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Isolation, Serotyping, Pathogenicity and Antibiotic Sensitivity Testing of *Escherichia Coli* from Broiler Chickens in Egypt

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ABSTRACT

Escherichia coli infections are of significant concern to the poultry industry. It is one of the most important and frequently encountered bacterial avian pathogens causing a wide variety of disease syndromes in birds, inducing up to 30% mortality. In this study, samples from the liver, heart blood, lung, air sacs, yolk sac and joints from diseased and freshly dead chickens from broiler and layer flocks were collected for the isolation and serotyping of *E. coli*. Nine *E. coli* serotypes were isolated and identified from 8 broilers and 1 layer flocks and they were O8 (3 strains), O27 (2 strains) and the other 4 isolates were O29, O115, O148 and O169, respectively. The *in-vitro* antibiotic sensitivity testing for these *E. coli* isolates revealed that all isolates were highly sensitive to cefotaxime, mostly higher sensitivity to amoxicillin + clavulanic acid and intermediate sensitivity to doxycycline, and spiramycin.

1. INTRODUCTION

Avian pathogenic *Escherichia coli* (APEC) represent a pathotype of enteric bacteria that affects avian species causing colibacillosis, a severe and most common infectious disease of farmed poultry. Colibacillosis is one of the serious problems affecting poultry industry because it is responsible for a variety of disease conditions such as colisepticemia, air sacculitis, peritonitis, perihepatitis, pericarditis, omphalitis, coligranuloma, enteritis, synovitis, swollen head syndrome and osteomyelitis (Nolan, L.K. *et al.*, 2013; Rahimi & Haghighi, 2012; Trampel *et al.*, 2007).Certain strains of APEC spread into various internal organs causing systemic fatal colibacillosis (La Ragione and Woodward, 2002; Nolan *et al.*, 2013).

More than 1000 *E. coli* serotypes have been reported but only a small percentage have been implicated in poultry diseases (Cloud *et al.*, 1985). The most important reservoir of *E. coli* is the intestinal tract of poultry. In chickens, there are about 10^6 CFU of *E. coli* per gram of feces. It has been commonly isolated from the upper respiratory tract, bird's skin, and feathers. These strains always belong to both pathogenic and non-pathogenic types (Harry & Hemsley, 1965).

Every damage to the respiratory system favors infection with APEC. Several pathogens, like NDV, IBV and MG, both wild type and vaccine strains, may play a part in this process. An unfavorable housing climate, like an excess of ammonia or dust, renders the respiratory system more susceptible to APEC infections through deciliation of the upper respiratory tract (Nolan *et al.*, 2013).

Treatment strategies include the control of predisposing infections or environmental factors and the early use of antibiotics. Unfortunately, a high frequency of resistance to some antibiotics exists, with - more than 93% of *E. coli* isolates being resistant to erythromycin and tetracyclines due to the well-developed plasmid, transposons, and class 1 integrons (Singh *et al.*, 2005).

The objective of this study was the isolation, biochemical and serological identification, pathogenicity detection and antibiotic sensitivity testing of APEC isolates.

2. MATERIAL AND METHODS

2.1. Samples

A total of 26 commercial chicken flocks (25 broilers flocks and 1-layer flock) from 3 Egyptian governorates (Beheira, Alexandria, and Kafr-Elsheikh) were used for *E. coli* isolation during the year 2015. Samples were collected from diseased broiler chickens suffering from respiratory manifestations, lamness and from layer chicks suffering from omphalitis.

2.2. Isolation, cultivation and biochemical testing of *E. coli*

Liver, heart blood, lung, air sacs, yolk sac and joints were sampled from ailing birds Different isolation medias were used: Nutrient, MacConkey's, Eosin Methylene Blue (EMB), Brilliant Green (BG) and Xylose Lysine Deoxycholate (XLD) agar medias. Also, Gram's staining and different biochemical tests were applied on the isolated strains (Konemann *et al.*, 1997; Quinn *et al.*, 2002).

2.3. Serological identification of the isolates

Nine biochemically positive *E. coli* isolates were subjected to serological identification according to (Edwards & Ewing, 1986) using slide agglutination test and specific polyvalent and monovalent sera. Group O-somatic antisera (Denka Seiken Co., Ltd.Tokyo, Japan) obtained from Animal Health Research Institute, Dokki, Egypt, were used. The kit contained 51 vials (2ml each) 8 of polyvalent sera and 43 of monovalent sera as shown in Table (1).

2.4. Determination of the pathogenicity and virulence of *E. coli* isolates

a) Congo red binding assay: Congo red positive (CR+) in all *E. coli* isolates was indicated by the development of red colonies due to the binding with Congo red dye (Berkhoff & Vinal, 1986).
b) Detection of hemolytic activity: Blood agar base containing 10% citrated sheep blood was streaked with overnight cultures of the isolated *E. coli* strains and incubated at 37^{O}C for 24 hours. β-hemolytic colonies confirmed the hemolysin production of all *E. coli* isolates (Nakazato *et al.*, 2009).
c) Embryo lethality assay: was done to determine the pathogenicity of the respective 6 designed isolates (O27, O148, O8, O169, O115, and O29). Each isolate was inoculated into 8 embryonated SPF eggs aged 10 days, through the allantoic sac route with 0.1 ml (containing 500 CFU/ml), and candled daily for 7 days post inoculation. The number of deaths were recorded (Nolan, L. K. *et al.*, 1992).

2.5. Antimicrobial susceptibility of *E. coli* isolates in vitro

In-Vitro sensitivity testing for each *E. coli* isolate to 9 different antibiotics as ampicilline, spiramycin, streptomycin, florfenicol, cefotaxime, oxytetracyclin, doxycycline, amoxycillin+ clavulonic acid and colistine sulphate (Oxoid Laboratories, Basingstoke, Hampshire, England. Lot No. 457221) was done according to (Finegold & Martin, 1982). Muller Hinton broth was inoculated with at least 4-5 pure *E. coli* colonies and incubated at 37 °C for 24 hrs.

One ml of the inoculated broth was streaked with a sterile blunt glass rod onto Muller Hinton agar plates (two plates for each isolate). The cultured plates were kept for 30 minutes at 37°C to dry. The standardized disks were placed on each plate and incubated at 37°C for 24 hrs. The inhibition clear zones around the disc were visually measured.

Table (1): Antisera used in serological identification of *E. coli*

Polyvalent Sera	Monovalent sera						
Polyvalent 1	O1	O26	O86	O111	O119	O127	O128
Polyvalent 2	O44	O55	O125	O126	O146	O166	
Polyvalent 3	O18	O114	O142	O151	O157	O158	
Polyvalent 4	O6	O27	O78	O148	O159	O168	
Polyvalent 5	O20	O25	O63	O153	O167		
Polyvalent 6	O8	O15	O115	O169			
Polyvalent 7	O28	O112	O124	O136	O144		
Polyvalent 8	O29	O143	O152	O164			

3. Results

3.1. Isolation and identification of APEC

The investigation of diseased chickens suffering from colisepticemia and air sac disease revealed perihepatitis, airsacculitis, pericarditis, arthritis or omphalitis revealed 9 *E. coli* isolates. The age of positive flocks ranged between 3 and 30 days and the mortality rate ranged between 1.1 - 7% within 3 days of the natural infection with respiratory troubles, lamness and unhealed navel (Table 2).

Table (2): History of the positive *E. coli* examined in chicken flocks

Flock No	Breed	Locality	Total No	Age (days)	Clinical signs	PM lesions	Mortality% on the sampling week
1	Layer	Beheira	55000	3	Water belly with sticky feces	Omphalitis	1.1 in 3 days
2		Beheira	5000	21	Arthritis and Resp. signs	CCRDa	5.2
3		Beheira	3000	22	Lamness and Resp. signs	CCRDa	7
4		Beheira	6000	25	Lamness and Resp. signs	CCRDa	2.5
5	Broilers	Beheira	5000	19	Lamness and Resp. signs	CCRDa	2.6
6		Beheira	4500	30	Lamness and Resp. signs	CCRDa	2.9
7		Alexandria	6000	12	Lamness and Resp. signs	CCRDa	3
8		Alexandria	10000	23	Resp. signs	CCRDa	3.4
9		Alexandria	12000	27	Resp. signs	CCRDa	2.9

a: Fibrinous pericarditis, perihepatitis and airsacculitis

E. coli isolates in this study appeared as rounded, non-pigmented colonies white on nutrient agar medium, while on MacConkey's agar medium showed rounded, non-mucoid bright pink colonies (lactose fermenter) on the surface of the medium. On EMB, the isolates showed a distinctive greenish metallic sheen colony (Fig. 1). Yellow colonies on BG and bright yellow colonies XLD agar. Biochemical profile of the isolated strains was recorded (Table 3). Microscopically *E. coli* strains were Gram-negative, pleomorphic medium sized rods, non-spore forming.

Serological identification of these 9 *E. coli* isolates revealed that 3 strains were serotyped as O8, 2 strains were O27 and the other 4 isolates were O29, O115, O148 and O169 respectively (Table 4).

3.2. Pathogenicity testing of *E. coli* isolates:

Detection of Congo red binding assay: All isolated *E. coli* showed Congo red binding activity (100%) with red colonies (CR+) as shown in (Fig. 2). The degree of redness of the colonies varied from one isolate to another.

Detection of haemolytic activity: All the 9 isolates were β- haemolytic to sheep blood agar.

Embryo lethality assay: The 9 isolates recorded 75-100% mortality which was recorded as highly virulent strains (Table 5).

Table (3): Biochemical reaction of MacConkey's positive *E. coli* isolates

Biochemical test	Reaction
1. Indole test	Positive
2. Methyl red test	Positive
3. Voges-Proskauer test (VP)	Negative
4. Citrate utilization test	Negative
5. Urease test	Negative
6. Triple sugar iron test (H2S production test)	Negative
7. Catalase test	Positive
8. Oxidase test	Negative
9. Sugar fermentation test	Positive
10. Nitrate Reduction test	Positive

Table (4): Serotyping of *E. coli* isolates

Polyvalent	Isolates No	Results
4	1&2	O27
4	3	O148
6	4,5&6	O8
6	7	O169
6	8	O115
8	9	O29

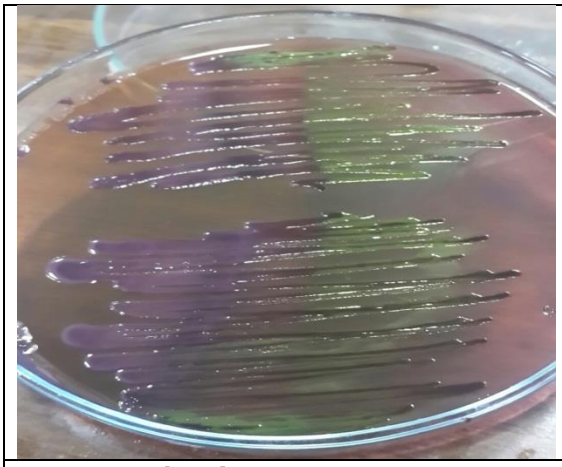

Fig. 1: *E. coli* isolates On EMB

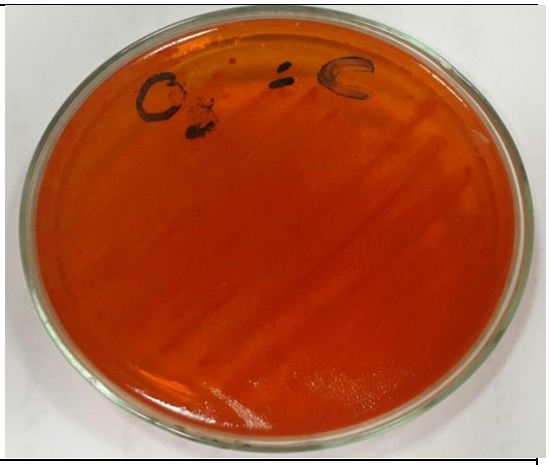

Fig. 2: Congo red binding assay

3.3. The *in- Vitro* sensitivity testing for the isolated *E. coli* strains:

All serotypes were highly sensitive to cefotaxime, 4 serotypes showed high sensitivity to amoxicillin + clavulanic acid, and - serotype O27 and O148 isolates showed a moderate sensitivity to amoxicillin+ calvulanic Acid. - The 4 serotypes (O8, O27, O148 and O169) isolates showed moderate sensitivity to oxytetracycline and doxycycline, respectively. Serotype O148 isolates demonstrated complete resistance to colistine sulfate (Table 6).

Table (6): The *in- vitro* sensitivity testing results for the isolated *E. coli* serotypes (one isolate for each serotype).

***E coli* serotype and (Isolate No)** / **Antibiotic (Oxoid)**	**O27 (1)**	**O148 (3)**	**O8 (4)**	**O169 (7)**	**O115 (8)**	**O29 (9)**
1. Cefotaxime (CTX-30 µg)	+++	+++	+++	+++	+++	+++
2. Amoxycillin+ Clavulanic acid (AMC-10 µg)	++	++	+++	+++	+++	+++
3. Doxycycline (DO-30 µg)	++	++	++	++	+	+
4. Spiramycin (SP-100 µg)	++	+	++	+	++	+
5. Florfenicol (FFC-30 µg)	+	+	++	+	++	+
6. Ampicillin (AMP-10 µg)	+	+	++	+	++	+
7. Streptomycin (S-100 µg)	+	+	++	+	+	++
8. Oxytetracycline (OT-100 µg)	++	+	++	++	-	++
9. Colistine sulphate (CT-100 µg)	+	-	++	++	++	+

Resistant +: Low sensitivity ++: Intermediate sensitivity +++: High sensitivity

4. DISCUSSION

E. coli strains is of importance due to its high economic impact on poultry production. Nine *E. coli* isolates (34.6%) were found in the examined samples collected from 25 broiler flocks and only one layer flock from 3 Egyptian governorates. A similar prevalence rate of *E. coli* in chickens was reported by Epidemiological tracking of *E* (Abd El Tawab *et al.*, 2015; Hussein *et al.*, 2013; Khalid, 1990; Roshdy *et al.*, 2012). While, a higher incidence of *E. coli* (81.46%) was recorded and published in Yugoslavia by Prukner (1986) and an incidence of (88.2%) was recorded in Jordan by El-Sukhon *et al.* (2002). This gives an indication of the

massive distribution of *E. coli* in different poultry flocks with different prevalence based on many factors such as management, epidemiology of respiratory viral diseases and the immune status of the bird.

Regarding to the biochemical characteristics of *E. coli* isolates recovered from examined chickens, all isolates of *E. coli* were positive for the typical biochemical tests for APEC (Khafaga, 1995; Sedhom, 2000).

Serological identification of the nine *E. coli* isolates, revealed 3/9 of the isolates were serotyped as O8, 2/9 of the isolates were serotyped as O27 and 1/9 of the *E. coli* isolates was serotyped as O29, O115, O148 and O169 for each serotype. In a study done by Tawfik *et al.* (2017), 28 *E. coli* were isolated out of 40 joint samples (70%). Six serogroups were identified as O128, O125, O146, O27, O114 and O158 demonstrating the diversity in the prevalence of serotypes in different geographical areas. Various *E. coli* serotypes have been reported by several researches. The different serotypes identified were O1, O2, O8, O11, O12, O14, O18, O19, O21, O20, O26, O53, O54, O57 O60, O65, O68, O78, O80, O81, O83, O89, O91, O101, O103, O106, O109, O111, O115, O123, O147, O148, and O162 (Mashood *et al.*, 2007; Mishra *et al.*, 2002; Vounba *et al.*, 2018; Wijesurendra *et al.*, 2017).

A direct correlation had been proved between the Congo red binding activity of clinically isolated *E. coli* strains and their ability to cause septicaemic infection in chickens and there were no CR negative *E. coli* isolates recovered from internal organs of colisepticaemic birds (Berkhoff & Vinal, 1986). On the contrary, Panigrahy and Yushen (1990) reported that not all the pathogenic *E. coli* serogroups were CR+ and added that Congo red binding did not correlate with pathogenicity and it may be an identifiable property for some serotypes of *E. coli*. In the present study, all isolates of different serogroups were Congo red positive (CR+).

Concerning embryo lethality assay, all tested isolates, 1 from each serotype, resulted in 75-100% mortalities revealing that all the isolates were highly pathogenic (Wooley *et al.*, 2000). The highly pathogenic serotypes were O27 and O148 induced 100% and 87.5% respectively. Serotype O27 isolated from 3 day old layer chickens suffering from enlarged belly with watery diarrhea and omphalitis, while, O148 was isolated from joints of 22 days old broiler chickens suffering from lamness, respiratory signs and CCRD lesions.

Mitra et al. (2009) tested 5 representative serotyped isolates of *E. coli* (O2, O27, O29, O39 and O119) for pathogenicity in mice and recorded that 4 of them were highly pathogenic (83.33%) and serotype O2 was the least pathogenic.

Kumar *et al.* (1996) stated that clinicopathological examination of 2 day old chicks infected with *E. coli* strains belonging to serogroups O45, O73, O75, O78, O84, O88, O103, O112, O128, O147 and O148 revealed disease manifestation 24 hours earlier in intraperitoneal route as compare to oral route. The affected birds showed respiratory distress accompanied by congestion, hemorrhages and mild increase in pericardial fluid. The lungs revealed congestion, pulmonary edema and air saculites and myocardites. Strains O75, O78, O112 and O148 were found to be more pathogenic particularly when the infection was given by intraperitoneal route.

The *in- Vitro* sensitivity testing for the isolated *E. coli* serotypes, indicated that the highest sensitivity was to cefotaxime in all serotypes, while 4 serotypes showed high sensitivity to amoxicillin + clavulanic acid, with serotype O27 and O148 demonstrating a moderate sensitivity to amoxicillin +clavulanic acid. - 4 of the serotypes demonstrated a moderate sensitivity to oxytetracycline and doxycycline. Serotype O148 had complete resistance to colistin sulfate**.**

Helal (2012) reported that the sensitivity test of *E. coli* isolated from the respiratory system of chickens (serotypes, O1, O114, O119) revealed sensitivity to cefotax, enrofloxacin, colistin, and amoxicillin, O1 isolate revealed sensitivity to doxycycline, and gentamycin. While the *E. coli* isolates from the intestine (O146) was sensitive to cefotax, enrofloxacin, amoxicillin and doxycycline and O157 was sensitive to colistin only.

These findings confirm the significant increase in the continuous evolution of antimicrobial resistance in the *E. coli* isolates especially in broiler flocks due to misuse of antibiotics without routine sensitivity testing and also, the weak biosecurity programs in broiler farms.

5. CONCLUSION

9 *E. coli* isolates (34.6%) representing 6 pathogenic *E. coli* serotypes were recovered from chickens and proved high pathogenicity represented by embryo lethality test and Congo Red binding activity. Also, the antibiotic sensitivity of these isolates revealed highest degree to cefotaxime for all isolates.

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