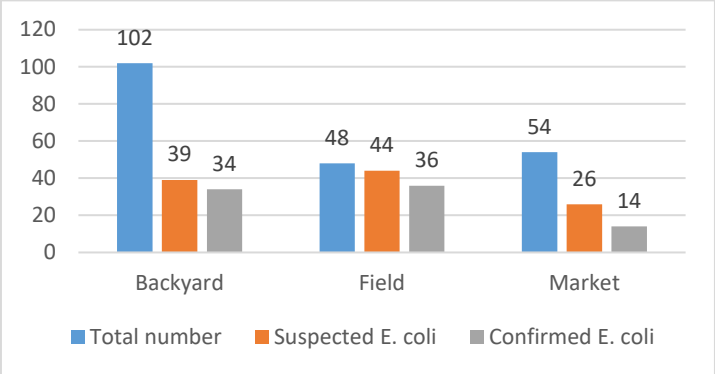

Figure (2): Total number of samples, suspected and confirmed isolates according to sample source.

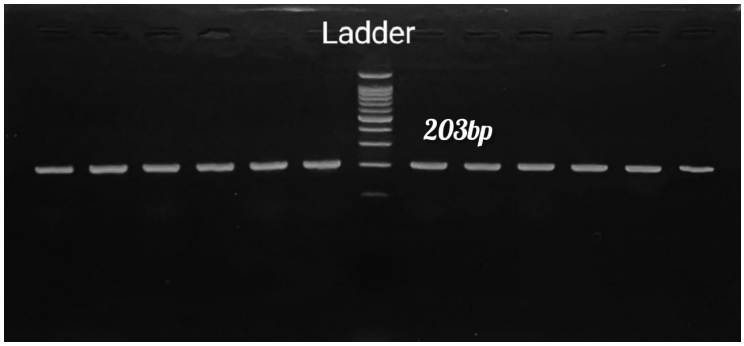

Figure (3): Product of PCR of *uidA* gene on 1.5% agarose gel, where (L) 100bp DNA ladder.

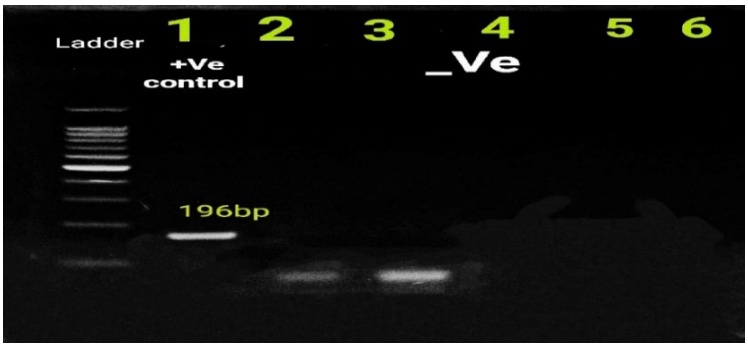

Figure (4): The *stx1* gene electropherogram. Lane (1) included a positive control sample, whereas lanes 2–6 had negative samples.

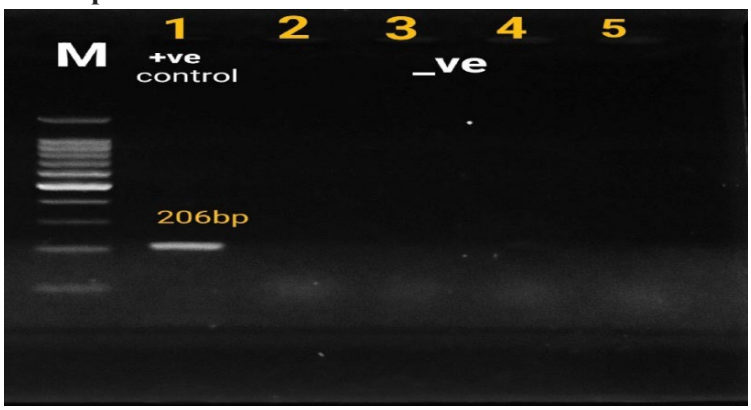

Figure (5): The *stx2* gene electropherogram. Lane 1 included a positive control sample, while 2–5 were negative.

Discussion

Escherichia coli is the main infectious pathogen in poultry (21). Shiga toxins (*Stx*), a class of cytotoxins made up of two primary kinds (*Stx1 and Stx2*), are produced by STEC strains (22). However, cattle and sheep are expected to be the natural reservoirs of these microorganisms, as they are the primary sources from which the STEC strains have been obtained. (23; 24).

The features and existence of pathogenic *E. coli* in healthy chickens may have implications for the health of animals and humans. This study aimed to evaluate the *E. coli* isolates from the feces of local chickens to see if they carried Shiga toxin. Out of all the samples, the net isolation rate of *Escherichia coli* was 53.4%. This result was found to be lower than 80% by (25). On the other hand, this rate is considered higher than 36% by (7).

The present study revealed the absence of stx1 and stx2 in 84 Escherichia coli isolates tested by the PCR technique in Basrah province. This is consistent with the observations of (26), who also found that fecal samples from 500 chickens had no STEC, and (27), who reported no STEC in 199 chicken fecal samples. This is in contrast to the results of (28), who detected

STEC genes in 7.3% of 422 chicken samples and 12.24% of isolates positive for both stx1 and stx2 genes (29). The main sources of Shiga toxin-producing *E. coli* are not chickens, but ruminants, particularly cattle and sheep, are more commonly associated with STEC, according to (19).

Conclusion

In the present study, genes (stx1 and stx2) were not found in any *E. coli* isolates obtained from chicken fecal samples from various sources in the Basrah province.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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الكشف عن وجود جينات سموم الشيجا في الإشريكية القولونية المعزولة من عينات براز الدواجن

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الخلاصة

صممت الدراسة الحالية لعزل وتشخيص بكتيريا الإشريكية القولونية من فضلات الدجاج واستكشاف وجود بكتيريا الإشريكية القولونية المنتجة لسموم الشيجا (STEC) في الدجاج في محافظة البصرة للفترة الممتدة من 18 تشرين الأول 2023 إلى 13 كانون الثاني 2024. من بين 204 عينة، كانت 102 عينة من دجاج الفناء الخلفي، و48 عينة من حقول الدواجن، و54 عينة من محلات الدجاج، وكانت 109 (53.4%) إيجابية بالنسبة للإشريكية القولونية باستخدام التقنيات الميكروبيولوجية التقليدية. تم استخدام تفاعل البوليميراز المتسلسل لتأكيد العزلات التي استهدفت جين *uidA* للإشريكية القولونية، أشارت نتائج هذه التقنية إلى أن 84 (77%) من العزلات كانت من الإشريكية القولونية من العينات التي تم اختبارها. تم تعريض جميع العزلات لتفاعل البلمرة المتسلسل للكشف عن جينات ذيفان الشيجا *(stx1 &stx2)*. لم تظهر أي من عزلات الإشريكية القولونية المدروسة وجود جينات *stx1* أو *stx2*. أشارت هذه النتائج إلى أن عزلات الإشريكية القولونية من الدجاج كانت سلبية لجينات ذيفان الشيجا (*stx1*، *stx2*).

الكلمات المفتاحية: دواجن، الإشريكية القولونية، جينات سموم الشيجا.