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Isolation, Characterization and Genetic Analysis of Antibiotic Resistance Genes and Virulence Factors of *Escherichia Coli* Isolated From Chicken Farms in Egypt

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Abstract

AVIAN PATHOGENIC *Escherichia coli* (APEC) causes severe diseases in broiler chicken farms. This bacterial pathogen is associated with high mortalities in chickens and is considered the leading cause of human zoonotic disease. The present study aimed to investigate the prevalence and antimicrobial resistance profile of APEC in broiler chicken farms in Gharbia and Menofeya Provinces in Egypt. The study also evaluates the virulence associated factors and the genetic mechanisms of β-lactam antibiotics that are commonly used in the poultry industry in Egypt. In this study, 100 samples were collected randomly from 25 broiler chicken farms. Clinical symptoms and mortality rates were reported in the two districts of the central region of the Egyptian Delta. In total, 100 samples were collected from 25 broiler farms, and the overall prevalence of APEC infection reported in the study region was 30%. Isolated strains were serotyped as EHEC (8 O111: H4, 8 O128: H2 and 5 O127: H6) and ETEC (4 O44: H18 and 5 O125: H21). . The results revealed that APEC infection was significantly high in chickens older than four weeks old compared to younger than four weeks old. All tested isolates were sensitive to colistin and amikacin while all tested isolates were resistant to amoxicillin/clavulanic acid, ampicillin and sulfamethoxazole-trimethoprim. All tested isolates were resistant to spectinomycin except six strains of serotypes EHEC O111: H4 and O128: H2. On the contrary, all tested isolates were sensitive to cefotaxime except for six strains of EHEC O111: H4 and O128: H2. Furthermore, gentamycin was sensitive to all tested isolates except for EHEC O111: H4, O128: H2, and ETEC O44: H12. The phenotypically resistant *E. coli* against ampicillin, amoxicillin and cephalosporins harboured bla_{TEM}, bla_{SHV}, and bla_{CMY2} as well as the following virulence genes, *iss, eaeA, stx1* and *stx2* were amplified in 12 (85.71%), 8 (57.14%), 2 (14.29%) and 1 (7.14%) isolate, respectively. Our findings suggest that farmers and veterinarians in the study area should use a standard treatment protocol for APEC to reduce the prevalence and antimicrobial resistance of this endemic disease. The efforts aimed at controlling and preventing such diseases will consequently lead to the minimal use of antibiotics on poultry farms.

Keywords: Avian Pathogenic *E. coli*, Prevalence, Antimicrobial resistance, Virulence genes, bla_{CMY2}

Introduction

The industry of poultry is regarded as the agricultural sub-sector with the greatest rate of growth worldwide, especially in developing countries. With the continuous increase in populations and the demand for meat and eggs, the supply of both needs to be increased [1]. In broiler chicken, *Escherichia coli (E. coli)* is a disease-causing pathogen [2]. It is responsible for high mortality and morbidity in poultry farms, causing high economic losses to the poultry industry around the world [3]. For the *E. coli*

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outbreak in poultry farms, economic costs have been estimated to be €3.7 million in some German cities [4] and $405 million in the USA [5]. In birds, *Escherichia coli* often lives in the lower gastrointestinal tract. It is also present in the trachea and pharynx [6]. The majority of *E. coli* strains are benign and non-pathogenic, however certain strains are aggressive and capable of infecting birds with illnesses [3]. These were known as avian pathogenic *E. coli* (APEC), and their reservoir was the gut. [7]. The disease is caused by avian pathogenic *E. coli* (APEC), a subtype of extraintestinal pathogenic *E. coli* with zoonotic potential [8].

The strains of avian pathogenic *E. coli* threaten global food security and bird welfare by causing severe respiratory and systemic diseases [7]. Through increased exposure of birds to pathogens and stress, the intensification of poultry production and rapid development of free-range production systems will raise the prevalence of *E. coli* infection or colibacillosis [9]. Yolk sac infection, swelling head syndrome, respiratory tract infection, polyserositis, salpingitis, coli granuloma, enteritis, pericarditis, and perihepatitis are all symptoms of the complicated disease known as colibacillosis [10]. Colibacillosis has become an economically significant disease phenomenon in broiler chickens due to higher condemnation, downgrading at processing, and increased costs associated with trimming and reprocessing of infected carcasses [10]. Colibacillosis can be identified by "plaques," which are often referred to as a diffuse, spreading, edematous, and suppurative inflammation of the deep subcutaneous tissues that occasionally extends into the muscle [11]. Lesions are not seen until the birds are processed since clinically, the affected birds seem normal. Numerous bacteria can cause colibacillosis, however, APEC is the most often isolated organism [12], still, compared to non-cellulitis-derived strains, E. coli isolated from cellulitis lesions was more likely to cause cellulitis lesions in experimentally infected birds. [10].

APEC strains use a variety of strategies to colonies different environments. Infections resulting from APEC pose a significant threat to broiler chicken farms. APEC infections cause significant mortality, ineffectual medication, low weight gains in broiler birds, decreased laying, fertilizations, and hatchability, and a rise in the number of carcasses or parts removed due to pathological alterations [13].

Antimicrobials are crucial to both human and animal health care. To treat E. coli infections in the community and hospitals, a variety of antibiotic medicines are utilized. The majority of antibiotics are utilized in human medicine, as well as in animals [14].

Several chicken farms in many low and middle-income countries (LMICs) lack waste or litter treatment systems, so they are frequently used as biofertilizers or as feed additives, particularly in fish ponds [15, 16]. This may increase the occurrence of human beings being exposed to antimicrobial-resistant bacteria in the feces. As a result, continuing to monitor and screen resistant bacteria at the human–animal–environmental interface is one of the greatest ways of creating decisions and reducing the impact of antimicrobial resistance on public health [17]. According to the World Health Organization's Global Antimicrobial Resistance Surveillance System (GLASS), *E. coli*, a common bacterium, is one of the best indicators for integrated antimicrobial resistance screening. It is the most likely vector for resistance gene transmission from animal to human and a reservoir of multidrug-resistant *E. coli* from farm to environment [18, 19]. However, limiting the number of resistant strains discharged into the environment will ease the burden of resistant bacteria in all One Health settings, reducing the impact of antimicrobial-resistance on public health [20]. The widespread presence of E. coli as a foodborne pathogen in raw beef and poultry meat in Egypt's Nile Delta. In Egypt, public health officials are concerned about the growth of antibiotic resistance in E. coli isolates [21].

Some bacterial genes that code for virulence factors have previously been found in a previous study found. The *Iss* gene wAPEC strains, as first discovered in a human septicemic *E. coli* strain [22, 23] and was related to a 20-fold increase in enhanced resistance and a 100-fold increase in virulence against 1-day-old chicks [22-24]. APEC's iss protein has been isolated, expressed, and extracted. [25], monoclonal antibodies have been induced against it [26], and it has been revealed that it protects against bird immunity [27]. Shiga toxins 1 and 2, which are produced by the *Stx1* and *Stx2* genes, respectively, or combinations of these toxins, are to be responsible for the majority of clinical signs of APEC [28]. *E. coli* intimin, an *eaeA* gene product associated with the adhesion traits of APEC, is one of the other primary key virulence factors [29].Shiga toxin-producing *E. coli* (STEC) are infectious organisms that can infect people and cause a variety of illnesses, from asymptomatic carriage to mild diarrhoea to haemolytic uraemic syndrome and haemorrhagic colitis (HUS) [30]. *E. coli* strains resulting in the more severe pathology of haemorrhagic diarrhoea and HUS are further designated enterohaemorrhagic *E. coli* (EHEC). Infection with STEC is through ingestion of contaminated food and water or through contact with infected animals. The pathogen migrates through the gut to the terminal ileum where adherence factors like intimin, the intimin receptor and EspA and EspB proteins aid colonisation through the formation of attaching and effacing lesions [31]. Enterotoxigenic

E. coli (ETEC) is a kind of *E. coli* that is known to cause diarrhoea in humans and is a significant cause of diarrhoea in children in underdeveloped nations. ETEC is linked to animal diarrhoea as well. By colonising the intestinal mucosa through non-intimate diffuse adhesion with fimbriae and producing enterotoxins (heat stable and/or heat labile toxins) that inhibit gut absorption and enhance intestinal secretion, ETEC results in watery diarrhoea. ETEC also causes diarrhoea in animals, and infections of pigs and cattle with ETEC are linked to the F4 (K88) and F5 (K99) antigens, respectively. Examples of ETEC serotypes include O6:H16 and O8:H9 [32]. The purpose of this research is to ascertain the frequency of avian pathogenic *Escherichia coli* (APEC) strains in broiler chicken farms in the Egyptian provinces of Gharbia and Menofeya. The study also assesses the genes linked with virulence, the susceptibility of isolated strains to eight regularly used antibiotics in Egypt's veterinary industry, and if genes contribute to the separated strains' antibiotic resistance traits.

Material and Methods

Sampling strategy and E. coli Isolation

In this study, one hundred samples were collected from diseased birds selected randomly from 25 broiler chicken farms (Avian 48, Abdelsalam Hegazy company) (four chickens from each farm) located in Gharbia and Menofiya provinces in Egypt from May to August 2021. The most common clinical symptoms of *E. coli* infection were coughing, decreased body weight, depression, high mortality rate, high respiratory sound, inappetence, loud respiratory sound, low feed intake, respiratory manifestations, and ruffled feathers, which were gathered at the time of sampling, and mortalities were screened for each farm, as shown in Table 2. The diseased birds were sacrificed humanly. At post-mortem, sections from the heart, liver, lung, air sac, trachea, spleen, and gallbladder were collected under aseptic conditions and directed to *E. coli* isolation. All samples were preserved on ice till being transported in not over four hours to the microbiology laboratory at the Faculty of Veterinary Medicine, Kafrelsheikh University. One gramme of each sample was enriched in 10ml of tryptic soy broth (TSB) (Oxoid, UK) and incubated overnight at 37°C. Incubated samples were streaked on MacConkey agar media (Oxoid, UK) and were incubated overnight at 37°C. The colonies suspected to be *E. coli* were sub-cultured on selective media eosin methylene blue agar (EMB) (Oxoid, UK) [33].

Biochemical identification of E. coli

For confirming The bacterial colonies that were grow on EMB agar to be *E. coli*, biochemical identification was used according to the method described by [34]. Catalase, Triple Sugar Agar TSI, Indole synthesis, Methyl Red, Urease, Voges Proskauer, Simmon's citrate, and H_2S were only a few of the biochemical assays used to find *E. coli*.

Serological identification of E. coli

E. coli isolates were identified by serology using the technique described by [35] Using quick diagnosis *E. coli* antisera kits for identifying *E. coli* serotypes are available from (DENKA SEIKEN Co. in Japan). A part of the colony from the suspicious culture was emulsified with the saline solution to create a smooth, dense suspension after two separate drops of saline were placed on a glass slide. One loopful of saline was put to one suspension control and stirred. One loopful of undiluted antiserum was added to the other suspension, and it was titled back and forth for one minute. On a dark background with indirect lighting, agglutination was seen. A second part of the colony was put onto a nutrient agar slant and cultured at 37 °C for 24 hours to grow as a culture for testing with mono-valent sera when a colony produced a strongly positive agglutination with one of the pools of polyvalent serum. To identify the O-antigen, slide agglutination tests were carried out using the diagnostic sera on a heavy suspension of bacteria from each slope culture.

DNA extraction and purification

Following the overnight inoculation of a single colony from each plate into TSB (Oxoid), the DNA extraction and purification for isolated *E. coli* was conducted using the QIAamp DNA mini kit (QIAGEN Co., USA) in accordance with the manufacturer's instructions.

Molecular identification of the E. coli virulence associated genes.

In order to test the virulence genes that are important for the pathogenicity of *E. coli*, we randomly selected 14 strains as representative samples from the isolated *E. coli* strains and tested them for virulence factors associated with pathogenicity attributed genes *Stx*1*, Stx*2*, eae*A *and iss* by polymerase chain reaction (PCR) following the procedure described by [36] using primers as shown in Table 2. In a brief, the primers were employed in a 25 l uniplex PCR mix that included 12.5 l of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 l of each primer (20 pmol), 5.5 l of water, and 5 l of DNA template. Thermal cycler 2720 from Applied Biosystems was used to conduct the process. The initial denaturation phase of the cycling condition began at 94 °C for 5 min, was followed by 35 cycles, and was finished with an extension at 72 °C for 10 min. Field samples that had previously been determined to be positive or negative by PCR for the associated genes in the Reference laboratory for veterinary quality control on chicken production,

Animal Health Research Institute, served as positive and/or negative controls (Dokki- Cairo, Egypt).

The antimicrobial Susceptibility Test

The test for antimicrobial susceptibility was conducted on 14 *E. coli* which tested for virulence genes using the Kirby–Bauer disc diffusion method, as recommended by the CLSI [37]. *E. coli* ACTC25922 and *E. coli* NCTC10418 were used as quality control strains during AST. The Egyptian poultry industry's clinically utilized antibiotics were used in the antimicrobial susceptibility test for *E. coli* isolates. Among these are Streptomycin (SC 10µg), Gentamicin (GN 30µg), Amikacin (AK 30µg), Amoxicillin/Clavulanic Acid (AMC 20µg), Ampicillin (AMP 20µg), Cefotaxime (CFM 30µg), Colistin (CL 10µg) and Sulfamethoxazole-Trimethoprim (SXT 1.25/23.75µg). A direct saline suspension was used to prepare the bacterial inoculum. of a Tryptic soy broth culture from an isolated colony on the EMB agar plates. The suspension was adjusted by measuring the turbidity using a McFarland Densitometer (Biomérieux Biotechnology, UK) until it contained around 1-2 x 108 CFU/ml for use with sterile saline, matching the 0.5 McFarland standard. Using a sterile swab, the saline suspension was applied to the Mueller-Hinton Agar plate's surface. Antibiotic-containing antimicrobial discs were strewn around the Mueller-Hinton agar surface (Oxoid, UK). Overnight, the agar plates were incubated at 37 °C. Sliding callipers were used to measure the diameters of the inhibited zones, including the diameter of the discs, and standard break points were used for interpretation in accordance with the Clinical Laboratory Standards Institute (Table 1) [38].

Molecular detection of antimicrobial resistance associated genes.

The *E. coli* isolates that were screened for AST were investigated for the presence of three genes known to be responsible for the antibiotic resistance to cephalosporins and β-lactamase. These genes are *CIT* (bla_{CMY2}), bla_{SHV} and bal_{TEM}. The Polymerase Chain Reaction (PCR) reaction and conditions were performed using primers as shown in Table 2, according to the method described by [36]

Statistical analysis

Using the Microsoft Excel, Student's t testing was conducted to determine the percentage of fatalities attributable to *E. coli* infection, according to the method described by [39].

Results

Clinical signs, incidence and mortalities

The most common clinical symptoms of *E. coli* infection were coughing, decreased body weight, depression, high mortality rate, high respiratory sound, inappetence, loud respiratory sound, low feed intake, respiratory manifestations, and ruffled feathers, which were gathered at the time of sampling, and mortalities were screened for each farm, as shown in Table 3.

Isolation and identification of E. coli

Following the isolation of E. coli from chicken samples, it was discovered that 30 (30%) E. coli were isolated from 100 samples, which corresponded to 8 (32%) of the 25 studied broiler chicken farms (Table 2).

A total of 700 clinical samples from various organs were gathered from 25 broiler chicken farms (heart, liver, lung, air sac, trachea, spleen, and gallbladder). The symptoms of E. coli infection are vague and vary depending on the age, organs involved, and other diseases, with symptoms ranging from one day to 23-days-old, including death, omphalitis, inappetence, ruffled feathers, and decreased body weight. The clinical symptoms of over-24-days-old chickens included ruffled feathers, less feed intake, respiratory manifestations, coughing, loud respiratory sounds, and a high mortality rate. From individual chickens, 30 putative E. coli strains were isolated, which showed that the rate of E. coli isolation was 4.2% out of the 700 samples. With E. coli infection, the mortality rate in the birds older than 28-days old compared to birds older than or equivalent to 28 days, was substantially higher ($P < 0.01$) (2.60 %), as shown in Table 4.

Bacterial identification and serotyping

The suspected pure yellow green or metallic sheen color colonies on EMB agar were confirmed to be *E. coli* biochemically as all isolated strains were positive for catalase, triple sugar agar (TSI), indole production, and methyl red while was negative for urease, Voges Proskauer, Simmon's citrate, and H_2S production.(Table 5**)**.

The incidence rate of the serotyped strains in different selected organs was as follows: The serotype O111: H4 incidence rate was 11.11%, 16.67, 40%, 16.67%, 50%, 25%, and 28.57% in the heart, liver, lung, spleen, air sac, gall bladder, and trachea, respectively. While the serotype O44: H18 incidence rate was 33.33%, 25%, negative, 33.33%, 25%, negative, and 28.57% in the heart, liver, lung, spleen, air sac, gall bladder, and trachea, respectively. Moreover, the serotype O128: H2 incidence rate was 22.22%, 25%, 20%, 33.33%, negative, 75%, and 14.29% in the heart, liver, lung, spleen, air sac, gall bladder, and trachea, respectively. Furthermore, the serotype O125: H21incidence rate was 22.22%, 25%, 20%, 16.67%, 25%, negative, and 28.57% in the heart, liver, lung, spleen, air sac, gall bladder, and trachea, respectively. Finally, the serotype O127: H6 incidence rate was 11.11%, 8.33%, and 20% in the

heart, liver, and lung, and negative for other investigated organs.

Detection of virulence associated genes.

In Fourteen E. coli isolates, iss, eaeA, *stx*1 and *stx*2 were amplified in 12 (85.71%), 8 (57.14%), 2 (14.29%) and 1 (7.14%) isolate, respectively as shown in Fig.1**.**

Phenotypic antimicrobial resistance profiles

All tested isolates were sensitive to colistin and amikacin. On the contrary, all tested isolates were resistant to Amoxicillin/Clavulanic Acid, Ampicillin and Sulfamethoxazole-Trimethoprim. All tested isolates were resistant to spectinomycin except for six strains of EHEC O111: H4 and O128: H2. On the contrary, all tested isolates were sensitive to cefotaxime except for six strains of EHEC O111: H4 and O128: H2. Furthermore, gentamycin was sensitive to all tested isolates except for EHEC O111: H4, O128: H2, and ETEC O44: H12, as shown in Table 6.

Prevalence of antimicrobial resistance genotypes

The phenotypically resistant *E. coli* isolates against ampicillin, amoxicillin, and cephalosporins were found to coincide with the presence of *blaCMY2, blaTEM*, and *blaSHV,* as shown in Figure 2 and Table 7. While the Percentage of distribution of the virulence attributed genes detected in *E. coli* isolated strains from broiler chicken's farms in Egypt was 2 positive sample for *stx1*(14.29%), 1 positive sample for *stx2*(7.14%), 12 positive sample for *iss* (85.71%), and 8 positive sample for *eaeA* (57.14%)

Discussion

This study found 32% of the broiler chicken farms were infected with *E. coli*. There were significantly higher levels of mortalities for farms infected at an age of over four weeks ($P < 0.01$) as compared to those younger than this age. Another study by Rahman M. A. et al., 2004 reported a similar result [40]. Furthermore, we report coughing, sneezing, and nasal mucous discharge as the most common clinical signs among the surveyed positive farms. Our results agree with the study documents by [41].

In this study, we present the patterns of susceptibility of *E. coli* among the eight antibiotics used. The highest antimicrobial resistance of *E. coli* to the antibiotics used in the survey was 100% to Amoxicillin/Clavulanic Acid, Ampicillin, and Sulfamethoxazole-Trimethoprim. All tested *E. coli* were sensitive to colistin and amikacin, reflecting the broiler chicken disease treatment habits by the farmers and veterinarians in the study region. Similar trends are reported by [42].

In the current study, PCR amplification of the genes encoding for β-lactamase resistance, bla_{CMY2}, was found in 7 (50%) of the isolates. bla_{TEM}, and bla_{SHV} genes were detected in 13 (92.85%) and 9 (64.28%) isolates, respectively. All strains isolated use at least one of these mechanisms of β-lactamases. However, one strain was negative for all of them. We suggest that it may use another resistance mechanism [43].

Multiple bacterial determinants have been implicated in the pathogenesis of EHEC infection [44]. Shiga toxin (*stx*) and intimin (*eae*A*)* are major virulence factors that have been implicated in many of the intestinal and systemic pathological consequences [28]. Importantly, it was recently shown that *stx* additionally promotes EHEC attachment to cells of human origin. This activity was attributed to *stx*-induced redistribution of nucleoli to the EHEC attachment sites (Robinson et al., 2006), where they serve as an intimin receptor (Sinclair and O'Brien, 2002).

In this study, 14.3%, 7.1%, 85.7%, and 57.1% of isolated *E. coli* were harboured virulence genes *Stx* 1*, Stx* 2*, iss*, and *eae*A, respectively. Similar results were shown by [13].

The results of the current study indicated that 86% of *E. coli* isolates contain the *iss* gene. This indicates its of avian origin which in agreement with those by [26].

Conclusions

The frequency of mortalities of broiler chickens was higher at older ages as compared to younger ages of less than 4 weeks. The antimicrobial resistant *E. coli* patterns showed that most of the isolates were resistant to most of the new antibiotics. The fast and wide spread of antimicrobial resistance in different types of *E. coli* to most of the new antibiotics necessitates the asylum to use an old generation of antibiotics like colistin.

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Conflict of interest

The authors declare that they have no competing interests.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

All authors participated in the conception of the research idea and the design of the methodology, supervision, performed data analysis and interpretation. All authors drafted and prepared the manuscript for publication and revision. All authors read and approved the final manuscript.

Declarations

Ethics approval

All experimental protocols were approved by the Committee on Research, Publication, and Ethics of the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt, which complies with all relevant Egyptian legislation in research and publications.

ARRIVE guideline statement.

The study was conducted in compliance with the ARRIVE guidelines.

TABLE 1. Break point values of each antimicrobial agent according to CLSI 2016

Antimicrobial agent(s) tested	Disc concentration	*E. coli* isolates		
		Resistant mm	Intermediate mm	Sensitive mm
Ampicillin (AM)	10 µg	≥ 13	14:16	**≤ 17**
Amikacin (AK)	30 µg	≥ 14	15:16	**≤ 17**
Amoxicillin/Clavulanic Acid (AMC)	20 µg	≥ 13	14:17	**≤ 18**
Cefotaxime (CFM)	30 µg	≥ 22	23:25	**≤ 26**
Colistin (CT)	10 µg	≥ 10	11:13	**≤ 14**
Gentamycin (CN)	30 µg	≥ 12	13:14	**≤ 15**
Streptomycin (SC)	10 µg	≥ 11	12:14	**≤ 15**
Sulfamethoxazole-Trimethoprim (SXT)	1.25/23.75 µg	≥ 10	11:15	**≤ 16**

TABLE 2. Oligonucleotide primer sequences and their corresponding genes are used for the detection of antimicrobial resistant genes in *E. coli* isolates.

Gene	Sequence	Amplified product	Annealing temp (°C)	cycles	Reference
Stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614 bp	58˚C	35	[45]
Stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779 bp	58˚C	35	
iss	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATACCC	266 bp	54˚C	35	[46]
eaeA	ATG CTT AGT GCT GGT TTA GG GCCTTCATCATTTCG CTT TC	248 bp	51˚C	35	[47]
CIT (bla_{CMY2})	TGGCCAGAACTGACAGGCAAA TTTCTCCTGAACGTGGCTGGC	462 bp	55˚C	35	[48]
blaTEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	54˚C	35	[49]
blaSHV	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	392 bp	54˚C	35	

TABLE 3. General characteristics and clinical signs of farms under surveillance.

Farm no.	No. of birds	Age by day	No. of collected samples	Mortality rate	*E. coli* isolation (+/-)	Clinical signs
1	6000	23	4	170 (2.8%)	-	The chicken showed no signs and grew normally
2	5000	28	4	100 (2%)	+	Low feed intake, depression, and high respiratory sound
3	10000	30	4	150 (1.5%)	-	The chicken showed no signs and grew normally
4	3000	1	4	70 (2.3%)	-	The chicken showed no signs and grew normally
5	4000	31	4	120 (3%)	-	The chicken showed no signs and grew normally
6	5000	27	4	140 (2.8%)	+	Respiratory manifestations, coughing, low feed intake and high mortality.
7	5000	15	4	100 (2%)	-	The chicken showed no signs and grew normally
8	8000	38	4	700 (8.75%)	+	Ruffling feathers, less feed intake and respiratory manifestations
9	3000	40	4	500 (16.67%)	+	Coughing, loud respiratory sound and high mortality rate

10	5000	3	4	90 (1.8%)	-	The chicken showed no signs and grew normally
11	12000	40	4	400 (7.5%)	-	The chicken showed no signs and grew normally
12	10000	11	4	430 (3%)	+	Inappetence, ruffled feather and high mortality
13	15000	39	4	1500 (10%)	+	Loud respiratory sound and high mortality
14	3000	37	4	325 (10.3%)	+	Low body weight and high mortality
15	5000	4	4	70 (1.4%)	-	The chicken showed no signs and grew normally
16	50000	17	4	1640 (3.3%)	-	The chicken showed no signs and grew normally
17	7000	37	4	190 (2.7%)	-	The chicken showed no signs and grew normally
18	15000	34	4	250 (1.7%)	-	The chicken showed no signs and grew normally
19	4000	32	4	100 (2.5%)	-	The chicken showed no signs and grew normally
20	7500	19	4	110 (1.5)	-	The chicken showed no signs and grew normally
21	9000	36	4	290 (3.2%)	-	The chicken showed no signs and grew normally
22	4000	16	4	80 (2%)	-	The chicken showed no signs and grew normally
23	5000	9	4	95 (1.9%)	-	The chicken showed no signs and grew normally
24	20000	42	4	400 (2.7%)	-	The chicken showed no signs and grew normally
25	5000	39	4	400 (8%)	+	Decreased body weight and high mortality

TABLE 4. Incidence of *E. coli* infection in broiler chicken and identifying characteristics.

Samples	**No. of examined birds**	***E. coli* on MacConkey and EMB Agars**		**Colony Characteristics**		**Morphology**
		Positive	**%**	**McConkey Agar**	**EMB Agar**	
Apparently healthy	68	0	0	Red or bright pink colonies color	Yellow green or metallic sheen colonies color	Single rods, single, pair or short chain
Diseased	15	13	86.7			
Freshly dead	17	17	100			
Total	**100**	**30**	**30.0**			

TABLE 5. Association between age and mortality rates in the twenty-five broiler chicken farms in Egypt.

Age	**Number of farms**	**Average of Mortality rate (%)**
Birds older than four weeks	5	9.43
Birds smaller than or equal to four weeks	3	2.60

Serological identification of 30 isolated *E. coli* confirmed that five different serotypes were found under two categories of *E. coli:* the Enterohemorrhagic (EHEC) and Enterotoxigenic (ETEC) *Escherichia coli*, shown in **Table 6**.

TABLE 6. Serotyping of some *E. coli* isolates (n=30) obtained from broiler chicken under two categories of *E. coli:* the Enterohemorrhagic (EHEC) and Enterotoxigenic (ETEC) *Escherichia coli*.

***E. coli* serotype**	**Characterization**	**No.**	**Percentage (%)**
O_{111}: H_4	EHEC	8	26.7
O128: H_2	EHEC	8	26.7
O44: H_{18}	ETEC	4	13.3
O125: H_{21}	ETEC	5	16.7
O127: H_6	EHEC	5	16.7
Total		30	100.0

TABLE 7. Results of Antibiotic sensitivity test for *E. coli* isolated strains. and PCR that was performed to detect the resistance and virulance genes.

Strain ID	*E. coli* serotype	Strain character	Antimicrobial susceptibility testing								Antibiotic resistance genes				Virulence genes		
			AK	AMC	AMP	CFM	CL	GN	SC	SXT	*blaCMY2*	*blaSHV*	*balTEM*	*Stx1*	*Stx2*	*eaeA*	*iss*
ID 1	**O_{111}:H_4**	**EHEC**	S	R	R	R	S	S	S	R	+	+	+	–	–	–	+
ID 2	**O_{111}:H_4**	**EHEC**	S	R	R	R	S	R	S	R	–	_	+	–	–	–	+
ID 3	**O_{128}:H_2**	**EHEC**	S	R	R	R	S	R	S	R	–	+	+	+	–	+	–
ID 4	**O_{44}:H_{18}**	**ETEC**	S	R	R	S	S	R	R	R	–	+	+	–	–	+	+
ID 5	**O_{44}:H_{18}**	**ETEC**	S	R	R	S	S	S	R	R	–	_	+	–	–	–	+
ID 6	**O_{125}:H_{21}**	**ETEC**	S	R	R	S	S	S	R	R	+	+	+	–	–	–	+
ID 7	**O_{128}:H_2**	**EHEC**	S	R	R	R	S	R	S	R	+	+	+	–	+	+	+
ID 8	**O_{111}:H_4**	**EHEC**	S	R	R	R	S	S	S	R	–	_	+	–	–	+	+
ID 9	**O_{125}:H_{21}**	**ETEC**	S	R	R	S	S	S	R	R	–	_	+	–	–	+	–
ID 10	**O_{128}:H_2**	**EHEC**	S	R	R	R	S	S	S	R	+	+	+	–	–	–	+
ID 11	**O_{127}:H_6**	**EHEC**	S	R	R	S	S	S	R	R	+	+	+	_	_	+	+
ID 12	**O_{44}:H_{18}**	**ETEC**	S	R	R	S	S	S	R	R	+	+	+	+	_	+	+
ID 13	**O_{125}:H_{21}**	**ETEC**	S	R	R	S	S	S	R	R	_	_	_	_	_	_	+
ID 14	**O_{128}:H_2**	**EHEC**	S	R	R	S	S	S	R	R	+	+	+	_	_	+	+

Abbravation of Antibiotic discs: streptomycin (SC 10µg), gentamicin (GN 30µg), amikacin (AK 30µg), amoxicillin/clavulanic acid (AMC 20µg), ampicillin (AMP 20µg), cefotaxime (CFM 30µg), colistin (CL 10µg), and sulfamethoxazole-trimethoprim (SXT 1.25/23.75µg).

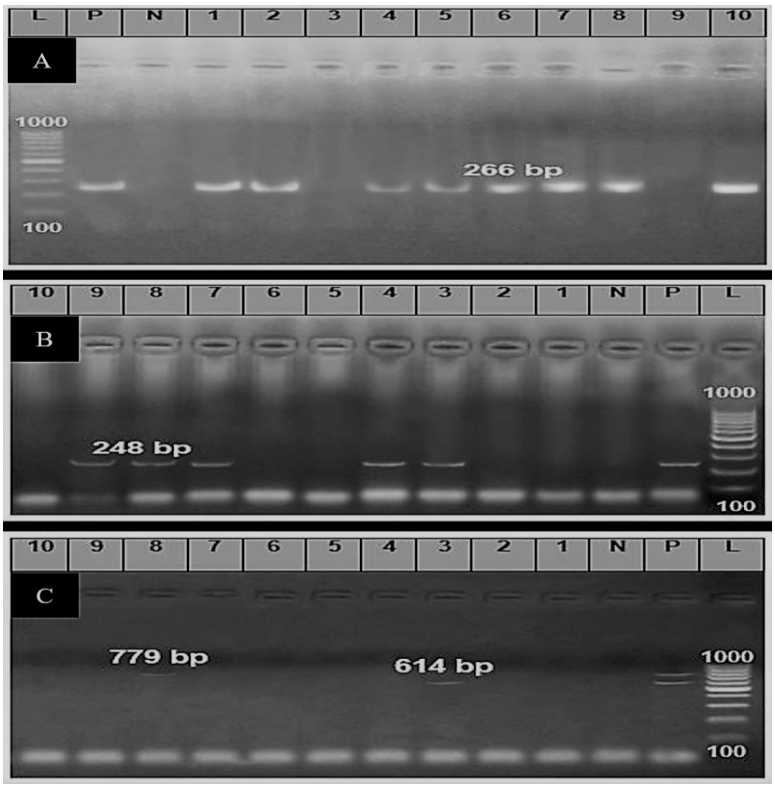

Fig. 1. PCR amplification of virulence genes from different E. coli serotypes isolated from broiler chicken flocks. Samples were run on 2% agarose gels as described in materials and methods. Lane L, a 100bp DNA ladder. (A) *iss* of various APEC serotypes (B) *eaeA*. While in (C) lanes 3 for *stx1* and 8 for *stx2* and p for positive control.

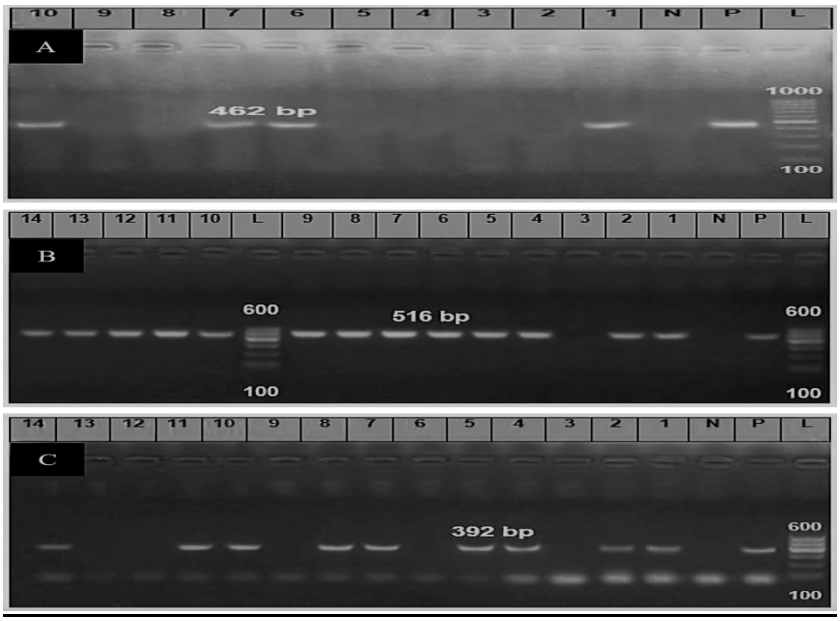

Fig. 2. Electrophoresis of β-lactamases associated with β-lactams resistance in APEC isolated from Egyptian broiler chicken farms. In (A) PCR amplification of bla_{CMY2} gives an expected band segment at 462 bp, (B) the expected band segment of bla_{TEM} at 516bp, while in, (C) the amplification of bla_{SHV} gives an expected band at 392 bp. Lane L, is a 100bp DNA ladder. Agarose electrophoresis was run on 2% agarose as described in materials and methods.

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عزل وتوصيف وتحليل جيني للجينات المسؤولة عن مقاومة المضادات الحيوية وعوامل الضراوة في بكتيريا الإشريكية القولوني المعزولة من مزارع الدجاج في مصر

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الملخص

تسبب بكتريا الإشريكية القولونية (APEC) أمراضًا خطيرة في مزارع دجاج التسمين. يرتبط هذا العامل الممرض البكتيري بارتفاع معدلات الوفيات في الدجاج ويعتبر السبب الرئيسي للأمراض المشتركة بين الإنسان. تهدف الدراسة الحالية إلى التحقيق في انتشار والمقاومة الحيوية لمضادات الميكروبات لـ APEC في مزارع دجاج التسمين في محافظتي الغربية والمنوفية في مصر. كما تقيم الدراسة العوامل المرتبطة بالضراوة والآليات الوراثية لمضادات حيوية بيتا لاكتام المستخدمة بشكل شائع في صناعة الدواجن في مصر. في هذه الدراسة، تم جمع 100 عينة عشوائيًا من 25 مزرعة دجاج تسمين. تم الإبلاغ عن الأعراض السريرية ومعدلات الوفيات في منطقتين من المنطقة الوسطى من دلتا مصر. في المجموع، تم جمع 100 عينة من 25 مزرعة دجاج تسمين، وكان معدل انتشار عدوى APEC المبلغ عنها في منطقة الدراسة 30٪. تم تصنيف السلالات المعزولة على أنها EHEC (8 O111: H4, 8 O128: H2 AND 5 O127: H6) ETEC (4 O44: H18 AND 5 O125: H21) وكشفت النتائج أن عدوى APEC كانت مرتفعة بشكل ملحوظ في الدجاج الذي يزيد عمره عن أربعة أسابيع مقارنة بأصغر من أربعة أسابيع. كانت جميع العزلات المختبرة حساسة للكوليستين والأميكاسين بينما كانت جميع العزلات المختبرة مقاومة لأموكسيسيلين / حمض الكلافولانيك والأمبيسلين والسلفاميثوكسازول - تريميثوبريم. كانت جميع العزلات المختبرة مقاومة للسبيكتينوميسين باستثناء ستة سلالات من الأنماط المصلية EHEC O111: H4 و O128: H2. على العكس من ذلك، كانت جميع العزلات المختبرة حساسة للسيفوتاكسيم باستثناء ستة سلالات من EHEC O111: H4 و O128: H2. علاوة على ذلك، كان الجنتاميسين حساسًا لجميع العزلات المختبرة باستثناء EHEC O111: H4 و O128: H2 و ETEC O44: H12. تحتوي الإشريكية القولونية المقاومة ظاهريًا ضد الأمبيسلين والأموكسيسيلين والسيفالوسبورينات على BLA_{TEM} وBLA_{SHV} وBLA_{CMY2} بالإضافة إلى جينات الضراوة التالية، ISS وEAEA وSTX1 وSTX2 والتي تم العثور عليها في 12 (85.71٪) و8 (57.14٪) و2 (14.29٪) و1 (7.14٪) من العزلات على التوالي. تشير نتائجنا إلى أن المزارعين والأطباء البيطريين في منطقة الدراسة يجب أن يستخدموا بروتوكول علاج قياسي لـ APEC لتقليل انتشار ومقاومة مضادات الميكروبات لهذا المرض المتوطن. وبالتالي فإن الجهود التي تهدف إلى السيطرة على مثل هذه يجب ان تؤدى الى الحد من استخدام المضادات الحيوية.

الكلمات الدالة: بكتيريا الإشريكية القولونية الممرضة للطيور، انتشار، مقاومة مضادات الميكروبات، جينات، BLACMY2 .