



Prevalence and Molecular Characterization of Avian Pathogenic *E. Coli* in Ducks

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Abstract | Avian pathogenic *E. coli* (APEC) represents a major threat of the Egyptian poultry industry in and worldwide leading to high economic losses. This study aims to investigate the prevalence of APEC strains and its tissue distribution in diseased ducks of different ages in Sharkia governorate, Egypt, between June and September 2018. Detection of virulence-associated genes among the identified serotypes was carried out using multiplex PCR. Also, the antimicrobial susceptibility of the isolated serotypes was examined using the disk-diffusion method. Our results revealed that *E. coli* isolation rate was 69 out of 200 samples (34.5%) and the highest prevalence was recorded at ducklings of 1-2 weeks old. Liver had the highest prevalence rate among the examined tissue (50%), followed by spleen (42.5%), heart (30%), lungs (25%) and gizzards (25%), respectively. Six serotypes were successively identified; *E. coli* O55:H7, *E. coli* O127:H6, *E. coli* O111:H4, *E. coli* O114:H21, *E. coli* O78:H- and *E. coli* O26:H11 at which *E. coli* O55:H7 serotype was the highest isolation rate 27.54% (19/69). Such pathogenic serotypes harbored virulence-associated genes including *tsh*, *astA* and *iroN*. Finally, antimicrobial sensitivity testing indicated that the isolated *E. coli* serotypes had multidrug-resistant profiles while gentamycin, neomycin and lincomycin are considered as promising candidates for the control of *E. coli* infection in ducks in Egypt.

Keywords | *E. coli*, Ducks, Virulence genes, Antimicrobial sensitivity test

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INTRODUCTION

Escherichia coli (*E. coli*) is a major threat affecting poultry industry in Egypt and worldwide. *E. coli* is normally inhabited in the intestinal tract of birds. However, *E. coli* O26, O78, O114 and O127 serotypes have acquired some virulence-associated genes that enabled them to cause intestinal diarrhea and hemorrhage (Darwish *et al.*, 2015). Such serotypes are known as avian pathogenic *E. coli* (APEC) (Ruttler *et al.*, 2006; Xia *et al.*, 2010). APEC may cause colisepticemia, yolk sac infection, cellulitis, coligranuloma and omphalitis in ducks and other bird species (Salehi and Ghanbarpour, 2010). However, there is a lack of information about both the incidence and

virulence-associated genes characterization for the extra-intestinal *E. coli* infections in ducks at Egypt.

Darwish *et al.* (2013) mentioned that antibiotics such as amoxicillin is regularly used in hen farms for prevention and control of bacterial diseases in Egypt. Moreover, several classes of the antimicrobials are used as feed additives to stimulate the growth and improve bird's feed conversion ratio. However, the extensive and abuse of such antimicrobials may lead to development of drug resistant bacterial strains. Therefore, it is of value to monitor the antibiogram of such pathogenic organisms.

Ying *et al.* (2010) used PCR assay for detection of APEC

isolates and isolates from healthy ducks for characterization colonization factors F1-fimbriae (*fimC*), resistance serum survival protein (*iss*), invasion of brain endothelium protein A (*ibeA*), outer membrane protein A (*ompA*), the iron acquisition-related factors ferric yersiniabactin (*fyuA*), iron repressible protein (*irp2*), haemolysin A and E (*blyA* and *blyE*), Shiga toxin 1 and 2 (*stx*₁ and *stx*₂), intimin (*eaeA*), transcription antiterminator (*rfaH*), as well as *betA* gene (encoding choline dehydrogenase).

Moreover, Nokukhanya and Joshua (2018) have been studied *E. coli* virulence genotyping from 45 different birds by using four different multiplex PCRs to investigate the presence of 12 virulence genes associated with APEC. Ewers et al. (2009) noted that virulence genotyping was considered to be the best way for differentiation between APEC and avian fecal *E. coli* (AFEC), while serotyping might not be particularly useful due to the overlap in serogroups not only between APEC and AFEC but also between APEC and other extra-intestinal pathogenic *E. coli* (ExPEC) isolates.

The present study aims to study the prevalence of the APEC strains in diseased duck flocks in Sharkia governorate, Egypt. Detection of the virulence-associated genes among the identified serotypes was conducted using PCR followed by the antibiogram examination using the disk-diffusion method.

MATERIALS AND METHODS

SPECIMENS

Two hundred specimens were collected from heart, lungs, liver, spleen and gizzards (40 each) from 40 ducks (morbid or freshly dead) between June and September 2018. The age of ducks ranged between one to eight weeks (W) with history of anorexia, poor growth associated with diarrhea. In some cases, respiratory symptoms were observed in some cases. The specimens were transported without delay in a cooled icebox to the Laboratory of Microbiology, Educational Veterinary Hospital, Faculty of Veterinary Medicine, Zagazig University, Egypt.

BACTERIOLOGICAL EXAMINATION AND SEROTYPING OF *E. COLI* ISOLATES

Using Irwin et al. (2010) method, the collected tissue samples were homogenized in sterile saline solution at 10%. Then 0.1 mL of the homogenate was plated on MacConkey agar plates (Difco, Detroit, MI, USA), followed by incubation at 37 °C for 18-24 h. Lactose-fermenting colonies (Pink, round medium-sized colonies) were re-inoculated onto eosin methylene blue agar plates (Difco, Detroit, MI, USA). Typical colonies of *E. coli* appeared greenish, metallic with dark purple center. Colonies were kept on Nutrient agar slants at 4 °C for

further identification. Based on staining and biochemical tests, identification of *E. coli* isolates was done (Cloud et al., 1985).

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

DNA EXTRACTION AND DETECTION OF VIRULENCE ASSOCIATED GENES BY MULTIPLEX POLYMERASE CHAIN REACTION (PCR)

Bacterial DNA was extracted from each of glycerol stock serotyped *E. coli* according to the previously described method (Ghanbarpour et al., 2010). The concentration and the purity of the extracted DNA was evaluated by Nanodrop.

A multiplex PCR was conducted to detect the virulence-associated genes of *E. coli*. The tested virulence-associated genes were included temperature-sensitive hemagglutinin (*tsb*), Arginine succinyltransferase (*astA*) and iron outer membrane receptor (*iroN*). Primer sets (Table 1) Eldin (2019). PCR assays were performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany) using the method of Eldin (2019). Amplified DNA fragments were analyzed by 2% of agarose gel electrophoresis (Applichem, Germany, GmbH). The reference strains were *E. coli* O157:H7 Sakai (positive control strain) and *E. coli* K12DH5a (a nonpathogenic negative control strain) kindly provided by Professor Mohamed Hassan, Food Control Department, Benha University, Egypt.

ANTIBIOGRAM OF THE IDENTIFIED *E. COLI* SEROTYPES

Identified *E. coli* serotypes were subjected to antibiogram susceptibility test using the disk diffusion technique and antimicrobial resistance profile were examined according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2013). The inhibition zone diameter for each antimicrobials was measured and interpreted according to CLSI guidelines (2013).

RESULTS AND DISCUSSION

PREVALENCE OF *E. COLI* IN DUCKS

The affected ducklings under investigation have a history of poor growth and diarrhea with respiratory signs in some cases with clinical postmortem findings of air sacculitis, pericarditis, perihepatitis, peritonitis and erosions on the gizzards. The overall isolation rate of *E. coli* in the examined ducks was 34.5% which considered high compared to the isolation rates of *E. coli* from diseased ducks in Egypt (22.8%) in pervious study Eid et al. (2019), while lower than Na et al. (2019) who reported 36.13% in ducks in

Table 1: Sequence and specificity of PCR primers and their product sizes

Target	Primer sequence (5'-3')	Product size (bp)	Tm (°C)	Accession number	Reference
Temperature-sensitive hemagglutinin (<i>tsh</i>)	F-5'-AATAATGCGCCGTCCTGG-3' R-5'-AAGGAGCGCTATCCTGTTT-3'	284	60	JX466850.1	Eldin (2019)
Arginine succinyltransferase (<i>astA</i>)	F-5'-CCAAAAACCTCAAAACCCC-3' R-5'-TATGCCAAAGGGATGACCA-3'	422	60	NC_000913	
iron outer membrane receptor (<i>iroN</i>)	F-5'-TCGGTATGGTTTGATTCC-3' R-5'-CAATGGCCGTACGTCCTA-3'	617	60	NC_014615	

South Korea. In China *E. coli* isolation rate in ducks was (47.1%) Liu et al. (2018) while higher prevalence rate (69.1%) was reported in chicken Wu et al. (2014). The higher isolation rate was recorded in ducklings aged 1-2 weeks 66.66% (12/18), followed by 3-4 weeks 50% (5/10), 5-6 weeks 42.86% (3/7) and ducks of 7-8 weeks 40% (2/5), respectively (Figure 1). This rate indicates that the incidence of the disease is high in the young age compared with the older ducks. Similar results were reported in ducks from Italy (Sgariglia et al., 2019).

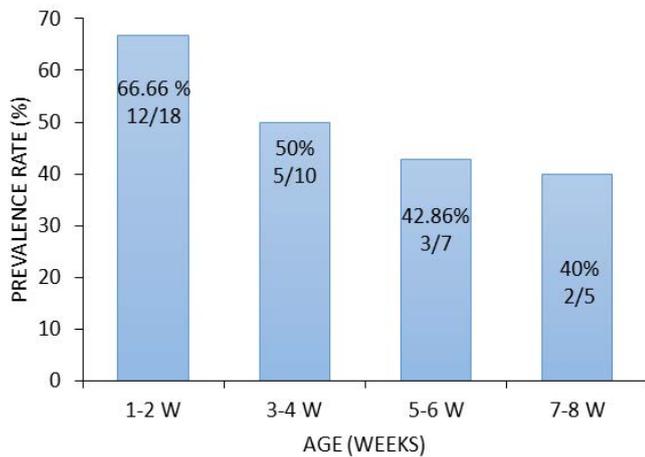


Figure 1: Prevalence rate of *E. coli* among different ages of the examined ducks

The prevalence rate of APEC among different examined tissues revealed that liver had the highest detectable rate 50% (20/40), followed by spleen 42.5% (17/40), heart 30% (12/40), lungs 25% (10/40) and gizzards 25% (10/40), respectively (Figure 2). The dissemination of *E. coli* in different organs such as liver, lungs and heart is verified by 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 tissue distribution of APEC is in correspondence with that reported in ducks (Darwish et al., 2015) and quails in Egypt (Eldin, 2019) reported that liver harbored the highest rate of the extra-intestinal APEC. 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). The high prevalence rate of

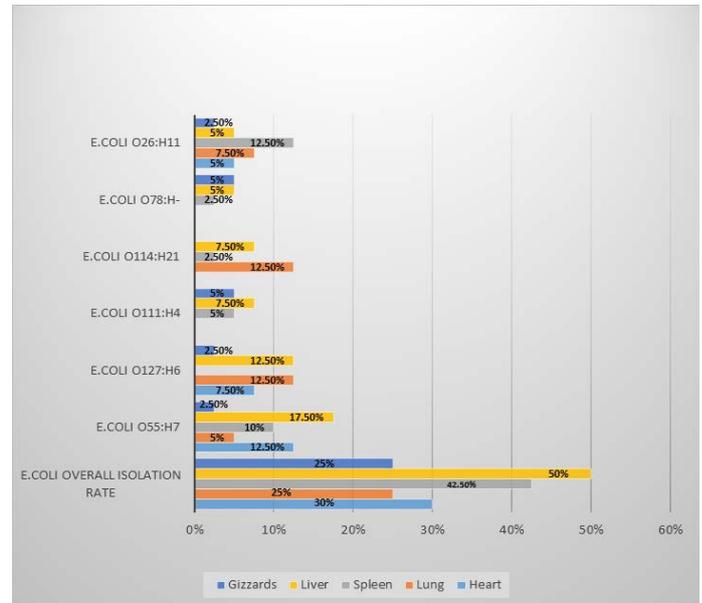


Figure 2: Tissue distribution of the identified *E. coli* serotypes isolated from ducks. Incidence (%) of the identified *E. coli* serotypes from different tissues of the diseased ducks collected from Sharkia Governorate, Egypt (n=40).

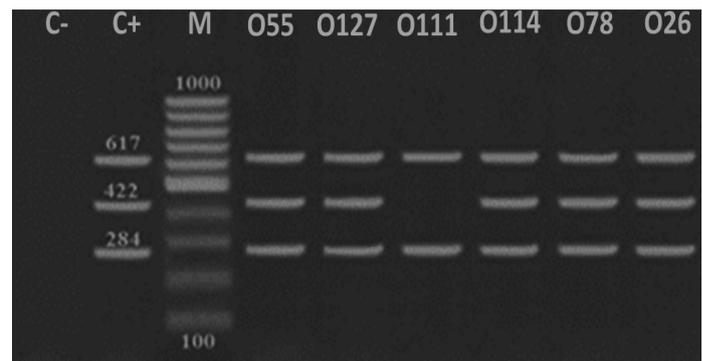


Figure 3: Detection of DNA virulence-associated genes in *E. coli* serotypes isolated from ducks. A representative DNA gel electrophoresis image for a multiplex PCR reaction for virulence-associated genes including *tsh* (284 bp), *astA* (422 bp) and *iroN* (617 bp) in *E. coli* serotypes (O55, O127, O111, O114, O78 and O26). M refers to a 100 bp DNA marker, C- refers to a negative control while C+ refers to a positive control.

E. coli in the liver might be attributed to the translocation

Table 2: Antimicrobial resistance profile among the identified *E. coli* serotypes

	<i>E. coli</i> O55:H7		<i>E. coli</i> 127:H6		<i>E. coli</i> O111:H4		<i>E. coli</i> O114:H21		<i>E. coli</i> O78:H-		<i>E. coli</i> O26:H11	
Ampicillin	17	89.47	12	85.68	6	85.68	8	72.72	5	100	13	100
Ceftiofur	6	31.58	2	14.28	2	28.56	1	9.09	2	40	6	46.15
Chloramphenicol	3	15.79	2	14.28	3	42.84	2	18.18	1	20	2	15.38
Ciprofloxacin	5	26.32	1	7.14	2	28.56	1	9.09	2	40	5	38.46
Enrofloxacin	4	21.05	1	7.14	3	42.84	2	18.18	2	40	3	23.07
Erythromycin	4	21.05	3	21.42	1	14.28	2	18.18	2	40	3	23.07
Gentamicin	2	10.53	2	14.28	1	14.28	1	9.09	1	20	2	15.38
Lincomycin	7	36.84	2	14.28	3	42.84	1	9.09	0	0	2	15.38
Nalidixic acid	14	73.68	12	85.68	5	71.4	8	72.72	3	60	9	69.23
Neomycin	4	21.05	2	14.28	1	14.28	1	9.09	1	20	2	15.38
Oxytetracycline	8	42.10	4	28.56	3	42.84	3	27.27	2	40	8	61.54
Penicillin	19	100	12	85.68	7	99.96	10	90.9	5	100	13	100
Polymyxin B	15	78.94	10	71.4	4	57.12	8	72.72	5	100	10	76.92
Sulpha-trimethoprim	8	42.10	5	35.7	2	28.56	4	36.36	3	60	7	53.84
Number of isolates	19	100	14	100	7	100	11	100	5	100	13	100

of *E. coli* pathotypes from intestine to the liver through the mesenteric lymph nodes leading directly for failure of the immune defense (MacFie, 2004).

Sixty-nine *E. coli* strains were isolated in the current study. Serotyping of the isolated *E. coli* revealed six serotypes, namely *E. coli* O55:H7, *E. coli* O127:H6, *E. coli* O111:H4, *E. coli* O114:H21, *E. coli* O78:H- and *E. coli* O26:H11. *E. coli* O55:H7 had the highest isolation rate 27.5% (19/69 strains), followed by *E. coli* 127:H6 20.3% (14/69), *E. coli* O26:H11 18.8% (13/69), *E. coli* O114:H21 15.9% (11/69), *E. coli* O111:H4 10.1% (7/69) and *E. coli* O78:H- 7.24% (5/69), respectively (Figure 2). Similarly, Darwish et al. (2015) isolated and identified *E. coli* O26, O78, O114 and O127 serotypes from ducks in Egypt. On the other hand, Cloud et al. (1985) noted that *E. coli* O2, O35 and O78 are frequently associated with avian colibacillosis.

DETECTION OF VIRULENCE-ASSOCIATED GENES

Avian pathogenic *E. coli* strains that cause colibacillosis harbor a peculiar set of pathogenicity genes that called virulence factors which facilitate the dissemination of the bacteria into different organs and colonization (Alizade et al., 2017; Dho-Moulin and Fairbrother, 1999). Temperature-sensitive hemagglutinin (*tsb*) is a member of the autotransporter group of protein strains harbored this virulent factor are characterized by their high pathogenicity and lethality as it contributes to the development of air sacculitis, pericarditis, perihepatitis and peritonitis (Dozois et al., 2000). Our results indicated that all identified pathotypes harbored *tsb* and *iroN* clarifying the importance of these genes for pathogenicity while *E. coli*

O55, O127, O111, O114, O78 and O26 serotypes conveyed *tsb* and *iroN* (Figure 3). On the other hand, Darwish et al. (2015) and Dozois et al. (2000) detected these virulent factors at *E. coli* O1, O2, O26, O78, O86, O114 and O127 serotypes that isolated from chickens and ducks. Iron outer membrane receptor (*iroN*) is another virulent factor that facilitate iron uptake, pathogenicity and biofilm formation (Magistro et al., 2015). Also Darwish et al. (2015) and Eldin (2019) highlighted that *E. coli* strains isolated from different organs of ducks and quails including O26, O78, O86, O114 and O127 extracted *iroN*. Likewisem arginine succinyltransferase (*astA*) is associated with faster growth and rapid multiplication of different species of bacteria including *E. coli* (Schneider et al., 1998). In the present study, *astA* was expressed in *E. coli* O55, O127, O114, O26 and O78 serotypes (Figure 3). Similarly, *astA* was expressed in *E. coli* O157 serotype (Shirai and Mizuguchi, 2003), *E. coli* O78, O86 and O114 serotypes (Darwish et al., 2015).

ANTIBIOGRAM OF THE IDENTIFIED *E. COLI* SEROTYPES

The excessive use of antimicrobials in poultry farms in Egypt had led to development of antimicrobial-resistant bacterial strains that resulted in a clear difficulty in the control of such pathogens (Darwish et al., 2013).

The resistance profiles for the identified *E. coli* pathotypes in the present work was reflected that *E. coli* O55 was resistant 100% to penicillin; 89.47% to ampicillin 78.94% to polymyxin B; 73.68% nalidixic acid and less than 50% to other tested antimicrobials (Table 2). *E. coli* O127 had a resistance profile as follows: 85.68% to ampicillin, penicillin, nalidixic acid; 71.4% to polymyxin B, while this

serotype showed resistance percentage to the other tested antimicrobials from 7.14% to 35.7% (Table 2). *E. coli* O111 was resistant 100% to penicillin, 85.68% to ampicillin and 57.12% to polymyxin B. While were less resistant to erythromycin, gentamicin and neomycin (14.28%). The resistance profiles of *E. coli* O114 were as following: 90.9% to penicillin; 72.72% to each of ampicillin, nalidixic acid and polymyxin B; 36.36% to sulpha-trimethoprim and 9.09% to each of ceftiofur, ciprofloxacin, gentamycin, lincomycin and neomycin (Table 2). *E. coli* O26 and O78 showed complete resistance to ampicillin, penicillin and polymyxin B (Table 2). It is clear from our results that most identified *E. coli* serotypes showed marked sensitivity to some of the tested antibiotics including gentamycin, neomycin, enrofloxacin and erythromycin. Our results are comparable with that reported by Darwish et al. (2015) who reported that *E. coli* O26 and O78 were highly sensitive to cefotaxime and norfloxacin but resistant to amoxicillin. Na et al. (2019) isolated extended-spectrum β -lactamase producing *E. coli* isolated from ducks in South Korea. Furthermore, Farghaly et al. (2017) 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%). The excessive and massive use of the antibiotics in the poultry production might explain the emergence of such resistance to *E. coli* strains. Therefore, the misuse of antibiotics should be stopped in duck farms in Egypt. Future approaches are also needed to investigate the expression of genes related to antimicrobial resistance in the identified *E. coli* serotypes.

CONCLUSION

The current study indicates the prevalence of *E. coli* infection in ducklings and ducks in Egypt. Ducklings are highly susceptible to *E. coli* infection compared with old ducks and liver is the most affected organ. The prevalent *E. coli* serotypes were *E. coli* O55:H7, *E. coli* O127:H6, *E. coli* O111:H4, *E. coli* O114:H21, *E. coli* O78:H- and *E. coli* O26:H11, respectively, and harbored virulence-associated genes *tsb* and *iroN*. All extracted *astA* gene except *E. coli* O111:H4. The identified *E. coli* had multidrug resistance profiles while gentamycin, neomycin and lincomycin are considered as promising candidates for the control of *E. coli* infection in ducks in Egypt.

AUTHORS CONTRIBUTION

All authors contributed equally.

CONFLICT OF INTEREST

There is no conflict of interest.

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