Isolation and Molecular Identification of Avian Pathogenic 
*Escherichia coli* in Broiler Chickens Suffering from Colibacillosis in Some Governorates in Egypt

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This study aimed to evaluate the most predominant strain of avian pathogenic *Escherichia coli* (APEC) that causes colibacillosis with respiratory manifestation in broiler chickens. Isolation and serotyping identification for the isolates of *E. coli* were performed. Prevalence of APEC differentiated by detection of the 8 virulence-associated genes "astA, iss, irp2, papC, iucD, tsh, vat, and cva/cvi" using multiplex PCR technique in the most important isolates of *E. coli* was also investigated. Antibacterial sensitivity test for antibiotics disc and oregano at different concentrations (20%, 15%, 10%, and 5%), were estimated for the most important isolates of *E. coli*. A total of 50 broiler farms were located in Gharbia, Giza, Menoufa, Bahaira, and Dakahliah Governorates were examined. Specimens from lung, liver, spleen, and kidney from diseased birds were used for bacteriological examination. Results revealed that *E. coli* O27 was the most predominant serotype strain (11 out of 35 isolates), followed by the serotypes of O146 (4/35), O Untypable serotypes (4/35), and the (1/35) of each serotype of O8, O20, O44, O78, O114, O125, O142, O152, O153, O158, O164, and O166. One isolate of APEC O27 out of 11 isolates has 6 virulence-related genes "astA, iss, papC, iucD, tsh, vat, and cva/cvi". Difloxacin and oregano at a concentration of 20% have the highest percentage (100%) of sensitivity against 11 isolates of *E. coli* O27 strains. In conclusion, *E. coli* O27 was the most predominant serotype infecting farms of broiler chickens. Difloxacin and oregano 20% could be used for the treatment of APEC O27.

**Keywords:** Molecular, Avian pathogenic *E. coli* O27, Antibacterial sensitivity test, Broiler chickens.

**Introduction**

The poultry sector remains invested in promoting food sustainability in Egypt. Unfortunately, different challenges, including diseases, have resulted in considerable losses in this segment. *Escherichia coli* (*E. coli*), is harmless, normal inhabitants of the gastrointestinal tract of animals and human beings [1]. Specific strains of *E. coli* can invade multiple birds’ organs, causing peritonitis, periretibitis, air sacculitis, pericarditis and other extraintestinal infections [2]. Avian colibacillosis is the most common disease leading to significant economic losses to the poultry industry in the world [3]. Collectively, experts have termed extraintestinal conditions as colibacillosis. Avian pathogenic *E. coli* (APEC) is a causative agent for colibacillosis. The pathogenic capability of *E. coli* is mainly due to multiple virulence...
factors. The presence of at least six or more of eight virulence-associated genes determines the presence of APEC [4]. The infection relevant by colibacillosis in fowls is pathogenic, might primary or secondary and consequently the symptoms become complicated as a reason of mixed infection. In birds, particularly APEC is the cause strain agents of colibacillosis and this is considered as a serious problem affecting the poultry industries [5]. The categorization of APEC strains is an extraintestinal pathogenic E. coli, this category has a specification characterized by presence of virulence factors that encourage the ability to survive extra-intestinally [6]. Those genes include iron acquisition genes (iucD, iroN), adhesion genes (tsh), toxin genes (vat, hlyF), serum resistance genes (iss), housekeeping proteases (ompT), and CoV Operon genes (cvi/cva). The APEC virulent “genes are enterohaemagglutinin (aest), increased serum survival protein (iss), iron-repressible protein (irp2), P fimbriae (papC), aerobactin (iucD), temperature-sensitive hemagglutinin (tsh), vacuolating autotransporter toxin (vat), and colicin V plasmid operon genes (cva/cvi)” [7]. “astA gene encodes for EAST1 (enterohaemagglutinin E. coli heat-stable enterotoxin 1), which was first observed in EAEC (enterohaemagglutinin E. coli) strains, that had been recognized as an agent of diarrhea” [8]. Despite the role of EAST1 in pathogenesis still established incompletely, the existence of this toxin was found in high frequency [9-10]. In chickens, iss gene attributes to increased serum survival protein that has been explained as a significant character of virulent E. coli [11]. “iss and the protein which iss encodes were useful targets of colibacillosis” as the reported previous studies [7, 12]. Additionally, in both CoV plasmid and bacterial chromosome, the location of iss gene tends to exist [6, 9, 12].

Oregano essential oil (Origanum vulgare L.) is an aromatic herbal product that has been used as an antibiotic for treatment of E. coli due to its phenolic compounds as carvacrol and thymol and their pre-cursors (p-cymene and γ-terpinene). Organo essential oil and its components have biological and pharmacological properties that included antimicrobial, antifungal, antiproliferative, anti-inflammatory, hepatoprotective, spasmylytic, and vasorelaxant [13-19], microflora balance in the intestine [20] antitoxiccidal agent [21] immunomodulatory [22], acaricidal activity [23], and antioxidant [24,25] properties. Also, carvacrol-rich oregano oil has high antibiofilm and antivirulence activities against uropathogenic E. coli [26]. Oregano essential oil is as an antimicrobial and antioxidant additive in food products. Moreover it has been used as a growth promoter which has less feed intake and a good feed conversion ratio in broiler chickens [21]. Oregano supplementation in diet improves the growth performance, carcass characteristic and some physiological and immunological parameters of broiler chicks [27]. In addition, oregano modulates microbial species in the intestine and improves their capacity for nutrient absorption, and promotes integrity of the intestinal barrier [28] and it reduces pathogen contamination [29]. So, oregano has a beneficial effect on the function of gut mucosa and digestion.

Therefore, this study aimed to evaluate the most predominant strain of avian pathogenic Escherichia coli that causes colibacillosis with respiratory manifestation in broiler chickens. Isolation and serotyping for E. coli isolates were performed. Detection of virulence related genes using multiplex polymerase chain reaction (PCR) technique in the most predominant isolates of E. coli was also investigated. Moreover, antibacterial sensitivity test for antibiotics disc and oregano at the concentrations of 20%, 15%, 10%, and 5%, were estimated for the most important isolates of E. coli.

Materials and Methods

Broiler Farms Locality

A total number of 50 broiler farms were examined for isolation and identification of avian pathogenic E. coli in chickens suffering from colibacillosis during the period from 2016 to 2017. The age of these birds ranged from the 20th to 37th day old. The capacity of farms was ranging from 1000-27000 birds/farm. These farms are located in 5 Governorates; Gharbia, Giza, Menofia, Bahaira, and Dakahliah (10 Farms/Governorate) in Egypt.

Sampling

Specimens were collected from lung, liver, spleen, and kidneys of broiler chickens suffering from loss of body weight, mortalities and respiratory manifestations from freshly dead or sacrificed. All samples were given a serial number and detailed information about age, clinical manifestations, postmortem lesions as well as the sample type. This study was conducted regarding to the guidelines of animal experiments and the Institutional Animal Care and Use Committee approval statements protocol, “National Research Centre, Dokki, Giza, Egypt, the protocol approval No.”: 1276072021.
Bacteriological examination

The isolation methods of *E. coli* from various samples were performed in the current study matching the standards of ISO-9308-1:2014 [30]. Specimens from lung, liver, spleen, and kidney were taken under aseptic condition and incubated for 24-48 hrs over night at 37°C. The inoculated colonies had a diameter of 2-3 mm, and light pink color (Lactose positive) on MacConkey’s agar and shiny metallic green colonies on Eosin Methylene Blue agar [31]. Morphological and biochemical identification of the isolates were carried out according to the method of Quinn et al. [32] using the oxidase, catalase, urease, H2S production on TSI, and citrate utilization tests. Other tests including motility test and hemolysis onto blood agar were performed.

Serological detection of *E. coli*

Isolation relevant for typing of *E. coli* was conducted in the Central Laboratory, Veterinary Quality Control- Poultry Production at Animal Health Research Institute, Dokki, Giza, Egypt, according to the method of Ørskov and Ørskov [33].

Antibacterial Assay

Preparation *E. coli* O27 strain suspensions

A suspension of each isolate (11) of *E. coli* O27 strain was freshly prepared by inoculating fresh stock culture (cultivated for 16 hours) from each isolate into tubes, each containing 7 ml of Muller Hinton Broth (Oxoid, UK). The inoculated tubes (11) were incubated at 37 °C for 24 h. Serial dilutions were carried out for each isolate, till dilution matching with 0.5 Mc- Farland scale standard which selected for screening of antimicrobial activities (~ each inoculum containing about 1×10⁶ colony-forming unit (CFU/ml)).

Antibacterial sensitivity test

According to the method of Quinn et al., disc diffusion procedure using Muller Hinton agar was illustrated to determine the antibacterial sensitivity of 11 isolates of *E. coli* O27 [32] and the Clinical and Laboratory Standard Institute (CLSI) recommendations [34, 35]. Routine susceptibility of sixteen antibacterial discs (Oxoid, UK), included difloxacin, gentamycin, cephalin, norfloxacin, amoxicillin, erythromycin, sulphamethoxine & trimethoprim, spiramycin, lincomycin, apramycin, ceftaxione, oxytetracycline, ampicillin, cefotaxime, streptomycin, and colisint sulphate. The sensitivity degree was detected by estimation the growth inhibition zone produced by the diffusion of the antibiotic into the surrounding medium followed by the incubation for 24 hours at 37 °C under aerobic status.

Oregeno solution 20%

Oregeno solution 20% (Ropadiar®) is derived from Oregano essential oil. It was purchased from ROPA Pharm International Co., Netherlands.

Agar-well diffusion method

The antibacterial activity of different concentrations of oregano (20% (stock), 15%, 10%, and 5%) against 11 isolates of *E. coli* O27 was evaluated using agar-well diffusion method according to the procedure of Katircioğlu and Mercan [36]. A 100 μl of cell culture suspension (about 1×10⁶ CFU/ml) of each isolate was spread onto each agar nutrient plate. For the investigation of the antibacterial activity, 100 μl of oregano 20%, 15%, 10% and 5%, were poured into the wells of agar plates directly, and were left for 1 h at 25°C. The plates were re-incubated at °C for 24 h. After incubation, the plates were investigated for antimicrobial activity by estimating the diameters of the inhibition zones for each concentrations of oregano. The tests were run in triplicate for each isolate.

Prevalence of APEC Differentiated using Multiplex PCR

The isolates of *E. coli* O27 strain were examined for detection of 8 virulence associated genes (“astA, iss, irp2, papC iucD, tsh, vat, and cva/cvi”). By using QIAamp DNA Mini kit (Qiagen, Germany, GmbH), bacterial DNA was purified from pure cultures. Primers of specific PCR virulent genes are used and illustrated in Table (1), the primers were supplied via Multiplex PCR Master Mix –Takara- Japan, 1 μl of 20 pmol conc. of each primer, 4.5 μl of water and 6 μl of template DNA were carried out in each PCR reaction. In Biometra T3 thermal, the cycling was performed. In this study, eleven of *E. coli* O 27 strains isolates were subjected to 8 virulent genes to detect virulence of the most isolated strain.

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Results

Prevalence of E. coli isolates

The prevalence of E. coli isolates among the examined samples revealed that broiler chickens which were located in Gharbia Governorate showed the highest percentage of infection with E. coli isolates followed by chickens located in Menoufia, Behira, Giza and Dakahlia Governorates.

The total number of samples collected from broiler chickens were 553, from which 114, 92, 111, 129, and 107 samples were collected from Gharbia, Giza, Menoufia, Bahaira, and Dakahlia Governorates, the positive samples for E. coli were 79, 71, 86, 102 and 80, respectively.

Bacterial isolation and identification

Positive samples of E. coli isolates showed typical pink colonies on MacConkey agar and showed characteristic green metallic sheen on EMB agar. Biochemical characters showed citrate utilization test negative, H$_2$S production in TSI agar negative, indole production positive, urease activity negative, methyl red test positive, Voges-Proskauer test negative and Lysine decarboxylase positive.

Serological identification of E. coli

Results of the serological identification showed 14 serotypes of E. coli as shown in Table (2). The isolated E. coli serotypes are O8, O20, O27, O44, O78, O114, O125, O142, O146, O152, O153, O158, O164, O166, and O Untypable. The isolates of E. coli O27 strain were predominant with a percentage of 31.42% (11 out of 35). So, all the eleven E. coli O27 isolates were subjected to the antibacterial sensitivity test and multiplex PCR technique for detection of genes associated with virulence.

Antibacterial sensitivity test

Antibacterial sensitivity test for 11 isolates of E. coli O27 revealed that difloxacin antibiotic showed sensitive (100%) to all the tested isolates. Colistin sulphate was sensitive with a percentage of 63.6%. The most antibiotics used have resistance as shown in Table (3) where a lot of antibiotics showed resistant and intermediate resistance.

Oregano at concentration of 20% showed the highest percentage (100%) of sensitivity against 11 isolates of E. coli O27 strains. While, oregano 15% was less sensitive with a percentage of 45.5% (Table 3).

| No. | Virulent genes | Primer sequence (5’-3’) | Location within gene | GenBank Accession No. | Size (bp) |
|-----|----------------|-------------------------|----------------------|----------------------|-----------|
| 1   | astA           | TGCCATCAACACAGTATATCC   | 797–817              | AF143819             | 116       |
|     |                | TCAAGTCCGAGTACGCGGC     | 912–894              |                      |           |
| 2   | Iss            | ATCACATAGGATTCTGCCG     | (-10)–(-28)          | X52665               | 309       |
|     |                | CAGCCGATATAGATGCA       | 282–264              |                      |           |
| 3   | irp2           | AAGGATTCCGTCTTGACCGGAC  | 22–42                | L18881               | 413       |
|     |                | AACTCTGATACGAGTTGCC     | 434–416              |                      |           |
| 4   | papC           | TGATATACCGCAGTCTAGGC    | 1284–1304            | Y00529               | 501       |
|     |                | CCGCCATATTCACATATAA     | 1784–167             |                      |           |
| 5   | iucD           | ACAAAAAGTCTATAGCTTTC    | 239–259              | M18968               | 714       |
|     |                | CCTGATCCAGATGATGTC      | 952–934              |                      |           |
| 6   | Tsh            | ACTTTTCTGCAGGAAGTC      | 132–151              | AF218073             | 824       |
|     |                | CTTCCAGTGTCAGAAGT       | 955–937              |                      |           |
| 7   | Vat            | TCCGGACATAATGGTCAG      | 1076–1095            | AY151282             | 981       |
|     |                | GTCTCAAGACCAGATTGT      | 2056–2038            |                      |           |
| 8   | cva A/B        | TGGTAGAAGTGCCAGAGCAAG   | 10745–10764          | AJ223631             | 1,181     |
|     | cvi cvaC       | GAGCTGTCTGGTACGAAGGCCC  | 11925–11904          |                      |           |

Table 1. Oligonucleotide primers sequences (Kwon et al. [4] and Ewers et al. [7])
TABLE 2. Serotypes of Escherichia coli and their detected numbers

| Serotypes of Escherichia coli | Number | Percentage (%) |
|------------------------------|--------|----------------|
| O8                           | 1      | 2.86           |
| O20                          | 1      | 2.86           |
| O27                          | 11     | 31.42          |
| O44                          | 1      | 2.86           |
| O78                          | 2      | 5.71           |
| O114                         | 1      | 2.86           |
| O125                         | 2      | 5.71           |
| O142                         | 1      | 2.86           |
| O146                         | 4      | 11.43          |
| O152                         | 1      | 2.86           |
| O153                         | 1      | 2.86           |
| O158                         | 2      | 5.71           |
| O164                         | 1      | 2.86           |
| O166                         | 2      | 5.71           |
| O Untypable                  | 4      | 11.43          |
| **Total**                    | **35** | **100**        |

TABLE 3. Antibacterial sensitivity test and MIC of Oregano

| Antibacterial | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | % of sensitivity |
|---------------|---|---|---|---|---|---|---|---|---|----|----|-----------------|
| Difloxacin    | S | S | S | S | S | S | S | S | S | S  | S  | 100             |
| Gentamycin    | S | S | S | I | R | R | 1 | S | R | 1  | R  | 36.4            |
| Cephradine    | R | R | R | R | R | I | S | R | R | I  | I  | 9               |
| Norfloxacin   | S | R | R | I | S | I | R | R | S | I  | I  | 27.3            |
| Amoxicillin   | R | I | S | R | R | R | S | R | I | S  | I  | 27.3            |
| Erythromycin  | S | R | R | S | R | R | R | R | S | R  | S  | 36.6            |
| Sulphamethoxocin & trimethoprim | I | I | I | S | I | S | R | R | S | S  | S  | 36.4            |
| Oregano 20%   | S | R | R | S | R | S | S | S | R | S  | R  | 36.4            |
| Lincomycin    | R | R | R | R | R | R | R | R | R | R  | R  | 0               |
| Apramycin     | S | R | R | S | R | S | R | R | S | R  | R  | 36.4            |
| Ceftriaxone   | S | R | S | I | S | I | S | I | S | I  | I  | 54.5            |
| Oxytetracycline | R | R | R | R | R | R | R | R | R | R  | R  | 0               |
| Ampicillin     | R | R | R | R | R | R | R | R | R | R  | R  | 0               |
| Cefotaxime    | S | R | S | R | S | S | R | R | S | R  | R  | 45.5            |
| Streptomycin  | R | R | R | R | R | R | R | R | R | R  | R  | 0               |
| Colistine sulphate | S | R | R | S | S | S | S | R | S | R  | S  | 63.6            |
| Oregano 20%   | S | S | S | S | S | S | S | S | S | S  | S  | 100             |
| Oregano 15%   | S | S | I | I | S | I | S | I | S | S  | I  | 45.5            |
| Oregano 10%   | R | R | R | R | R | R | R | R | R | R  | R  | 0               |
| Oregano 5%    | R | R | R | R | R | R | R | R | R | R  | R  | 0               |

S= Sensitive.  I= Intermediate resistant.  R= Resistant.
Detection of Virulence-associated genes

Results revealed that one isolate APEC O27 has 6 virulence related genes “\textit{astA, iss, papC, iucD, vat} and \textit{cva/cvi}” from eleven isolates of \textit{E. coli} O27 serotype.

In this illustrated Fig.1, there were three isolates of \textit{E. coli} O27 that have virulence-associated genes; Two isolates have 3 virulence-associated genes –non APEC O27- (Fig. 1: lanes 1 & 2) and one isolate only has 6 virulence-associated genes –APEC O27- (Fig. 1: lane 3). Control negative has no virulence-associated genes (Fig. 1: -ve).

Discussion

This study was conducted to evaluate the most predominant strain of APEC causing colibacillosis with respiratory manifestation in broiler chickens in farms located in the Governorates of Giza, Menofia, Bahaira, Dakahliah and Gharbia in Egypt. Isolation and molecular identification of \textit{E. coli} were performed by bacteriological, biochemical and serological diagnosis. Antibacterial sensitivity test of different types of antibiotics discs, and oregano at different concentrations (20%, 15%, 10%, and 5%) against \textit{E. coli} O27 were also investigated.

In this study, isolates of \textit{E. coli} O27 strains showed high level of resistance against amoxicillin, ampicillin, oxytetracycline, trimetoprim-sulfamethoxazole, lincomycin, streptomycin, norfloxacine, cephradine, and spiramycin but high sensitive to difloxacin. Similar result was recorded by Messaï et al. [37] who studied the antimicrobial resistance of APEC strains responsible for colibacillosis in broiler breeding farms in eastern Algeria and Sarker \textit{et al.} [38] reported resistance to ampicillin and tetracycline among multi drug resistance (MDR) \textit{E. coli} isolated from broiler chickens. Moawad \textit{et al.} [39] observed high resistant \textit{E. coli} isolates from raw chicken meat with the different antibiotics (ampicillin (71.4%), tetracycline (80.9%), streptomycin (61.9%), trimethoprim/sulphamethoxazole (61.9%), and cefotaxime 33.3%). Several international studies have investigated the phenotypic resistance profile of \textit{E. coli} isolates from chicken, such as those conducted in Saudi Arabia [40], Algeria [41], Canada [42], and the Netherlands [43].

In the present work, oregano 20% showed the highest percentage (100%) of antimicrobial activity against 11 isolates of \textit{E. coli} O27 strain due to presence of phenolic compounds as carvacrol and thymol and their pre-cursors (p-cymene and γ-terpinene) which have potential antimicrobial agents against several types of both Gram negative and Gram positive bacteria [44, 45]. The most important bacteriostatic component in oregano oil is carvacrol. The mechanism of action of carvacrol on cell membrane of the food-borne pathogen \textit{Bacillus cereus} reported by Ultee \textit{et al.} [46] revealed that carvacrol interacts with the cell membranes of bacteria by alteration its permeability for cations (as hydrogen ion (H\textsuperscript{+}) and potassium ion (K\textsuperscript{+})). The removal of ion gradients leads to impairment of essential processes in the cell lead finally to cell death. Farag \textit{et al.} [47] and Smink [48] recorded that the high antioxidant activity of thymol (a bioactive component presents in essential oil of oregano), is due to the presence of phenolic OH groups which act as hydrogen donors to the peroxy radicals that produced during the first step in lipid oxidation, thus, retarding the hydroxy peroxy formation. Antioxidant and antibacterial activity of oregano essential oil [49, 50] is due to free radical scavenging activity, total reducing capacity and prevent autoxidation of polyunsaturated fatty acid esters [51]. Moreover, oregano essential oil act as anti-inflammatory by inhibiting the inflammatory biomarkers levels; monocyte chemoattractant protein-1 (MCP-1), the vascular cell adhesion molecule-1 (VCAM-1) and the intracellular cell adhesion molecule-1 (ICAM-1) (MCP-1, VCAM-1 and ICAM-1), and decrease synthesis of cytokines; tumor necrosis factor- alpha (TNF-α), interleukin (IL)-6, IL-1β, and IL-6 and increased synthesis of cytokine IL-10 [22]. So, oregano has anti-inflammatory and antimicrobial effects [52], by modulating the cytokine levels and immunity-related transcription factors [28]. Also, carvacrol reduces the severity of inflammation by the production of mediators of inflammation and protein concentration as well as the gene expression modulation of interleukins [53]. Burt and Reinders [54] recorded that 625 µl of organo essential oil/L have antibacterial activity which causes irreversible damage within 1 min against \textit{E. coli} O157:H7. Da Costa \textit{et al.} [55] reported that the minimum inhibitory concentration (MIC) of \textit{Origanum vulgare} essential oil against \textit{E. coli} was inhibited (100%) at the concentration of 0.125%. While, Pei \textit{et al.} [56] found that thymol/carvacrol components have antibacterial activity against \textit{E. coli} at 400 mg/l.
The virulence related genes rates and the combinational abilities of APEC within targeted 8 virulence associated genes were studied [4]. In the present study, one isolate APEC O27 has 6 virulence related genes “astA, iss, papC, iucD, vat and cva/cvi” from eleven isolates of E. coli O27 serotype. Subedi et al. [1] found similar APEC-associated virulence genes in broiler chickens in Nepal. In previous study, Kwon et al. [4] found 4 virulence genes (iss, vat, iucD and cvi/cva) among 18 avian E. coli strains.

In this study, one isolate of APEC O27 has the most virulent genes of pathogenicity and could cause severe losses in the broiler farms.

**Conclusion**

*E. coli* O27 strains were the most predominant serotype infected chickens in broiler farms. APEC O27 possessed the six virulent associated genes of pathogenicity and could cause severe losses in the broiler farms. Difloxacin and oregano 20% could be used for the treatment of APEC O27. Further investigations should be performed experimentally to study the pathogenesis of local isolate APEC O27 strain in broiler chickens and trails for its treatment.

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**Conflict of Interest**

None.

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None.

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**ISOLATION AND MOLECULAR IDENTIFICATION OF A VIAN PATHOGENIC *Escherichia coli* …**

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**عزل وتصنيف جزيئي لبكتريا الإشريشيا كولاي الممرضة للطيور في بدارى التسمين التي تعاني من مرض العصيات القولونية والمتواردة في بعض محافظات مصر**

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**أجريت هذه الدراسة بهدف تقييم السلالة الأكثر انتشاراً لبكتريا الإشريشيا كولاي الممرضة للطيور في بدارى التسمين والتي تعاني من مرض العصيات القولونية مع وجود أعراض تنفسية، تم إجراء عزل وتصنيف سيرولوجي للعزلات الإشريشيا كولاي، وتتم تحديد مدى الاضرار للعِلات المعزولة من خلال الكشف عن “astA, iss, irp2, papC iucD, tsh, vat, and cva/cvi” وذلك باستخدام تقنيات تفاعل متعدد البلمرة المتسلسل، تم عمل اختبار الحساسية لأهم العِلات المتداولة لهذه البكتريا وذللك باستخدام أقراص مضادات الحيوية والأوريجانو بتركيزات مختلفة (20%, 15%, 10%, 5%), تم فحص عدد خمسون مزارع دواجن البديدة المشكوك فيها في محافظات القاهرة، الجيزة، الغربية، المنوفية، والبحيرة، وقد أخذت عينات من الرئة والكبد والطحال والكلى من الطيور المريضة للعِصيات البكتيرية، وقد أظهرت نتائج التصنيف السيرولوجي أن سلالة الإشريشيا كولاي O27 هي الأكثر انتشارًا بعدم O146 (3/35) وO124 (2/35) من أصل 35 عِلة، تتبعها (O146, O144, O124) من سلالة الغير معرفة سيرولوجيًّا (35/35) ، وقد وضع عدد 4 عِلة من سلالة S. enterica سيرولوجيًّا في كل من: “astA, iss, irp2, papC iucD, tsh, vat, and cva/cvi” وحدها من سلالة الأوريجانو O146، O144, O124. (0/35) من O146، O144, O124. ولها 11 عِلة ذات ضراوة حيث أحتوت على عدد 1 جين مرتبطة بالضراوة وهي "astA, iss, irp2, papC iucD, tsh, vat, and cva/cvi” كما أظهرت نتائج اختبار الحساسية لعدد 11 عِلة، "astA, iss, irp2, papC iucD, tsh, vat, and cva/cvi" نتائج متماثلة في كل من: O27, O164، O153. وقد أظهرت نتائج التحليل السيرولوجي للعِلات المعزولة الإشريشيا كولاي O27 أن ديفلوكساسين وأوريجانو بتركيز 20% هما الأعلى نسبة حساسية بنسبة 100%، ونستنتج من هذه الدراسة أن الإشريشيا كولاي O27 هي الأكثر انتشارًا في مزارع بدارى التسمين، ونستطيع استخدام ديفلوكساسين وأوريجانو 20% لعلاجها.

**الكلمات الدالة:** التصنيف السيرولوجي و الجزيئي، الإشريشيا كولاي الممرضة للطيور، اختبار الحساسية، بدارى التسمين.