Assiut University web-site: www.aun.edu.eg

# PREVALENCE OF VIRULENCE FACTORS AND ANTIBIOTIC RESISTANCE GENES IN SHIGA TOXIN-PRODUCING ESCHERICHIA COLI ISOLATED FROM QUAILS

GHADA ALLAM ABD EL –DAYEM [1]; AHMED H. RAMADAN [2] and HANAA S. ALI [3]

[1] Poultry Diseases Department, Animal Health Research Institute **(**AHRI**)** (Mansoura Branch), Agriculture Research Center (ARC), Dokki, Giza, Egypt.

[2] Bacteriology Department, Animal Health Research Institute **(**AHRI**)** (Mansoura Branch), Agriculture Research Center (ARC), Dokki, Giza, Egypt.

[3] Pathology Department Animal Health Research Institute **(**AHRI**)** (Mansoura Branch), Agriculture Research Center (ARC), Dokki, Giza, Egypt.

**Received:** 17 October 2020; **Accepted:** 28 October 2020

## ABSTRACT

This study aimed to determine the prevalence, pathological investigation and profiles of virulence and antibiotic resistance genes in Shiga toxin-producing Escherichia coli (*E. coli*) isolated from quails. A total of 450 samples were collected from 150 quails (50apparently healthy, 50 diseased and 50 recently dead) for bacterial isolation. The isolated *E.coli* was tested for antimicrobial susceptibility and was screened for the presence of virulence genes (*stx1*, *stx2*, *eaeA*, and *hly*) and antibiotic resistance genes (*tetA*and*sul1*) using polymerase chain reaction (PCR). Seven-day old quails were challenged with *E. coli* to determine the pathogenicity of the isolated strains. A total number of 93 (20.67%) *E. coli* isolates were recovered. The most prevalent serogroups O125, O20, and O78. *E. coli* were highly sensitive to neomycin, norfloxacin, and, colistin, highly resistant to sulphamethoxazole, tetracycline, and amoxicillin. *Stx2* and *eaeA* genes were detected in (3/8) and (2/8) of isolates, respectively, while all isolates were negative for *stx1* and *hly* genes. Moreover, *tetA* and *sul1* genes were detected in 100% of examined isolates. The pathogenicity test revealed that *E .coli* (O125 STEC) was a highly pathogenic and induced mortality rate of 40%. Histopathological changes supported the post mortem lesions. In conclusion, these data can be considered alarming since quail may be a carrier of STEC that is highly pathogenic for humans. Also, these *E. coli* showed marked resistance to several antibiotics of clinical interest. So proper hygiene regime and biosecurity measures are necessary to minimize the risk of spread of infection to the human population.

***Keywords****:* Quails; STEC; antibiotic resistance; virulence genes

## INTRODUCTION

Quail farming is considered as one of the most alternative sources for meat to overcome protein shortage in Egypt. This bird species is characterized by rapid growth, short generation, low feed intake, nutritious meat, higher egg production potentiality, and less susceptible to bacterial diseases as compared to other poultry species *(Yusuf et al., 2016).* Avian pathogenic Escherichia coli (*E. coli*) strains are responsible for many cases of colibacillosis, yolk sac infection, cellulitis, and omphalitis in quails *(Salehi and*

*Corresponding author:* AHMED H. RAMADAN
*E-mail address:* ahmedhegazy711@yahoo.com
*Present address:* Bacteriology Department, Animal Health Research Institute **(**AHRI**)** (Mansoura Branch), Agriculture Research Center (ARC), Dokki, Giza, Egypt;

*Ghanbarpour, 2010).* Based on the virulence factors, *E. coli* has been classified into different pathotypes: enterotoxigenic *E. coli* (ETEC) enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), diffusely adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), and enterohemorrhagic or Shiga toxin-producing *E. coli* (EHEC/STEC). The latter is most commonly associated with outbreaks of foodborne diseases *(Bandyopadhyay et al., 2011)*, hemorrhagic diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura *(Shahreza et al., 2017).* STEC pathogenicity depends mainly on many virulence genes such as Shiga toxin type 1 (*stx1*), *stx2*, *eaeA*, and *hlyA*. The *eaeA* gene encodes intimin, which enables intimate attachment of bacteria to the lining epithelia of the intestine *(Ateba and Mbewe, 2014).* The *hlyA* gene encodes enterohemolysin, which causes hemolysis thereby facilitating iron uptake by bacteria to maintain their survival in the gut *(Dontorou et al., 2003).*

Antibiotic resistance constitutes a major threat to human and animal health, particularly in developing countries, where antibiotics administrated without regulation from authorities (Spellberg and Gilbert, 2014; El-Domany *et al.,* 2017; El-Adawy *et al.,* 2018). Another major cause of higher pathogenicity for STEC strains is abundant antibiotic resistance to a large variety of most commonly used antibiotics including aminoglycosides, penicillin, streptomycin, cephalosporins, sulfonamides, tetracycline, and quinolones (Wang *et al.,* 2016).

Some published reports investigated STEC in many poultry species such as chicken, duck, quails, and goose (Farooq *et al.,* 2009; Dutta *et al.,* 2011; Sekhar *et al.,* 2017). However, little is known regarding the pathogenicity, virulence, and drug resistance genes of STEC in quails. Therefore, the present study aimed to determine the incidence of Shiga toxin-producing *E. coli* and the prevalence of their virulence and antibiotic resistance genes in quails. We also investigated the pathogenicity of STEC on the experimentally infected quails.

## MATERIALS AND METHODS

### *Samples collection and culture*

A total number of 150 quails were used in the present study. We collected 450 samples as follows: 50 cloacal swabs from 50 healthy quails and 400 samples from heart blood, liver, lung, and intestine of 50 freshly dead birds and 50 diseased birds. Samples were first inoculated into buffer peptone water (with incubation at 37˚C for 18 -24 h under aerobic conditions) and then cultured on 5% sheep blood and MacConkey agar (Merck, Germany) media (with incubation at 37 °C for 24 h). Suspected colonies of *E. coli* were re-cultured on blood agar plates and EMB agar (Merck, Germany), and colonies with green metallic sheen were selected as *E. coli*. These colonies were further confirmed by the following biochemical tests: urease, indol, methyl red, lactose and glucose fermentation (using TSI medium), Voges Proskauer, citrate, and lysine decarboxylase (Quinn *et al.*, 2002).

### Pathogenicity tests

The pathogenicity of E. coli isolates was tested by congo red (Berkhoff and Vinal, 1986) and hemolysis production (Livezey and Zusi, 2007) tests. To determine congo red binding activity, E. coli isolates were cultured on tryptic soy agar supplemented with 0.02% Congo red (Sigma, USA) and 0.15% bile salt (Difco, USA) at 37°C for 24 h and red colonies (positive) were selected. To determine hemolysis production, E. coli isolates were grown on tryptose blood agar (Difco) plates supplemented with 5% defibrinated washed sheep blood at 37°C for 24h and then suspected colonies were examined for hemolytic activity (Livezey and Zusi, 2007).

**Serological identification of E. coli**

E.coli isolates were serotyped in Animal Health Research Institute, Dokki, Giza using polyvalent and monovalent diagnostic E. coli antisera (Denka Seiken Co. Ltd,Japan) as previously described (Edwards and Ewing, 1972).

**Antimicrobial drug sensitivity test**

It was performed by disc diffusion method using Muller-Hinton agar using 7 antibiotic disc belongs to six different antimicrobial classes including sulfamethoxazole (100 µg/disk), norfloxacin (10 µg/disk), tetracycline (30 µg/disk), neomycin (30 µg/disk), doxycycline (30 µg/disk), amoxicillin (10 µg/disk), and colistin sulphate, (30 µg/disk) (Oxoid, Basingstoke, UK). Interpretation of the results was done following Clinical and Laboratory Standards Institute Guidelines **CLSI. (2011)**.

**Polymerase chain reaction (PCR)**

**DNA extraction:**

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56$^{O}$C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

**Oligonucleotide Primer.** Primers used were supplied from **Metabion (Germany)** are listed in table (1).

**For stx1, stx2 duplex PCR,** primers were utilized in a 50- µl reaction containing 25 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 13 µl of water, and 8 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

**Analysis of the PCR Products:**

The products of PCR were separated by electrophoresis on 1% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the uniplex PCR products and 40 µl of the duplex PCR products were loaded in each gel slot. Generuler 100 bp ladder (Fermentas, Thermo) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

**Table 1:** Primers sequences, target genes, amplicon sizes and cycling conditions.

| Target gene | Primers sequences | Amplified segment (bp) | Primary Denaturation | Amplification (35 cycles) | | | Final extension | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | Secondar denaturation | Annealing | Extension | | |
| ***hly*** | AACAAGGAT AAGCACTGTT CTGGCT / ACCATATAAG CGGTCATTCC CGTCA | 1177 | 94˚C 5 min. | 94˚C 30 sec. | 60˚C 50 sec. | 72˚C 1 min. | 72˚C 10 min. | ***(Piva et al., 2003)*** |
| ***eaeA*** | ATGCTTAGTG CTGGTTTAGG / GCCTTCATCA TTTCGCTTTC | 248 | 94˚C 5 min. | 94˚C 30 sec. | 51˚C 30 sec. | 72˚C 30 sec. | 72˚C 7 min. | ***(Bisi-Johnson et al., 2011)*** |
| ***Stx1*** | ACACTGGATG ATCTCAGTGG / CTGAATCCCC CTCCATTATG | 614 | 94˚C 5 min. | 94˚C 30 sec. | 58˚C 40 sec. | 72˚C 45 sec. | 72˚C 10 min. | ***(Dipineto et al., 2006)*** |
| ***Stx2*** | CCATGACAAC GGACAGCAG TT / CCTGTCAACT GAGCAGCAC TTTG | 779 | | | | | | |
| ***tetA(A)*** | GGTTCACTCG AACGACGTC A / CTGTCCGACA AGTTGCATGA | 576 | 94˚C 5 min. | 94˚C 30 sec. | 50˚C 40 sec. | 72˚C 45 sec. | 72˚C 10 min. | |
| ***Sul1*** | CGG CGT GGG CTA CCT GAA CG / GCC GAT CGC GTG AAG TTC CG | 433 | 94˚C 5 min. | 94˚C 30 sec. | 60˚C 40 sec. | 72˚C 45 sec. | 72˚C 10 min. | ***(Ibekwe et al., 2011)*** |

**Pathogenicity of E.coli isolates to quails**

A total of 95 one-day-old Japanese quails (*Coturnix japonica*) were used in this experiment. Five birds were randomly sacrificed for the bacteriological examination which proved that they were free from bacterial pathogens. The iron cages were fumigated with formaldehyde and KMnO4 (2:1) fifteen days before the arrival of birds. The cages were kept dry, clean, disinfected, and well ventilated during the entire experimental periods. Incandescent lights were used to brood baby birds at 34°C up to 7 days of age and the temperature was gradually reduced to 26°C by 21 days of age after which, no supportive heat was provided.

Quail chicks were randomly assigned into 3 groups GA, GB, and GC (n = 30 birds/group). Each group subdivided into 2 replicates of 15 birds for each.: GA non-infected, non-treated, GB infected with E. coli O125 at the age of 7 days, and GC infected with E. coli O125 at the age of 7 days and medicated with neomycin (20%) 1 g/liter for 5 successive days at the age from 9 to 13 days. The E. coli O125 (Shiga toxin-producing *E.coli*) used in the experimental infection was previously isolated from diseased quails. Birds in GB and GC were orally challenged with 1 ml of saline containing $10^8$cfu/ml *E. coli* O125 in the back of the oral cavity using a sterile syringe at the age of 7 days (Cao *et al.*, 2013).

The birds in the challenged groups were observed daily after the challenge for any symptoms and deaths throughout the period of the experiment (4weeks). Dead birds were necropsied immediately after the detection of their death and macroscopical lesion scores were registered. For bacterial

re-isolation, swabs from the lung, heart, liver, and intestine were collected from 4 sacrificed birds in each group at the 1st, 2nd, and 3rd weeks after the challenge. In addition, tissue samples from these organs were fixed in 10% neutral buffered formalin for histopathological examination. The fixed tissues were dehydrated in methanol, cleared in xylene, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin (Bancroft and Gamble, 2008). All tissues were microscopically examined and the histopathological alterations were blindly graded.

## RESULTS

### Clinical signs and post mortem findings in field cases

The symptoms of colibacillosis were declined food intake and body weight, weakness, ruffling of feathers, and yellowish diarrhea. While, the post mortem examination for dead and diseased birds showed congestion of liver, spleen, kidney, and lung, pericarditis, perihepatitis, air sacculitis, congestion and hemorrhage in the intestinal mucosa, and some cases showed catarrhal enteritis.

Out of 450 examined samples, *E. coli* was identified in 20.67% (93/450) of the total examined samples based on morphological and biochemical characteristics. Out of 93strains, ten strains (20%) were isolated from cloacal swabs of healthy birds, 38 strains (19%) from diseased birds and 45 strains (22.5%) from freshly dead. Congo red media was used for differentiation between pathogenic and nonpathogenic *E. coli*. Out of 93 identified strains, 40(43.01%) were pathogenic (table2)**.** Regarding to hemolysis on blood agar all isolated strains were non hemolytic.

The recovery rates of the pathogenic *E. coli* from different quail samples were 13%, 8%, 6%and 11% form liver, heart blood, lung and intestine, respectively (fig1)**.** *E. coli* isolates were serotyped into 7 serotypes including O125, O20, O78, O55, O44, O114,and O35 the most prevalent serotypes were O125, O20 and O78 with an incidence of 22.5%, 20% and15 % respectively (table3).

The antimicrobial susceptibility pattern of (40) pathogenic *E. coli* strains isolated from cloacal swabs and internal organs of the examined quail revealed variable results in susceptibility and zones of inhibition to the different antimicrobial drugs which commonly used in quail treatments. *E. coli* isolates demonstrated high rates of resistance to sulfamethoxazole, tetracycline (100%for each) and amoxicillin (75%). Conversely, neomycin, norfloxacin and colistin showed the lowest resistance rate against *E. coli* isolates (15%, 20% and 25% respectively) Table (4).

### Virulence and antibiotic resistance genes:

In this study, the presence of 4virulence genes, including *stx1, stx2, eae and hly* were verified by multiplex PCR analysis. Among the virulence genes detected, *stx2* was detected in (3/8) isolates and *eae* was detected in (2/8) isolates whereas none of the isolate was carrying *stx1* or *hly* gene. Also, multiplex PCR was used for the detection of antibiotic resistance genes including *tetA* and *sul1*. The antibiotic resistance genes profile revealed the presence of *tetA* and *sul1* in all examined isolates table (5).

**Results of experimental infection**:

### Clinical signs:

No clinical signs were observed in the uninfected-untreated group (GA) while in infected groups (B&C) clinical symptoms appeared within 48 hours following experimental *E .coli* infection. The clinical symptoms observed were depression, loss of appetite, ruffled feather, emaciation, conjunctivitis, rhinitis, and severe watery diarrhea. Within 24 hours following initiation of neomycin treatment, infected birds treated with neomycin (GC) improved clinically after 3-days treatment period and clinical signs disappeared within 5 to 7 days

following treatment. In contrast, the incidence of clinical disease in surviving infected-untreated birds (GB) was estimated as 20-50%, seven days following infection.

**Mortalities:** In *E. coli* challenged groups, mortalities started at the 3rd day post-challenge and were (40%) while in group(C) treated with neomycin reduced into (23.33%) (Table 6).

**Gross lesions:** Dead and sacrificed birds of infected-untreated group post infection were represented tracheitis with purulent exudates in the tracheal lumen and bronchi, air-saculitis on both the thoracic and abdominal air-sacs, hyperemia of intestinal mucosa, congestion of liver, pericarditis. While infected and treated groups with neomycin showed less severe lesions .Seven days following initiation of treatment lesions virtually disappear**.**

**Re-isolation of *E .coli*:**

All quails in uninfected-untreated group (GA) were negative for reisolation of the challenge bacteria. *E.coli* could be recovered from tissues in some birds which infected and treated with neomycin in the drinking water (GC) with re-isolation rate of 21.1%. While infected-untreated group (GB) had a higher re-isolation rate of *E.coli* (62.2%) from different organs. (Table7).

**Histopathological finding:**

Histopathological examination of the lung in GA showed normal lung histological structure (Fig. 3A). However, GB showed severe histopathological changes including congestion, hemorrhage (arrow), perivascular edema, bronchopneumonia (arrowhead), mild sero-fibrinous air saculitis (Fig. 3B). Sero-fibrinous exudate extended to interlobular pulmonary septae. GC demonstrated mild congestion of blood vessels (arrow) without exudate in bronchi or air saculitis (Fig. 3C). Livers in GA showed normal histological structure (Fig. 4A). In contrast, GB exhibited focal necrotic hepatic cells intermingled with microsteatosis and leukocytic infiltration in the hepatic parenchyma (Fig. 4B). Necrotic areas demarcated from adjacent degenerated hepatic cells by fibrous tissue containing erythrocytes and mononuclear cells (arrow, Fig. 4B). The majority of portal areas exhibited numerous bile duct with mononuclear cell infiltration. On the other hand, GC demonstrated mild vacuolation in few hepatocytes (arrow) and the majority of the hepatic parenchyma was normal (Fig. 4C). Hyperplastic Kuffer cells and mild interstitial lymphoid aggregation could be seen in livers of this group. Hearts in GA displayed normal histological architecture (Fig. 5A). However, Heart in GB revealed sero-fibrinous pericarditis (arrow) with edema and mononuclear cell infiltration (Fig. 5B). Meanwhile, the pericardium and myocardium restored their normal morphologic picture in GC (Fig. 5C). Histopathological examination of the intestine in GA showed normal histological structure (Fig. 6A). In contrast, the intestine in GB revealed necrotic and sloughed villi (arrow), intestinal exudate, and mononuclear cell infiltration in intestinal crypts (Fig. 6B). On the other hand, GC showed hyperplastic goblet cell (arrow) and proliferative intestinal crypts (arrowhead) to replace sloughed portions (Fig. 6C).

**Table 2:** Prevalence of E.coli in examined samples collected from quail.

| quails | Number of examined sample | No. of Escherichia coli positive samples | Prevalence (%*) | No. of E. coli positive Congo red | Prevalence (%**) |
|---|---|---|---|---|---|
| healthy | 50 | 10 | 20 | 2 | 20 |
| diseased | 200 | 38 | 19 | 16 | 42.11 |
| Recently dead | 200 | 45 | 22.5 | 22 | 48.89 |
| Total | 450 | 93 | 20.67 | 40 | 43.01 |

(%*) No of E.coli positive samples/No.of examined samples.

(%**) No. of E.coli positive congo red / No. of Escherichia coli positive samples

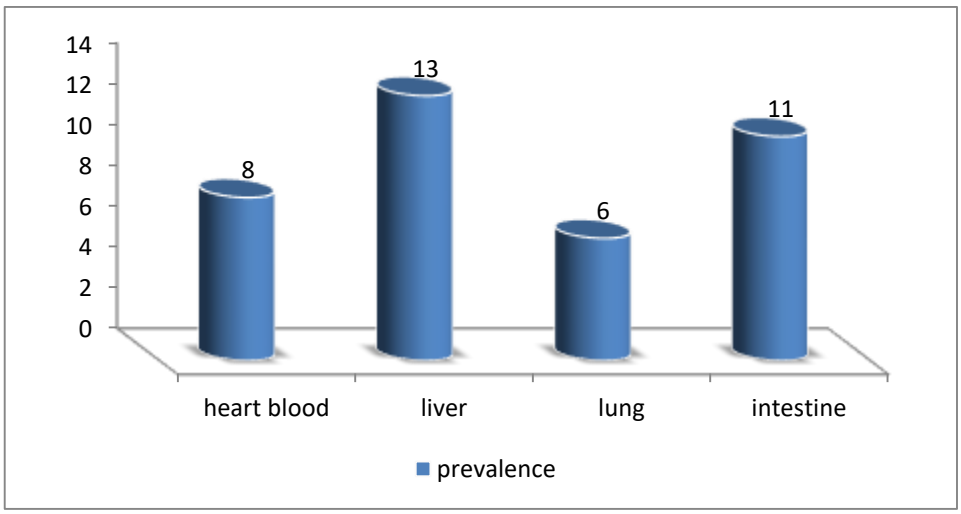


**Figure 1:** Prevalence of *E. coli* in different organs of the examined quails

**Table 3:** Serogrouping of 40 *E.coli* isolates recovered from examined samples.

| Serogroup | O125 | O20 | O78 | O55 | O44 | O114 | O35 | UNTYPED |
|---|---|---|---|---|---|---|---|---|
| Number | 9 | 8 | 6 | 5 | 3 | 3 | 2 | 4 |
| % | 22.5 | 20 | 15 | 12.5 | 7.5 | 7.5 | 5 | 10 |

**Table 4:** Results of antimicrobial susceptibility test for 40 E. coli isolates.

| Antibiotic class | Antimicrobial agent | resistant | | sensitive | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| β-lactams | Amoxicillin | 30 | 75 | 10 | 25 |
| Sulfonamides | Sulfamethoxazole | 40 | 100 | 0 | 0 |
| Aminoglycosides | Neomycin | 6 | 15 | 34 | 85 |
| Tetracycline | Tetracycline | 40 | 100 | 0 | 0 |
| | Doxycycline | 28 | 70 | 12 | 30 |
| Quinolones | Norfloxacin | 8 | 20 | 32 | 80 |
| polymxins | Colistin | 10 | 25 | 30 | 75 |

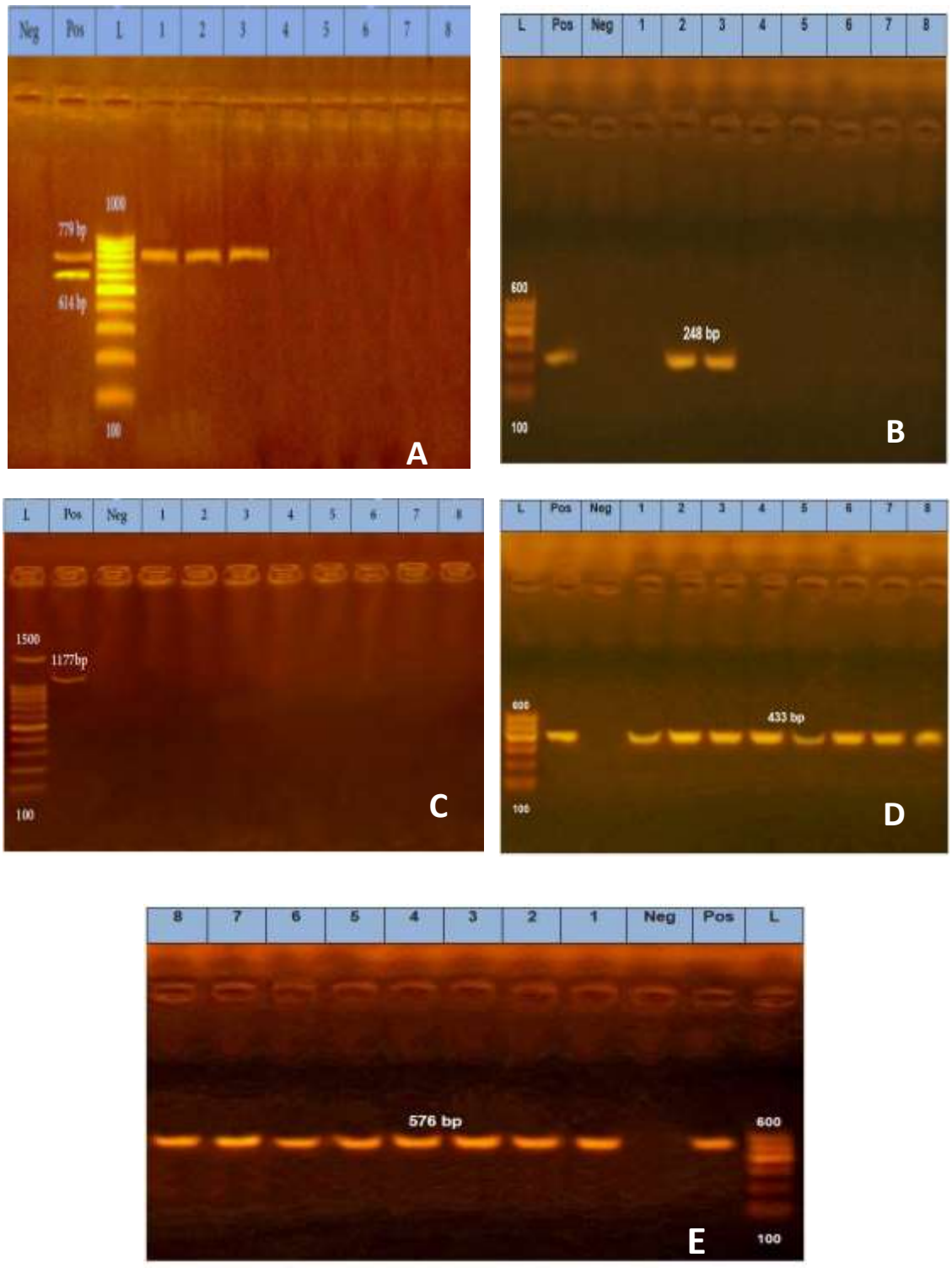


**Figure 2:** Agarose gel electrophoresis of PCR amplified products of *sxt1 &sxt2* **(A)**, *eae* **(B)**, and *hly* **(C)** virulence genes and *sul1* **(D)**, *tet*A **(E)** resistance gens. LaneL: DNA molecular size marker (100 bp), lanes 1- 8 *E. coli* isolates from quail organs. The size in base pairs (bp) of each PCR product is indicated on the right of the bands.

**Table 5:** Virulence and antibiotic resistance genes in 8 E. coli isolates.

| Sample number | serogroup | Virulence genes | | | | Antibiotic resistance genes | |
|---|---|---|---|---|---|---|---|
| | | Stx1 | Stx2 | eae | hly | Sul1 | Tet A |
| 1 | O20 | - | - | - | - | + | + |
| 2 | O125 | - | + | + | - | + | + |
| 3 | O55 | - | + | + | - | + | + |
| 4 | O78 | - | + | - | - | + | + |
| 5 | O44 | - | - | - | - | + | + |
| 6 | O114 | - | - | - | - | + | + |
| 7 | O35 | - | - | - | - | + | + |
| 8 | Untyped | - | - | - | - | + | + |

**Table 6:** The Mortality rate in experimentally infected quail.

| Experimental group | No.of examined quail | Mortality Weeks PC* | | | Mortality rate% |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| GA | 30 | 0 | 0 | 0 | 0% |
| GB | 30 | 6 | 4 | 2 | 40% |
| GC | 30 | 4 | 2 | 1 | 23.33% |

*post challenge

**Table 7:** Reisolation rate of E. coli from dead and scarified experimentally infected quail**.**

| Experimental group | Weeks PC *(positive / total examined birds**) | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| GA | 0/4 | 0/4 | 0/4 |
| GB | 8/10 | 5/8 | 2/6 |
| GC | 4/8 | 2/6 | 0/5 |

*post challenge ** Dead and scarified birds

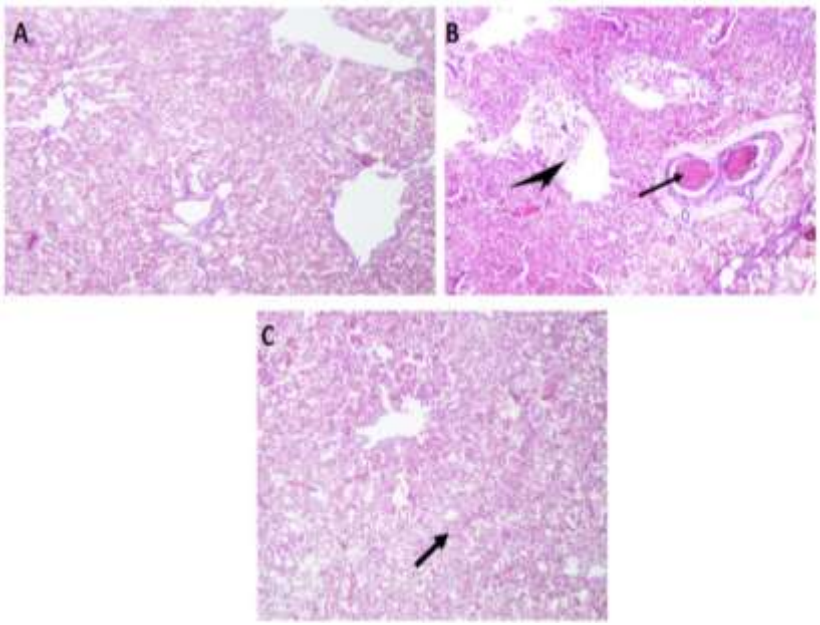


**Fig.3:** Photomicrograph of sections from lung of quail stained with H&E X120. (A) Histological picture of lung show normal picture in G1 (control group); (B) Severe congestion, hemorrhage, edema and bronchopneumonia in G2; (C) Mild congestion of pulmonary blood vessels.

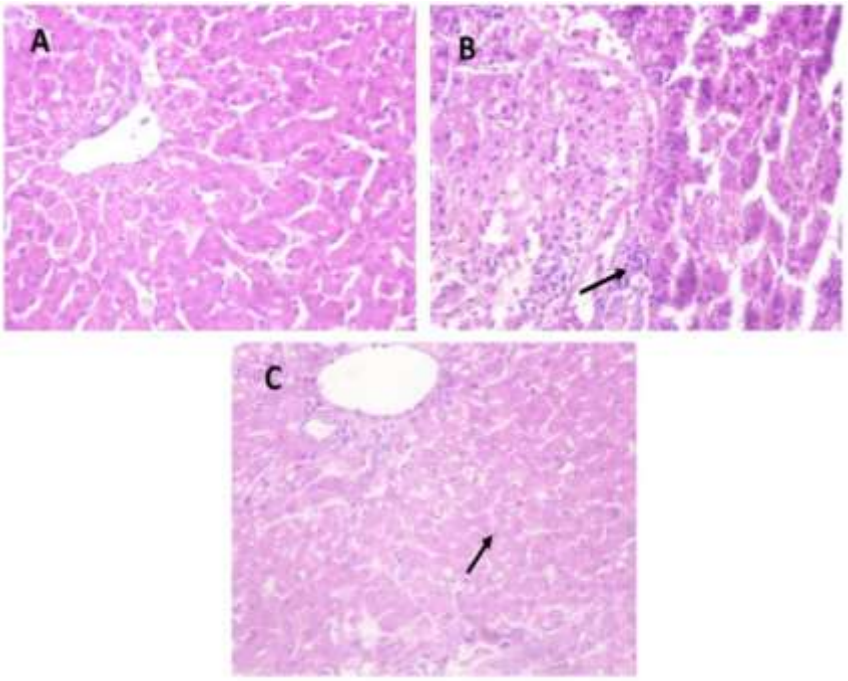


**Fig.4:** Photomicrograph of sections from liver of quail stained with H&E X400. (A) Histological picture of liver show normal picture in G1 (control group); (B) focal necrotic hepatic parenchyma encircled by fibrous tissue and inflammatory cells in G2; (C) Mild vaculation of a few hepatic cells.

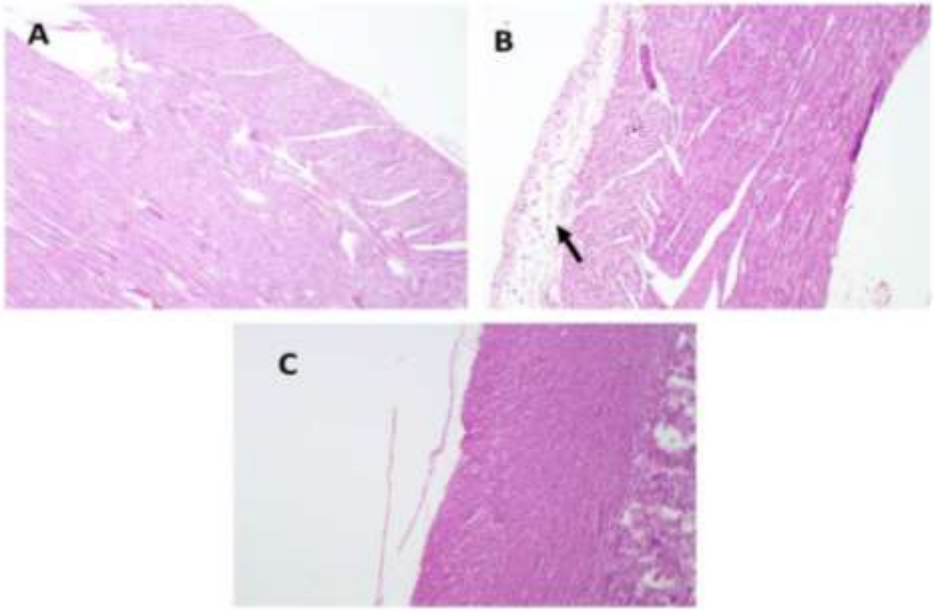


**Fig.5:** Photomicrograph of sections from heart of quail stained with H&E X100. (A) Histological picture of heart show normal picture in G1 (control group); (B) serofibrinous pericarditis containing extravasted erythrocytes and leukocytes inG2; (C) apparently normal myocardium.

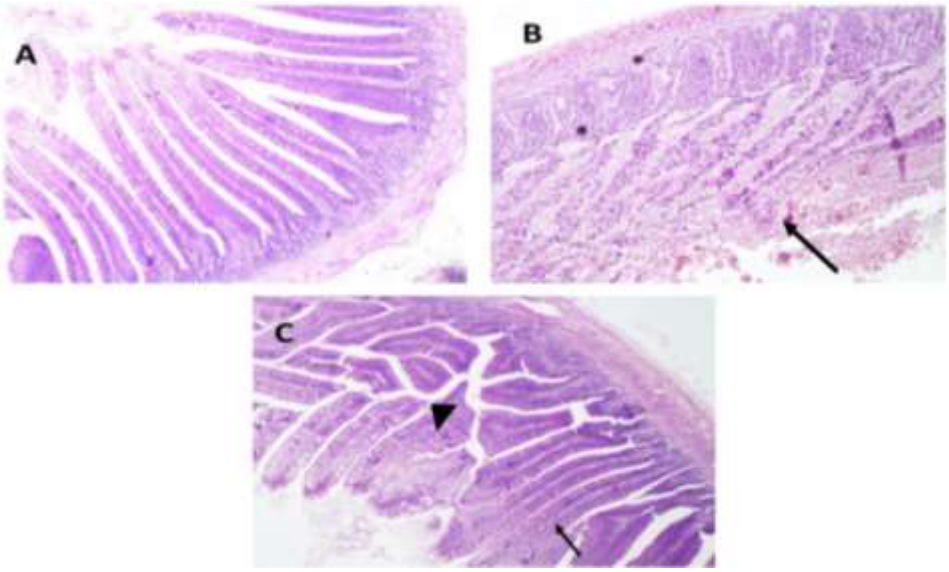


**Fig.6:** Photomicrograph of sections from intestine of quail stained with H&E X100. (A) Histological picture of intestine show normal picture in G1 (control group); (B) Partial necrosis and sloughing of superficial tips of intestinal villi beside intestinal exudate inG2; (C) Hyperplastic goblet cell and proliferative intestinal crypts.

## DISSCUSION

Based on morphological, biochemical and serological characteristics, the overall prevalence of E. coli was 20.67% (Table 2). This recovery rate was close to that obtained by Ibrahim (2019) (which was 28%) but higher than that reported by Farghaly *et al.* (2017) (8.3%) and lower than that found by Prihtiyantoro *et al.* (2019) (55.36%). The recovery rate of E. coli isolates differs according to the source of the examined samples. Indeed, our results revealed a higher incidence of isolates from freshly dead quails (22.5%) than diseased quails (19%) and healthy quails (20%) (Table 2). Noteworthy, these healthy quails act as asymptomatic carriers for E. coli and can be considered a possible source of colibacillosis to humans (Kabir, 2010). These carriers also increase the incidence of antimicrobial-resistant bacteria (Pruden *et al.*, 2013).

Congo red media was used for differentiation between pathogenic (pink/brick red colonies) and nonpathogenic E. coli (Singh and Gupta, 2001). The prevalence of the pathogenic strains was 43.01% with the following distribution: 20% from healthy birds, 42.11% diseased birds, and 48.89% recently dead birds (Table 2). Similarly, Azeem Riaz *et al.* (2016) also reported that 40% of avian E. coli isolates were Congo red positive. Regarding hemolysis on blood agar, all isolated strains were non-hemolytic. Similarly, Eid *et al.* (2016) did not find any E. coli isolates positive for hemolysis on 5%sheep blood agar.

The recovery rates of the pathogenic *E. coli* from different quail samples were 8%, 13%, 6%, and 11% from heart blood, liver, lung, and intestine, respectively (Fig.1). The dissemination of *E. coli* into these organs agreed with the postmortem lesions including fibrinous perihepatitis, pericarditis, enteritis, and pneumonia. This indicates that *E. coli* can lead to septicemia which subsequently can cause death (Kabir, 2010).

The pathogenic E. coli isolates were serotyped into seven O serotypes including O125, O20, O78, O55, O44, O114, and O35 with prevalence rates of 22.5% (9/40), 20% (8/40), 15% (6/40), 12.5% (5/40), 7.5% (3/40), 7.5% (3/40), and 5% (2/40), respectively. The prevalence of the untyped strains was 10% (4/40). Consistent with these findings, other Egyptian researchers also found similar serotypes in diseased quail with a predominance of O125 (Farghaly *et al.*, 2017; Ibrahim, 2019). Other serotypes of *E. coli* such as O9, O42, and O88 were isolated from dead Japanese quails in other countries (Roy *et al.*, 2006).

The misuse of antibiotics in animal farms induced the development of antibiotic-resistant strains that resulted in inefficient control of

bacterial diseases (Darwish *et al.,* 2013). The antimicrobial susceptibility pattern of pathogenic E. coli strains (n = 40) isolated from cloacal swabs and internal organs of the examined quail revealed variable results in susceptibility and zones of inhibition to the different antimicrobial drugs which commonly used in quail treatments (Table4, Fig.2). E. coli isolates were not 100% sensitive to any of the tested antimicrobial drugs. E. coli serotypes showed high sensitivity to neomycin (85%), norfloxacin (80%), and colistin (70%). These results are consistent with those obtained (Farghaly *et al.,* 2017) (Ibrahim *et al.,* 2019) (Ibrahim, 2019). E. coli isolates showed high resistance against sulphamethaxole, tetracycline (100% for each) and amoxicilin(75%). These findings were in agreement with (Youssef and Mansour (2014); Farghaly *et al.* (2017)). While another study documented lower resistance rates among E. coli isolated from quail against sulfamethoxazole (39.28%), and tetracycline (35%) (Ibrahim, 2019).

Shiga toxins play an important role as virulence factors in the pathogenicity and severity of STEC (Ojo *et al.,* 2010). The presence of stx1, stx2, eae, and hly virulence genes was determined by PCR in 8 pathogenic E. coli isolates including all detected serotypes. Stx2 and eae genes were detected in (3/8) and in (2/8) of examined isolates. Both virulent genes were detected in isolates 2 and 3. Whereas, none of the 8 isolates contained stx1 or hly gene (Table5, Fig.3). Similarly, Himi *et al.* (2015) also did not find stx1 in all examined E. coli serogroups. However, Prihtiyantoro *et al.* (2019) detected both sxt1 and sxt2 in 90% and 10 % of E. coli isolated from quails. The stx2 was detected in O125, O55, and O78 serotypes. Eid *et al.* (2016) also detected stx2 in the same serotypes isolated from broilers. Stx2 is 1000 times more cytotoxic than stx1 and therefore it is associated with many diseases in humans (Mir *et al.,* 2016). We also found another virulent gene, eae in O125 and O55 E. coli with an incidence rate of 25%. In agreement, Eid *et al.* (2016) also detected eae in 33.3% of E. coli isolates. Taken together, both O125 and O55 E. coli carried the two virulent genes eae and stx2. Dipineto *et al.* (2014) also detected both genes in E. coli isolated from quail. On the other hand, we and Ezzat *et al.* (2018) did not find the hly gene in the examined E. coli isolates. In contrast, Dutta *et al.* (2011) detected hly in 7 E.coli strains out of 10 samples (70%).

The possibility of the transmission of antibiotic resistance genes to humans should be considered when agricultural products, particularly chickens, are infected with bacterial strains that are resistant to most antibiotics (Manges *et al.,* 2007). Herein, we detected two antibiotic resistance genes, *tetA*, and *sul1*, among the 8 *E. coli* isolates with a prevalence rate of 100%, for each (Table5, Fig.3). These findings suggest that the results of the antibiotic disk diffusion study were generally compatible with the PCR findings for the identification of the related antibiotic resistance genes. These findings agree with Ammar *et al.* (2015) who detected *sul1* in 100% and Ibrahim *et al.* (2019) who detected *tetA* at an incidence rate of 91.18% among the examined *E. coli* isolates. In contrast, Younis *et al.* (2017) had reported a relatively lower prevalence of these antibiotic resistance genes, *sul1* 87%, and *tetA* 60%, among *E. coli* isolates.

In the present study, O125 and O55 E. coli harbored virulence and antibiotic resistance genes. This could explain the severe pathogenicity of these stains and underline the role of quails in transmission of antibiotic resistance bacteria to humans. Previous studies also reported the presence of virulent and antibiotic resistant E. coli strains in poultry meat (Ammar *et al.,* 2015; Himi *et al.,* 2015; Eid *et al.,* 2016; Prihtiyantoro *et al.,* 2019; Mousavi *et al.,* 2020).

Experimental infection of quails was conducted to evaluate the pathogenicity of an isolated virulent field strain of E. coli (O125 STEC) and to assess the efficacy of neomycin in controlling the adverse effects of colibacillosis. No clinical signs were observed in the uninfected-untreated group (GA) while in infected groups (GB and GC) clinical signs appeared within 48 hours following experimental E. coli infection. GB quails showed typical symptoms of E.coli O125 similar to those described by Shen-Orr *et al.* (2002), which were depression, loss of appetite, ruffled feather, emaciation, conjunctivitis, rhinitis, and severe watery diarrhea. These symptoms were relieved 3 days post-treatment with neomycin (GC) and clinical signs completely disappeared after 5 to 7 days of treatment. This agreed with the results obtained by Abeer *et al.* (2019). In contrast, the incidence of clinical disease in surviving

infected-untreated birds (GB) was estimated as 20-50%, seven days following infection.

The overall mortality rate was 40% and 23.33% in GB and GC, respectively (Table6). The highest death rate was noticed in GB in the 1st week after the challenge. Post-mortem lesions in dead and sacrificed birds of GB included tracheitis with purulent exudates in the tracheal lumen and bronchi, thoracic and abdominal air-saculitis, hyperemia of the intestinal mucosa, congestion of the liver, and pericarditis. Similar post-mortem lesions recorded by Ameh *et al.* (2011). These multiple lesions in different organs may be attributed to the ability of virulent E. coli O125 to penetrate the mucosal barrier of the respiratory tract and circulate in the blood to disseminate throughout the body (Norhan *et al.*, 2014). While neomycin-treated group (GC) exhibited less severe lesions which were completely disappeared on the 7th day of neomycin treatment. However, there were still mild lesions in some organs, which may be due to the long period needed by the inflamed tissues to heal. This result was in agreement with that recorded by Fernandez *et al.* (1998).

No E. coli isolates were re-isolated from all quails in GA over the three time points (1st, 2nd, and 3rd-week post-challenge). However, GB had a higher re-isolation rate of E .coli (80%, 62.5%, and 33.3%) at the three time points, respectively (Table7). E. coli was also recovered from GC but with lower re-isolation rates of 50% and 33.3%at 1st and 2nd w post-challenge. These results agreed with those recorded by Abeer *et al.* (2019). The presence of E. coli 2 w post-treatment explained by Toutain *et al.* (2002) who mentioned that infection caused inflammation and production of exudate and other debris, which inhibit penetration of the antibiotic or complete bacterial destruction.

The histopathological changes recorded in the lung, liver, heart, and intestine in the present study came in parallel with that recorded by Ghosh *et al.* (2006), Manimaran *et al.* (2003); Kilany *et al.* (2018) who reported that chicks experimentally infected with E. coli showed sloughing in (epithelial lining), infiltration of mononuclear cell and congestion of blood vessels in lung, liver, heart, and intestine. Improvement in the histopathological lesions of examined organs in the treated group was similar to that reported by El-Sawah *et al.* (2018).

## CONCLUSIONS

The presence of shiga toxin-producing *E. coli* in quails alarms the public health as quails may serve as a vector for transmission of STEC to the environment and humans. Therefore, eating raw or undercooked quail meat could result in STEC-associated diseases such as foodborne diseases, hemorrhagic diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, or thrombotic thrombocytopenic purpura. Moreover, STEC showed a notable resistance to several antimicrobial drugs that commonly used in poultry production in Egypt. So, further focus has to be given to biosecurity controls in quail farms to minimize infection and subsequently reduce the spread of antibiotic resistance genes between different bacterial populations**.**

## REFERENCES

*Abeer, E.M.B. and Ali, G. (2019):* Effect of Some Antibiotic Alternatives on Experimentally Escherichia Coli Infected Broiler Chicks. Alexandria Journal of Veterinary Sciences; 63: (1), 110.

*Ameh, J.; Adamu, J. and Ikpa, T. (2011):* Experimental infection of chicks with Avian Enteropathogenic Escherichia coli (APEC) Serotype O78: K80. African Scientist; 12: (1), 27-32.

*Ammar A.; El-Hamid, M. and Eid, S.E. (2015):* Insight into antimicrobial resistance and virulence genes of emergent multidrug resistant avian pathogenic Escherichia coli in Egypt: How closely related are they. Rev. Med. Vet; 166: (9-10), 304-314.

*Ateba, C. and Mbewe, M. (2014):* Genotypic Characterization of Escherichia coli O157:H7 Isolates from Different Sources in the North-West Province, South Africa, Using Enterobacterial Repetitive Intergenic Consensus PCR Analysis. International Journal of Molecular Sciences; 15: (6), 9735-9747.

*Azeem Riaz, M.; Aslam, A. and Rehman, M. (2016):* Pathological Investigation and Molecular Detection of Avian Pathogenic E. coli Serogroups in Broiler Birds. Journal of Veterinary Science & Technology; 7(5).

*Bancroft, JD. and Gamble, M. (2008):* Preface to the sixth edition. Theory and Practice of Histological Techniques. Elsevier. ix.

*Bandyopadhyay, S.; Mahanti A. and Samanta, I. (2011):* Virulence repertoire of Shiga toxin-producing Escherichia coli (STEC) and enterotoxigenic Escherichia coli (ETEC) from diarrhoeic lambs of Arunachal Pradesh, India. Tropical Animal Health and Production; 43: (3), 705-710.

*Berkhoff, HA. and Vinal, AC. (1986):* Congo Red Medium to Distinguish between Invasive and Non-Invasive Escherichia coli Pathogenic for Poultry. Avian Diseases; 30: (1), 117.

*Bisi-Johnson, MA.; Obi, CL. and Vasaikar, SD. (2011):* Molecular basis of virulence in clinical isolates of Escherichia coli and Salmonella species from a tertiary hospital in the Eastern Cape, South Africa. Gut Pathogens; 3: (1), 9.

C*ao, G T.; Zeng, XF. and Chen, AG. (2013):* Effects of a probiotic, Enterococcus faecium, on growth performance, intestinal morphology, immune response, and cecal microflora in broiler chickens challenged with Escherichia coli K88. Poultry Science; 92: (11), 2949-2955.

*Darwish, WS.; Eldaly, EA. and El-Abbasy, MT. (2013):* Antibiotic residues in food: the African scenario .Japanese Journal of Veterinary Research; 61: (Supplement), S13-S22.

*Dipineto, L.; Russo TP. and Gargiulo, A. (2014):* Prevalence of enteropathogenic bacteria in common quail (Coturnix coturnix). Avian Pathology; 43: (6), 498-500.

*Dipineto, L.; Santaniello, A. andFontanella, M. (2006):* Presence of Shiga toxin-producing Escherichia coli O157: H7 in living layer hens. Letters in Applied Microbiology; 43: (3), 293-295.

*Dontorou, C.; Papadopoulou, C. and Filioussis, G. (2003):* Isolation of Escherichia coli O157:H7 from foods in Greece. International Journal of Food Microbiology; 82: (3), 273-279.

*Dutta, T.; Roychoudhury, P. and Bandyopadhyay, S. (2011):* Detection & characterization of Shiga toxin producing Escherichia coli (STEC) & enteropathogenic Escherichia coli (EPEC) in poultry birds with diarrhoea. The Indian journal of medical research; 133: (5), 541.

*Edwards, PR. and Ewing, WH. (1972):* Identification of enterobacteriaceae. Identification of Enterobacteriaceae.; (Third edition).

*Eid, H.; Algammal, A. and Nasef, S. (2016):* Genetic variation among avian pathogenic E. coli strains isolated from broiler chickens. Asian J. Anim. Vet. Adv; 11: (6), 350-356.

*El-Adawy, M.; El-Aziz, MA. and El-Shazly, K. (2018):* Dietary propionic acid enhances antibacterial and immunomodulatory effects of oxytetracycline on Nile tilapia, Oreochromis niloticus. Environmental Science and Pollution Research; 25: (34), 34200-34211.

*El-Domany, R.; Emara, M. and El-Magd, M. (2017):* Emergence of Imipenem-Resistant Pseudomonas aeruginosa Clinical Isolates from Egypt Coharboring VIM and IMP Carbapenemases. Microbial Drug Resistance; 23: (6), 682-686.

*El-Sawah, AA. Dahshan, ALHM. and El-Nahass, E-S. (2018):* Pathogenicity of Escherichia coli O157 in commercial broiler chickens. Beni-Suef University Journal of Basic and Applied Sciences; 7: (4), 620-625.

*Ezzat, M.; Wahdan, A. and Yousef, F. (2018):* Genetic Variation of E. coli Strains Isolated from Poultry Slaughterhouses at Ismailia Governorate, Egypt. Advances in Animal and Veterinary Sciences; 6 : (12).

*Farghaly, E.; Samy, A. and Roshdy, H. (2017):* Wide prevalence of critically important antibiotic resistance in Egyptian quail farms with mixed infections. Vet. Sci. Res. Rev; 3: (1), 17-24.

*Farooq, S.; Hussain, I. and Mir, MA. (2009):* Isolation of atypical enteropathogenic Escherichia coli and Shiga toxin 1 and 2f-producing Escherichia coli from avian species in India. Lett Appl Microbiol; 48: (6), 692-7.

*Fernandez, A.; Lara, C. and Puyuelo, R. (1998):* Efficacy of phosphomycin in the control of Escherichia coli infection of broiler chickens. Research in Veterinary Science; 65: (3), 201-204.

*Ghosh, R.; Hirpurkar, S. and Suryawanshi, P. (2006):* Concurrent colibacillosis and infectious bursal disease in broiler

chicks. Indian veterinary journal; 83: (9), 101.١٠٢٠-٩

*Himi, HA.; Parvej, MS. and Rahman, MB. (2015):* PCR Based Detection of Shiga Toxin Producing E. coli in Commercial Poultry and Related Environments. Turkish Journal of Agriculture - Food Science and Technology; 3: (6), 361.

*Ibekwe, AM.; Murinda, SE. and Graves, AK. (2011):* Genetic Diversity and Antimicrobial Resistance of Escherichia coli from Human and Animal Sources Uncovers Multiple Resistances from Human Sources. PLOS ONE; 6: (6), e20819.

*Ibrahim, WA.; Marouf, SA. and Erfan, AM. (2019):* The occurrence of disinfectant and antibiotic-resistant genes in Escherichia coli isolated from chickens in Egypt. Veterinary World; 12: (1), 141-145.

*Ibrahim, W F. (2019):* Isolation, identification and antimicrobial susceptibility testing of recent E. coli serotypes from Japanese Quails reared in Sharkia Governorate, Egypt. Damanhour Journal of Veterinary Sciences; 1: (2), 14-17.

*Kabir, SML. (2010):* Avian Colibacillosis and Salmonellosis: A Closer Look at Epidemiology, Pathogenesis, Diagnosis, Control and Public Health Concerns. International Journal of Environmental Research and Public Health; 7: (1), 89-114.

*Kilany, O.; Youssef, F. and Mabrouk, M. (2018):* Clinicopathological studies on the effect of some antibacterial medicinal plants in broilers. J Clin Pathol Forecast. 2018; 1 (1); 1003.

*Livezey, BC. and Zusi, RL. (2007):* Higher-order phylogeny of modern birds (Theropoda, Aves: Neornithes) based on comparative anatomy. II. Analysis and discussion. Zoological Journal of the Linnean Society; 149: (1), 1-95.

*Manges, A R.; Smith, SP. and Lau, BJ. (2007):* Retail Meat Consumption and the Acquisition of Antimicrobial ResistantEscherichia coliCausing Urinary Tract Infections: A Case–Control Study. Foodborne Pathogens and Disease; 4: (4), 419-431.

*Manimaran, K.; Singh, S. and Shivachandra, S. (2003):* Haematobiochemical and pathological changes in experimental Escherichia coli infection in broiler chicks. Indian journal of animal sciences; 73: (9), 960-962.

*Mir, RA.; Weppelmann, TA. and Elzo, M. (2016):* Colonization of Beef Cattle by Shiga Toxin-Producing Escherichia coli during the First Year of Life: A Cohort Study. PLOS ONE; 11: (2), e0148518.

*Mousavi, R.; Rahimi, E. and Shakerian, A. (2020):* Incidence and Profiles of Antibiotic Resistance and Virulence Markers of The Escherichia coli O157 Bacteria Recovered From Poultry Meat. Egyptian Journal of Veterinary Sciences; 51: (2), 215-223.

*Norhan, K.; Ibrahim, E. and Ahmed, M. (2014):* Molecular studies on M. gallisepticumand avian pathogenic E. coli induced infections in broilers. European Journal of Veterinary Medicine; 4: 1-16.

*Ojo, OE.; Ajuwape, ATP. and Otesile, EB. (2010):* Potentially zoonotic shiga toxin-producing Escherichia coli serogroups in the faeces and meat of food-producing animals in Ibadan, Nigeria. International Journal of Food Microbiology; 142: (1-2), 214-221.

*Piva, IC.; Pereira, AL. and Ferraz, LR. (2003):* Virulence markers of enteroaggregative Escherichia coli isolated from children and adults with diarrhea in Brasilia, Brazil. Journal of Clinical Microbiology; 41: (5), 1827-1832.

*Prihtiyantoro, W.; Khusnan, K. and Slipranata, M. (2019):* Prevalensi Strain Avian Pathogenic Escherichia coli (APEC) Penyebab Kolibasilosis pada Burung Puyuh. Jurnal Sain Veteriner; 37: (1), 69.

*Pruden, A.; Larsson, DGJ. and Amézquita, A. (2013):* Management Options for Reducing the Release of Antibiotics and Antibiotic Resistance Genes to the Environment. Environmental Health Perspectives; 121: (8), 878-885.

*Quinn, P.; Markey, BK. and Carter, M. (2002):* Veterinary microbiology and microbial disease, Blackwell science .

*Roy, P.; Purushothaman, V. and Koteeswaran, A. (2006):* Isolation, Characterization, and Antimicrobial Drug Resistance Pattern of Escherichia coli Isolated from Japanese Quail and their Environment. Journal of Applied Poultry Research; 15: (3), 442-446.

*Salehi, M. and Ghanbarpour, R. (2010):* Phenotypic and genotypic properties of Escherichia coli isolated from colisepticemic cases of Japanese quail. Tropical Animal Health and Production; 42: (7), 1497-1504.

*Sekhar, M S.; Sharif, N M. and Rao, T S. (2017):* Genotyping of virulent Escherichia coli obtained from poultry and poultry farm workers using enterobacterial repetitive intergenic consensus-polymerase chain reaction. Vet World; 10: (11), 1292-1296.

*Shahreza, M H S.; Rahimi, E. and Momtaz, H. (2017):* Antibiotic resistance pattern of Shiga-toxigenic Escherichia coli isolated from ready-to-eat food stuffs. Bioscience Biotechnology Research Communications; 10: (2), 155-159.

*Shen-Orr, S S.; Milo, R. and Mangan, S. (2002):* Network motifs in the transcriptional regulation network of Escherichia coli. Nature Genetics; 31: (1), 64-68.

*Singh, S. and Gupta, R. (2001):* Pathogenicity and in-vitro Drug Resistance of Escherichia coli Isolated from Colibacillosis Cases in Chicks of Tarai Region .INDIAN JOURNAL OF COMPARATIVE MICROBIOLOGY IMMUNOLOGY AND INFECTIOUS DISEASES; 22: (2), 166-167.

*Spellberg, B. and Gilbert, D N. (2014):* The Future of Antibiotics and Resistance: A Tribute to a Career of Leadership by John Bartlett. Clinical Infectious Diseases; 59: (suppl 2), S71-S75.

*Toutain, PL.; Del Castillo, JRE. and Bousquet-Mélou, A. (2002):* The pharmacokinetic–pharmacodynamic approach to a rational dosage regimen for antibiotics. Research in Veterinary Science; 73: (2), 105-114.

*Wang, J.; Stanford, K.; McAllister, TA. (2016):* Biofilm Formation, Virulence Gene Profiles, and Antimicrobial Resistance of Nine Serogroups of Non-O157 Shiga Toxin–ProducingEscherichia coli. Foodborne Pathogens and Disease; 13: (6), 316-324.

*Younis, G.; Awad, A. and Mohamed, N. (2017):* Phenotypic and genotypic characterization of antimicrobial susceptibility of avian pathogenic Escherichia coli isolated from broiler chickens. Veterinary World; 10: (10), 1167-1172.

*Youssef, AI.; and Mansour, DH. (2014):* Potential Role of Migratory Quail in Spreading of Some Zoonotic Pathogens in Egypt. American Journal of Animal and Veterinary Sciences; 9: (4), 203-210.

*Yusuf, MS.; Nabtiti, ASE. and Cui, H. (2016):* Effects of NENP vs LELP Diets on Some Laying and Reproductive Performance Parameters of Japanese Quail'S Hens. Journal of Advanced Agricultural Technologies; 3: (2), 132-135.

## مدى تواجد عوامل الضراوه والجينات المقاومه للمضادات الحيويه فى الايشيريشيا كولاى المنتجه لسموم الشيجا المعزوله من السمان

***غاده علام عبد الدايم ، احمد حجازى رمضان ، هناء سعيد على***

E-mail: ahmedhegazy711@yahoo.com Assiut University web-site: www.aun.edu.eg

فى هذه الدراسه تم تجميع عدد ٤٥٠ عينه من عدد ١٥٠ سمان عمر١-٤ اسابيع (٥٠سليم ظاهريا و٥٠ مريض و٥٠ نافق حديثا) تم زرعها على الاوساط الغذائية المناسبة لعزل ميكروب الايشيريشيا كولاى٠ واظهرت النتائج تواجد ميكروب الايشيرشيا كولاى بنسبة (٢٠,٦٧%) فى تلك العينات٠ وقد تم تصنيف عدد سبع مجموعات مصليه وكانت المجموعه المصليه O125و O20 ,O78 الاكثر تواجد٠ تم اجراء اختبار الحساسيه على عدد من المضادات الحيويه المستخدمه فى علاج السمان واظهرت النتائج ان العترات المعزوله عاليه الحساسيه للنيومايسين نسبة ٨٥% وونورفلوكساسين ٨٠% والكولستين ٧٠%٠ بينما اظهرت مقاومه عاليه لكل من السلفاميزاكسول والتتراسيكلين بنسبة ١٠٠% لكل منهماو الاموكسيسللين٧٥%٠ وقد تم اجراءاختبار انزيم البلمره المتسلسل التعددى لثمانيه من المعزولات واظهرت النتائج وجود جينات الضراوه (stx2) فى ثلاث معزولات و(eae) فى معزولتين بينما لم يمكن تحديد وجود جينات (stx1,hly) فى اى من المعزولات٠ وكذلك اظهرت النتائج تواجد الجينات المقاومه للمضادات الحيويه (sul1,tetA) بنسبة ١٠٠% لكليهما فى المعزولات٠ وتم اجراء عدوى اصطناعيه باستخدام عترة الايشيريشياكولاى O 125 المعزوله حقليا (عالية الضراوه ومقاومه لمعظم المضادات الحيويه) لبيان مدى ضراوتها وكفاءه النيوميسين فى علاج داء العصيات القولونيه فى السمان٠ واثبتت التنائج كفاءة النيوميسين فى معالجه هذا الميكروب فى السمانة واشارت تلك الدراسة إلى وجود الميكروب القولونى الحامل لجينات الشيجا والمقاوم للعديد من المضادات الميكروبية فى السمان والتي تشكل خطرا على الصحه العامه حيث انها يمكن أن تنتقل من السمان إلى البشر. لذلك توصى هذه الدراسة بإدخال برامج رقابية لمقاومة البكتريا المسببة للأمراض والمقاومة للمضادات الميكروبية.