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# Isolation, identification and antimicrobial susceptibility testing of recent *E. coli* serotypes from Japanese Quails reared in Sharkia Governorate, Egypt

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**A B S T R A C T**

Quails are growing sources for animal-derived protein in Egypt. This study aimed to investigate of the prevalence of avian pathogenic *E. coli* (APEC) in quails reared in Sharkia Governorate, Egypt. Furthermore, serological identification of APEC strains was performed followed by screening of the expression of the virulence-associated genes and the antibiogram of the identified serotypes. The achieved results in the present study revealed that *E. coli* isolates were recovered at 28% of the examined quail samples. *E. coli* isolates were recovered from the different organs at variable percentages. Serological identification of the isolated *E. coli* demonstrated that five serotypes were identified, namely O2, O20, O35, O78 and O127. *E. coli* O 127 was the most predominant serotype (28.56%). *E. coli* O2 came second at 24.99%. The identified serotypes harbored virulence-associated genes including *tsh*, *astA* and *iroN* that facilitate microbial colonization, adhesion and dissemination into different tissue. Antimicrobial resistance profiling of the identified serotypes revealed their resistance to several antimicrobials commonly used in the poultry production in Egypt including ampicillin, ceftiofur, penicillin, polymixin B and nalidixic acid. On the same time, the identified *E. coli* serotypes showed marked sensitivity to gentamycin, neomycin, enrofloxacin and erythromycin indicating that these antimicrobials are promising candidates for the control of *E. coli* infection in quails.

**Keywords:** *E. coli*; quails; antibiogram; virulence-associated genes

## 1. Introduction

Japanese quails (*Coturnix coturnix japonica*) are considered as growing sources of meat and egg production worldwide. Quails are characterized by their high feed conversion ratio and rapid growth rates. In Egypt, this small bird is introduced and reared since centuries because of the low running costs and as an excellent source to fill the shortage in the animal-derived proteins. The meat of the quail is rich in protein, fats, vitamins and minerals (Darwish et al., 2018; FAO, 2003). Quails are considered as relatively resistant to diseases compared to chicken, therefore few reports had investigated the diseases affecting the quail's meat industry (Yusuf et al., 2016).

Avian pathogenic *Escherichia coli* (APEC) are major threats affecting poultry industry in Egypt and worldwide. APEC strains are responsible for many cases of colibacillosis, yolk sac infection, cellulitis, coligranuloma and omphalitis in quails (Salehi and Ghanbarpour, 2010). However, there is a clear lack of information about the incidence of the extra-intestinal *E. coli* infections in quails in Egypt.

Extra-intestinal pathogenic *E. coli* (ExPEC) depend mainly on several virulence-associated factors to facilitate bacterial invasion, colonization, spread and reducing the host immune response (Ghanbarpour et al., 2010).

The abuse of antibiotics in the field of veterinary Medicine and in poultry farms, in particular led to development of multidrug resistant pathogens, which make the prevention and control of the bacterial diseases of great difficulty affecting not only the poultry industry but also the public health through the spread of such pathogens via consumption of the contaminated products (Darwish *et al*., 2013).

In sight of the previous facts, the present study aimed at investigating the prevalence of ExPEC in quails suffering from colibacillosis symptoms and visiting private veterinary clinics or educational Veterinary Hospital at Faculty of Veterinary Medicine, Zagazig University, Egypt. The obtained *E. coli* isolated were further serologically identified and the expressions of three virulence-associated genes, including temperature-sensitive hemagglutinin (tsh), Arginine succinyltransferase (astA) and iron outer membrane receptor (iroN), were investigated using multiplex PCR. Finally, the antibiotic resistance profiles of the identified *E. coli* serotypes were screened using the disk-diffusion method.

## 2. Material and methods

### *2.1. Compliance with ethical standards*

All experiments using animals were conducted according to the guidelines for the use of animals adopted by Zagazig University, Egypt.

### *2.2. Specimens*

A hundred specimen including 20 each of heart, lungs, liver, gizzards and small intestine (duodenum) were collected from 20 quails (moribund or freshly dead) collected during their visits to private veterinary clinics or Educational Veterinary Hospital, Faculty of Veterinary Medicine, Zagazig University, Egypt during the period of July to October 2017. Birds were aged 1-3 weeks of age. The collected quails represent 20 quail flocks (average number per flock is 500 ± 125). Birds were suffering from poor appetite, respiratory distress, poor growth associated with diarrhea. Postmortem inspection revealed air sacculitis, pericarditis, perihepatitis, peritonitis and erosions on the gizzards. Specimens were transported without delay in a cooled icebox to the Laboratory of Microbiology, Educational Veterinary Hospital, Faculty of Veterinary Medicine, Zagazig University, Egypt.

### *2.3. Bacteriological examination:*

Tissue samples were directly platted on MacConkey agar plates (Difco, Detroit, MI, USA), followed by incubation at 37 °C for 24 h. Lactose fermentative colonies were re-inoculated to eosin methylene blue agar plates (Difco, Detroit, MI, USA). Typical colonies of *E. coli* appeared greenish, metallic with dark purple center. Such colonies were transferred into Nutrient agar slants and incubated at 37 °C for 24 h and then stored at 4 °C for further identification. Identification of isolates was done according to the method described before (Cloud et al., 1985) based on staining and biochemical tests.

### *2.4. Serodiagnosis of E. coli:*

The confirmed *E. coli* isolates were serologically identified by using rapid diagnostic *E. coli* antisera sets (Difco, Detroit, MI, USA) for diagnosis of the Enteropathogenic types (Kok et al., 1996).

### *2.5. DNA preparation*

Bacterial DNA was extracted from each of glycerol stock Serodiagnosis *E. coli* according to the method described before (Ghanbarpour et al., 2010). DNA concentration in supernatant was evaluated by Nanodrop (ND-1000, Nanodrop Technologies, Wilmington, DE, USA).

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*2.6. Detection of virulence factors by Polymerase Chain Reaction (PCR)*

Tested *E. coli* were examined for harboring different virulence-associated genes including tsh, astA and iroN using multiplex PCR. Primer sets used were displayed in Table 1 and they were designed using Primer3Plus software (https://primer3plus.com/ cgi-bin/ dev/ primer3 plus.cgi). The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). PCR assays were carried out using the method of Dhanashree and Mallya (2008), the amplification reaction was performed on a thermal cycler (Master cycler, Eppendorf, Hamburg, Germany). Amplified DNA fragments were analyzed by 2% of agarose gel electrophoresis (Applichem, Germany, GmbH). Finally, the gel was stained with ethidium bromide and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder was used as a marker. The reference strains were *E. coli* O157:H7 Sakai (positive control strain) and *E. coli* K12DH5α (a nonpathogenic negative control strain).

*2.7. Antibiogram of the identified E. coli serotypes*

Antimicrobial resistance profiles of the identified *E. coli* serotypes were tested by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2013). Antimicrobial sensitivity disks were purchased from Oxoid Limited, Basingstoke, Hampshire, UK. The choice of antimicrobial disks used and diameters of zones of inhibition for the tested strains were also conducted according to CLSI guidelines (2013). Fourteen antimicrobials were tested including ampicillin (10 µg), ceftiofur (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), lincomycin (15 µg), nalidixic acid (30 µg), neomycin (N) (30 µg), oxytetracycline (30 µg), penicillin (P) (10 IU), polymixin B (300 U) and trimethoprim/sulfamethoxazole (SXT) (25 µg). The antimicrobial inhibition zones were measured by a ruler and interpreted according to the recommendation of CLSI (2013).

## 3. Results and Discussion

*3.1. Prevalence of E. coli in quails*

The achieved results in the present study revealed that *E. coli* isolates were recovered at 28% of the examined quail samples. This *E. coli* overall prevalence rate is lower than that reported by Farghaly et al. (2017), who isolated *E. coli* at 50% from quails collected from Giza, Port Said, Cairo and Kafr El Shiekh. However, lower isolation rate (5.7%) for enterotoxigenic *E. coli* was recorded in common quails from Italy (Dipineto et al., 2014). In the current study, *E. coli* was isolated from both intestine and other extra-intestinal organs. The prevalence rates of *E. coli* in the collected samples were 15%, 15%, 20%, 40% and 50% from the examined heart, lungs, gizzard, liver and intestine, respectively (Figure 1). The dissemination of *E. coli* in to different organs such as liver, lungs and heart is going in agreement with the postmortem lesions including fibrinous perihepatitis, pericarditis, enteritis and pneumonia indicating that *E. coli* led to septicemia and followed by death of the birds (Kabir, 2010). The dissemination of *E. coli* into different organs is going in agreement with Darwish et al., (2015) who reported high prevalence of *E. coli* in the duck meat and giblets. Additionally, *E. coli* was isolated from different tissues (spleen, liver, kidney, trachea, lungs, skin, ovary, oviduct, intestine, and cloaca) of chicken and turkeys in Brazil (De Carli et al., 2015).

Serological identification of the isolated *E. coli* demonstrated that five serotypes were identified, namely O2, O20, O35, O78 and O127 as clear in Figure 2. *E. coli* O 127 was the most predominant serotype (28.56%). This serotype was identified at the different organs at 3. 57% (heart), 7.14% (lungs), 3.57% (gizzard), 7.14% (liver) and 7.14% (intestine). *E. coli* O2 came second to O127 at 24.99%. It was similarly isolated from the examined tissues at 3.57% from heart, lungs and gizzard and at 7.14% from both of liver and intestine. *E. coli* O78 was identified at 21.42% and isolated only from liver (7.14%) and intestine (14.28%. *E. coli* O20 was represented 17.85% of the identified *E. coli* serogroups. It was isolated at 3.57% from heart, gizzard and intestine; and at 7.14% from the liver. *E. coli* O35 was the least identified serotype in the present study at 7.14%; and detected at 3.57% from both of the examined liver and intestine. *E. coli* O2, O35 and O78 are frequently reported to be associated with avian colibacillosis (Cloud et al., 1985). In agreement with the results of the present study, Farghaly et al. (2017) reported that the most prevalent *E. coli* serotypes isolated from quails in Egypt were O125, O20, O127, O44 and O78 respectively. Other *E. coli* serotypes as O26 and O128 were also isolated from common quails in Italy (Dipineto *et al.*, 2014). Furthermore, *E. coli* O9, O42, and O88 were isolated and identified from freshly dead Japanese quails in India (Roy et al., 2006).

**Table 1: Primers used in the present study**

| Target | Primer sequence (5'-3') | Product size (bp) | Tm (°C) | Accession number |
|---|---|---|---|---|
| Temperature-sensitive hemagglutinin (*tsh*) | F-5'-aataatgcgccgtcactgg-3'<br>R-5'-aaggagcgctatcctgttt-3' | 130 | 60 | JX466850.1 |
| Arginine succinyltransferase (*astA*) | F-5'-ccaaaaacctcaaaacccc-3'<br>R-5'-tatgccaaagggatgacca-3' | 309 | 60 | NC_000913 |
| **Iron outer membrane receptor (*iroN*)** | F-5'-tcggtatggtttgattcc-3'<br>R-5'-caatggccgtacgtccta−3' | 356 | 60 | NC_014615 |

**Table 2: Antimicrobial resistance profiles of the identified *E. coli* serotypes from naturally-infected quails**

| | Inhibition zone (mm) | O2 | | O20 | | O35 | | O78 | | O127 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No | % | No | % | No | % | No | % | No | % |
| **Ampicillin** | ≤11 | 5 | 71.43 | 4 | 80 | 2 | 100 | 5 | 83.33 | 7 | 87.5 |
| **Ceftiofur** | ≤25 | 6 | 85.71 | 4 | 80 | 2 | 100 | 3 | 50 | 5 | 62.5 |
| **Chloramphenicol** | ≤12 | 2 | 28.57 | 1 | 20 | 0 | 0 | 1 | 16.67 | 2 | 25 |
| **Ciprofloxacin** | ≤15 | 3 | 42.86 | 1 | 20 | 1 | 50 | 1 | 16.67 | 2 | 25 |
| **Enrofloxacin** | ≤14 | 2 | 28.57 | 1 | 20 | 1 | 50 | 1 | 16.67 | 1 | 12.5 |
| **Erythromycin** | ≤13 | 1 | 14.29 | 2 | 40 | 1 | 50 | 1 | 16.67 | 2 | 25 |
| **Gentamicin** | ≤12 | 2 | 28.57 | 1 | 20 | 0 | 0 | 0 | 0 | 1 | 12.5 |
| **Lincomycin** | ≤9 | 4 | 57.14 | 1 | 20 | 1 | 50 | 2 | 33.33 | 2 | 25 |
| **Nalidixic acid** | ≤13 | 3 | 42.86 | 3 | 60 | 2 | 100 | 4 | 66.67 | 5 | 62.5 |
| **Neomycin** | ≤12 | 2 | 28.57 | 1 | 20 | 0 | 0 | 0 | 0 | 1 | 12.5 |
| **Oxytetracycline** | ≤11 | 4 | 57.14 | 2 | 40 | 1 | 50 | 1 | 16.67 | 2 | 25 |
| **Penicillin** | ≤11 | 7 | 100 | 5 | 100 | 2 | 100 | 6 | 100 | 8 | 100 |
| **Polymyxin B** | ≤11 | 5 | 71.43 | 4 | 80 | 2 | 100 | 6 | 100 | 8 | 100 |
| **Sulpha-trimethoprim** | ≤10 | 3 | 42.86 | 2 | 40 | 1 | 50 | 2 | 33.33 | 3 | 37.5 |
| **Total isolates** | | 7 | 100 | 5 | 100 | 2 | 100 | 6 | 100 | 8 | 100 |

The recorded inhibition zones are for resistance profiling according to guidelines of **CLSI** (2013)

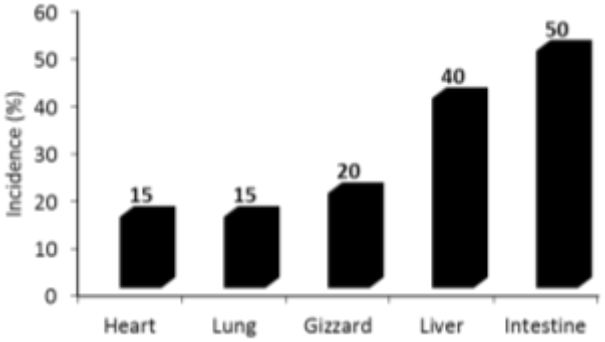


**Figure 1:** Prevalence of *E. coli* in different organs of the examined quails.

*3.2. Expression of virulence-associated genes in the identified E. coli serotypes*

Avian pathogenic *E. coli* strains are characterized by having a peculiar set of pathogenicity genes that called virulence factors which facilitate the dissemination of the bacteria into different organs, colonization and resistance to antimicrobials. These genes are usually expressed in *E. coli* strains associated with colibacillosis (Alizade et al., 2017; Dho-Moulin and Fairbrother, 1999). Among these, temperature-sensitive hemagglutinin (*tsh*) which is a member of the autotransporter group of proteins and was first identified in avian-pathogenic *E. coli* O78. Strains expressing this virulent factor are characterized by their high pathogenicity and lethality (Dozois et al., 2000). The protein, temperature-sensitive hemagglutinin (tsh gene autotransporter) contributes to the development of lesions and deposition of fibrin in the bird's air sacs and cleaves casein and exhibits mucinolytic activity and this may be related to the lesions of air sacculitis, pericarditis, perihepatitis and peritonitis (Henderson *et al*., 1998). The achieved results in the present study indicated that *E. coli* O2, O35, O78 and O127 expressed *tsh* as clear in Figure 3. This result agrees with Dozois *et al*. (2000) who reported that *E. coli* O1, O2 and O78 harbor *tsh* virulent factor and associated with high lethality in one-day old chicks. In addition, *E. coli* O26, O78, O86, O1145 and O127 isolated from the different organs of ducks expressed *tsh* virulent factor (Darwish *et al*., 2015). Arginine is used by various bacteria as nitrogen, carbon and energy sources. In *E. coli*, arginine succinyltransferase (*astA)* pathway plays the major role in arginine catabolism. Over expression of *astA* in *E. coli* isolates is associated with faster growth and rapid multiplication of the bacteria (Schneider et al., 1998). In the present study, *astA* was expressed in *E. coli* 02, O20, 035 and O78 (Figure 3). Similarly, *astA* was over expressed in the highly virulent strain of *E. coli* (O157) (Shirai and Mizuguchi, 2003). Likely, *E. coli* O78, 86 and O114 isolated from different tissues of ducks harbored *astA* (Darwish et al., 2015). Iron outer membrane receptor (*iroN*) is another virulent factor, which is a critical determinant for iron uptake and pathogenesis especially in enteropathogenic *E. coli* species and promotes biofilm formation in ExPEC serogroups (Magistro et al., 2015). In the current study, all identified *E. coli* serotypes including O2, O20, O35, O78 and O127 harbored *iroN* (Figure 3). Similarly, *E. coli* strains isolated from the different organs of ducks including O26, O78, O86, O114 and O127 expressed *iroN* (Darwish et al., 2015).

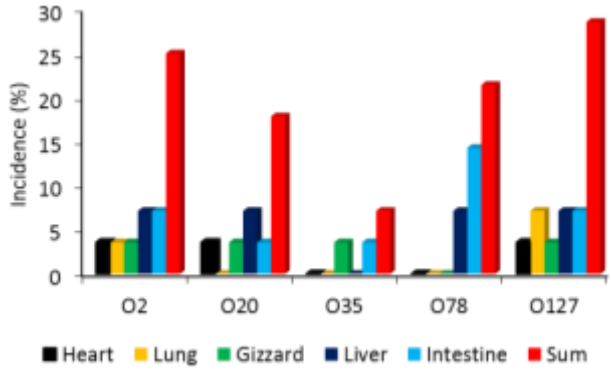


**Figure 2.** Prevalence of different *E. coli* serotypes in the different organs of the examined quails

*3.3. Antibiogram of the identified E. coli serotypes*

The abuse of antimicrobials in poultry farms had led to development of antimicrobial-resistant strains that resulted in a clear difficulty in the prevention and control of bacterial diseases affecting poultry industry (Darwish *et al*., 2013).

In the present study, the resistance profiles of the identified *E. coli* serotypes from naturally-infected quails were described in Table 2. *E. coli* O2 showed marked resistance to several antimicrobials that are commonly used in poultry farms in Egypt. The resistance profile of O2 was 100% to

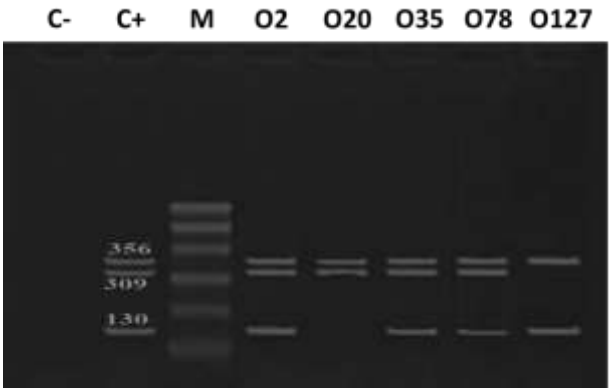


**Figure 3:** DNA expression of virulence-associated genes in *E. coli* serotypes isolated from naturally-infected quails. A representative DNA gel electrophoresis image for a multiplex PCR reaction for virulence-associated genes including *tsh* (130 bp), *astA* (309 bp) and *iroN* (356 bp) in *E. coli* serotypes (O2, O20, 035, O78 and O127). M refers to a 100 bp DNA marker, C- refers to a negative control while C+ refers to a positive control

penicillin; 85.71% to ceftiofur; 71.43% to ampicillin and polymyxin B; 57.14% to lincomycin and oxytetracycline; 42.86% to ciprofloxacin, nalidixic acid and sulpha-trimethoprim; 28.57% to chloramphenicol, enrofloxacin, gentamycin and neomycin; 14.29% to erythromycin. *E. coli* O20 had a resistance profile as follows: 100% to penicillin; 80% to ampicillin, ceftiofur and polymyxin B; 60% to nalidixic acid; 40% to erythromycin, oxytetracycline and sulpha-trimethoprim; 20% to chloramphenicol, ciprofloxacin, enrofloxacin, gentamycin, lincomycin and neomycin. The isolates of *E. coli* O35 had complete resistance to ampicillin, ceftiofur, nalidixic acid, penicillin and polymyxin B; while were completely sensitive to chloramphenicol, gentamycin and neomycin. The resistance profiles of *E. coli* O78 were as following: ampicillin (83.33%), ceftiofur (50.0%), chloramphenicol (16.67%), ciprofloxacin (16.67%), enrofloxacin (16.67%), erythromycin (16.67%), gentamycin (0%), lincomycin (33.33%), nalidixic acid (66.67%), neomycin (0%), oxytetracycline (16.67%), penicillin (100%), polymyxin B (100%) and sulpha-trimethoprim (33.33%). In case of *E. coli* O127, the resistance profiles were as following: ampicillin (87.5%), ceftiofur (62.5%), chloramphenicol (25%), ciprofloxacin (25%), enrofloxacin (12.5%), erythromycin (25%), gentamycin (12.5%), lincomycin (25%), nalidixic acid (62.5%), neomycin (12.5%), oxytetracycline (25%), penicillin (100%), polymyxin B (100%) and sulpha-trimethoprim (37.5%) (Table 2). It is clear from the achieved results that the identified *E. coli* serotypes showed marked sensitivity to some of the tested antibiotics including gentamycin, neomycin, enrofloxacin and erythromycin. These results go in agreement with Farghaly *et al*. (2017) who declared that *E. coli* O20, O78 and O127 isolated from quails showed marked resistance to amoxicillin (71.4%), ciprofloxacin (57.1%), and nalidixic acid (57.1%). Furthermore, *E. coli* serotypes O78 and 127 isolated from duck giblets were highly sensitive to cefotaxime and norfloxacin but resistant to amoxicillin (Darwish *et al*., 2015). The excessive and massive use of the antibiotics in the poultry production might explain the emergence of such resistance *E. coli* strains. Therefore, unnecessary use of antibiotics should be controlled in quail production farms in addition to adoption of strict hygienic measures during the intensive production of such important meat source. Future approaches are also needed to investigate the expression of genes related to antimicrobial resistance in the identified *E. coli* serotypes.

## 4. Conclusion

The results of the current investigation indicated the prevalence of *E. coli* infection in quails reared in Egypt, with the highest incidence recorded in the intestine and liver. The major identified *E. coli* serotypes were *E. coli* O2, O20, O35, O78 and O127. Such pathogenic serotypes harbored virulence-associated genes including *tsh*, *astA* and *iroN*. The identified *E. coli* showed marked resistance to several antibiotics that commonly used in the poultry production in Egypt. However, gentamycin, neomycin, enrofloxacin and erythromycin are considered as promising candidates for the control of *E. coli* infection in quails.

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## Conflict of interests

The authors have not declared any conflict of interests.

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