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Molecular determination of virulence and antimicrobial resistance genes in *Escherichia coli* recovered from broiler chickens

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ABSTRACT

This study was carried out to detect the prevalence of *Escherichia coli* (*E. coli*) strains in Cobb, Sasso, and Balady breeds of broiler chickens using conventional techniques and to evaluate the in-vitro susceptibility of the isolated strains to different antimicrobials. Besides, the presence of some virulence and antimicrobial resistance genes of *E. coli* strains was molecularly investigated. A total of 400 samples including liver, heart, spleen, lungs, and intestine were collected from freshly dead, diseased, and apparent healthy chickens in Damietta city, Damietta governorate, Egypt. The samples were subjected to conventional isolation and biochemical identification of *E. coli* and the results revealed a total prevalence of 20.5% (82/400) with the highest isolation rates from freshly dead breeds of chickens. The serological typing showed presence of O86:K61 and O26:K60 *E. coli* types. The results of the in-vitro antimicrobial susceptibility test of 82 *E. coli* strains revealed high degrees of resistances to cephradin, amoxicillin/clavulanic acid, sulfamethoxazole-trimethoprim, and doxycycline. The virulence genes including *phoA*, *iss*, and *tsh* were detected in 15 of *E. coli* strains with percentages of 100%, 33.33%, and 13.33%, respectively. Moreover, the results showed presence of bla_{TEM}, *sul*1, and *mcr*1 antimicrobial resistance genes in 15 of *E. coli* strains with a percentage of 33.33% for each gene. In conclusion, *E. coli* strains are still circulating in chicken flocks causing economic loses and they are resistant to most of the commonly used antimicrobials. Thus, adopting effective intervention strategies and better management systems are very critical to reduce *E. coli*-related hazards linked to poultry.

Introduction

The meat of poultry is regarded as the most affordable source of high-quality protein in the world. Poultry industry is usually subjected to bacterial affections that threat this massive production. One of the most important bacterial infections is *Escherichia coli* (*E. coli*). Strains of *E. coli* are mostly nonpathogenic; but, there are some strains that could spread from animals to humans and even cause diseases conditions in chicken's flocks (Ievy *et al*., 2022). Virulent or avian pathogenic *E. coli* (APEC) are considered as one of the most common bacterial threats which adversely affect the poultry industry worldwide. *E. coli* is a Gram-negative, rod-shaped, facultative anaerobic, and coliform bacterium of the family Enterobacteriaceae and genus *Escherichia* (Tenaillon *et al*., 2010). Strains of *E. coli* normally inhibit the intestines of people, animals, and birds (Levine, 1987); however other extra-intestinal APEC strains are common in all ages of poultry and cause a colibacillosis disease (Ahmed *et al*., 2013; Filho *et al*., 2015). Avian colibacillosis is associated with various systemic and localized affections such as colisepticemia, pericarditis, perihepatitis, airsacculitis, peritonitis, salpingitis, omphalitis, cellulitis, swollen head syndrome, panophthalmitis, coligranuloma, and arthritis (Dziva and Stevens, 2008; Kim *et al*., 2020). These diseases conditions are responsible for significant economic losses in poultry production system including high mortality, reduction of productivity, and increasing carcass condemnation rate and medication costs (Kabir, 2010; Guabiraba and Schouler, 2015). Furthermore, in the last decades, *E. coli* has acquired a dramatic relevance in human and veterinary medicine due to increasing prevalence of antibiotic resistant strains (Markland *et al*., 2015). Enterohemorrhagic and Shiga toxin-producing *E. coli* can cause epidemic diarrhea, enteritis, septicemia, endocarditis, meningitis, urinary tract infections, and hemolytic-uremic syndrome in humans (Rahman *et al*., 2020).

As a result of the expression of several putative virulence factors, some intestinal commensal *E. coli* become APEC and infect other extra-intestinal niches (Jeong *et al*., 2012; Mateus *et al*., 2020). There are interactions between molecular ligands on the surface of *E. coli* and cognate receptors on the host cell surface. Without such binding, the bacteria become powerless to resist being swept away and exiting the host (Pósfai *et al*., 2006). The virulence of *E. coli* is assisted by many virulence factors expressed by virulence-associated genes, including *phoA*, *tsh*, *iss*, iutA, *pap*C, *iuc*D, *omp*T, *hly*F, *iron*, and *ast*A (Barbieri *et al*., 2013; Mohamed *et al*., 2014; Awad *et al*., 2020; Hamed *et al*., 2023). The episomal increased serum survival (*iss*) gene, located on the large virulence plasmid ColV, has been detected and linked more often to APEC strains than the nonpathogenic ones (Pfaff-Mcdonough *et al*., 2000; Dissanayake *et al*., 2014; De Oliveira *et al*., 2015; Sarowska *et al*., 2019). Temperature-sensitive haemagglutinin (*tsh*) plays a role in the colonization of air sacs (Dozois *et al*., 2000). It has been assumed that the presence of two of these genes in one *E. coli* isolate indicates that this isolate is an APEC, while the absence or presence of a gene may disclose non-pathogenic *E. coli* (Schouler *et al*., 2012). Alkaline phosphatase (*phoA*) gene encodes for a hydrolase enzyme which is responsible for removing phosphate groups from molecule. This gene is common in *E. coli* strains (Hu *et al*., 2011; Yu and Thong, 2009; El-Demerdash *et al*., 2021). The *phoA* housekeeping gene was used to detect *E. coli* in seawater samples (Kong *et al*., 1999).

Application of antimicrobials is the primary step for controlling important infectious diseases affecting poultry (Talukder *et al*., 2021). The overuse and the indiscriminate or misuse of these antimicrobials can lead to selection and spread of multi-drug resistant strains of *E. coli* (Miles *et al*., 2006; Gyles, 2008; Islam *et al*., 2021), which may be transmitted to humans through food or direct contact with the diseased hosts (Founou *et al*., 2016). In the twenty first century, the spread of antimicrobial resistance genes of *E. coli* strains became the most divisive issue for humans, animals, poultry, and ecosystems (Mohamed *et al*., 2014; Poirel *et*

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al., 2018; Islam *et al*., 2022). Besides, APEC strains may carry certain resistance genes that makes other microorganisms resistant to antimicrobials (Guerra *et al*., 2018). The antimicrobial resistant genes including encoding resistance to β-lactams (bla_{TEM}) (Parvez *et al*., 2016), sulfonamides (*sul*1) (Parvin *et al*., 2020), and colistin (*mcr*1) (Amin *et al*., 2020; Dawadi *et al*., 2021) have been commonly found in *E. coli* strains. For instance, *E. coli* may produce extended-spectrum β-lactamase (ESBL) (Castanheira *et al*., 2021) which are enzymes that can degrade extended-spectrum β-lactam antibiotics, such as third generation cephalosporins, commonly used to control many serious infections. The transmission of ESBL-producing multidrug-resistant *E. coli* in chickens and humans is common (Dierikx *et al*., 2013; Kameyama *et al*., 2013; Badr *et al*., 2022; Lemlem *et al*., 2023). Human's consumption of chicken meat containing ESBL-producing *E. coli* represents an important food safety hazards and public health threats (Lazarus *et al*., 2014; Ramos *et al*., 2020). The achievement of ESBL-producing *E. coli* genes play a significant role in the spread of multidrug-resistant bacteria among humans, animals, poultry, and the environment via the food chain (Day *et al*., 2016; Van Hoek *et al*., 2018; Subramanya *et al*., 2021; Awawdeh *et al*., 2022). The recent study of Islam *et al*. (2023) indicated that strains of *E. coli* isolated from poultry and poultry environments showed a higher resistance to almost all classes of antimicrobials, indicating a significant one health hazard.

This study was designed to investigate the prevalence of *E. coli* strains in different chicken's breeds using conventional isolation, identification, and serotyping methods, and evaluate the *in-vitro* antimicrobial susceptibility of the isolated strains. Moreover, the presence of specific *E. coli* virulence genes (*phoA*, *tsh*, and *iss*) and antimicrobial resistant genes (bla_{TEM}, *sul*1, and *mcr*1) was molecularly detected.

Materials and methods

Sampling

A total of 400 chickens representing 199 Cobb, 148 Sasso, and 53 Balady breeds with ages vary from 30 to 55-days were collected from different farms in Damietta city, Damietta governorate, Egypt during the period from 2021-2022. Samples including liver, lung, heart, spleen, and intestine were randomly collected from sacrificed diseased chickens showing mortalities, loss of weight, diarrhea, and respiratory manifestations, as well as from apparent healthy and freshly dead birds with septicaemia and congestion of internal organs, pericarditis, perihepatitis, airsacculitis, peritonitis, ascites, and enteritis. Separate organs of each chicken were taken and put in polyethylene bags and then immediately transferred in an ice tank to the laboratory for bacteriological analysis.

Phenotypic characterization

For isolation of *E. coli*, 2 grams of each organ were directly inoculated into the nutrient broth (Oxoid®) and incubated aerobically for 18 h at 37°C. Then, a loopful from the previously inoculated broth was streaked onto MacConkey agar plates (Oxoid®) for 24 h at 37°C. Pink to dark pink, dry, and donut-shaped colonies surrounded by a dark pink area of precipitated bile salts were picked up and streaked on eosin methylene blue (EMB) (Oxoid®) agar, tryptone bile X-glucuronide (TBX) agar, and xylose lysine deoxycholate agar (XLD) agar and incubated overnight at 37°C (Collee *et al*., 1996). The suspected *E. coli* isolates were further identified morphologically and biochemically by observing their culture characteristics, Gram's staining morphology, as well as using biochemical reactions such as oxidase, indole, methyl-red, Voges-Preuskuar, citrate, and triple sugar iron agar tests (Prescott, 2003). Isolates of *E. coli* were stored at −80°C in brain heart infusion broth containing 25% glycerol until used.

The slide agglutination test was performed for the serological identification of *E. coli* isolates (Edwards and Ewing, 1986). Both polyvalent and monovalent *E. coli* antisera were used for the sero-grouping according to somatic (O) and capsular (K) antigens (Sifin diagnostic Gmbh, Berlin, Germany). Serotyping of *E. coli* isolates was carried out at the Serology Department of Animal Health Research Institute, Dokki, Giza, Egypt.

Antimicrobial susceptibility test

The in-vitro antimicrobial susceptibility test of 82 *E. coli* strains was carried out on the Muller-Hinton agar using disc diffusion method. Ten antimicrobial discs (Oxoid) include amoxicillin/clavulanic acid (AMC, 30 µg), cefotaxime (CTX, 30 µg), cephradin (CE, 30 µg), ciprofloxacin (CIP, 5 µg), gentamycin (CN, 10 µg), colistin sulphate (CT, 10 µg), streptomycin (S, 10 µg), doxycycline (DO, 30 µg), erythromycin (E, 15 µg), and sulfamethoxazole-trimethoprim (SXT, 25 µg) were used. The minimal inhibitory concentration (MIC) interpretation guideline was done according to the clinical and laboratory standards institute (CLSI, 2020).

Detection of virulence and antimicrobial resistance genes

Both virulence and antimicrobial resistance genes of 15 *E. coli* strains were detected using polymerase chain reaction (PCR) assay. The targeted virulence genes were *phoA*, *iss*, and *tsh* while the antimicrobial resistance genes were bla_{TEM}, *sul*1, and *mcr*1. The DNA extraction was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 20 µl of proteinase K and 200 µl

Table 1. Primers sequences, target genes, amplicon sizes, and cycling conditions virulence and antimicrobial resistance genes of *E. coli* isolates.

Target gene	Primers sequences	Amplified segment (bp)	Amplification (35 cycles)				Final extension	Reference
			Primary denaturation	Secondary denaturation	Annealing	Extension		
phoA	CGATTCTGGAAATGGCAAAAG	720	94°C	94°C	55°C	72°C	72°C	Hu *et al*. (2011)
	CGTGATCAGCGGTGACTATGAC		5 min.	30 sec.	40 sec.	45 sec.	10 min.	
tsh	GGT GGT GCA CTG GAG TGG	620	94°C	94°C	54°C	72°C	72°C	Delicato *et al*. (2003)
	AGT CCA GCG TGA TAG TGG		5 min.	30 sec.	40 sec.	45 sec.	10 min.	
iss	ATGTTATTTTCTGCCGCTCTG	266	94°C	94°C	54°C	72°C	72°C	Yaguchi *et al*. (2007)
	CTATTGTGAGCAATATACCC		5 min.	30 sec.	30 sec.	30 sec.	7 min.	
bla_{TEM}	ATCAGCAATAAACCAGC	516	94°C	94°C	54°C	72°C	72°C	Colom *et al*. (2003)
	CCCCGAAGAACGTTTTC		5 min.	30 sec.	40 sec.	45 sec.	10 min.	
*sul*1	CGGCGTGGGCTACCTGAACG	433	94°C	94°C	60°C	72°C	72°C	Ibekwe *et al*. (2011)
	GCCGATCGCGTGAAGTTCCG		5 min.	30 sec.	40 sec.	45 sec.	10 min.	
*mcr*1	CGGT CAGTCCGTTTGTTC	308	94°C	94°C	60°C	72°C	72°C	Newton-Foot *et al*. (2017)
	CTTGGTCGGTCTGTAGGG		5 min.	30 sec.	40 sec.	40 sec.	10 min.	

of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit. The used oligonucleotide primers were supplied from Metabion (Germany). The primers used in uniplex PCR were utilized in a 25-µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler. Primers sequences, target genes, amplicon sizes, and cycling conditions are listed in Table 1. Finally, the PCR products were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products were loaded in each gel slot. A generuler 100 base pair (bp) ladder (Fermentas, thermo, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Results and Discussion

In this study, suspected *E. coli* colonies appeared as greenish metallic sheen on EMB (Figure 1A), blue green on TBX (Figure 1B), and yellow on XLD agar (Figure 1C). Staining of suspected colonies showed Gram-negative rod shape bacteria under the microscope. The biochemical identification of suspected *E. coli* colonies revealed positive indole and methyl-red, as well as negative Voges-Preuskuar, citrate, and oxidase. On triple sugar iron agar test, positive *E. coli* isolates showed yellow slant and butt and gas with H2S production. Based on the morphological and biochemical characterizations, the overall prevalence rate of the isolated *E. coli* from the different chicken's breeds was 82/400 (20.5%). Moreover, the prevalence rates were 45 (22.6%) in Cobb, 31 (20.9%) in Sasso, and 6 (11.3%) in Balady breeds of chickens (Table 2). This result was similar to Eid and Erfan (2013), Peer *et al.* (2013); Mahmud *et al.* (2018), and Hassan *et al.* (2020) who showed that the prevalence rates of *E. coli* were 80%, 84%, 83.08%, and 80.5%, respectively. However, low incidences of *E. coli* isolation have been reported by Zhao *et al.* (2001) (38.7%); Sharada *et al.* (2010) (44.61%); Hasan *et al.* (2011) (36.20%), and Literak *et al.* (2013) (35.74%). The variable prevalence rates or serotypes of *E. coli* among the different studies may be related to the geographical localization of the tested flocks and the methods used for detection.

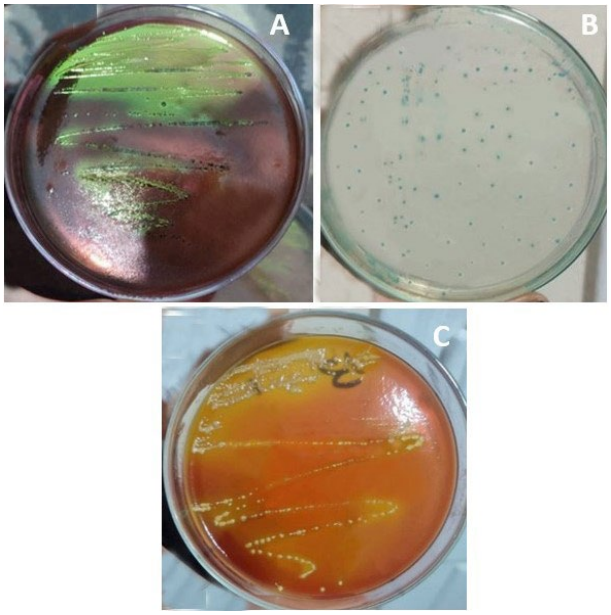

Fig. 1. A) Greenish metallic sheen colour suspected *E. coli* colonies on EMB agar plate. B) Blue green colour suspected *E. coli* colonies on TBX agar plate. C) Yellow colour suspected *E. coli* colonies on XLD agar plate.

Table 2. The prevalence of *E. coli* in the different breeds of chickens in Damietta governorate, Egypt.

Breeds of chickens	Total number	Number of positive samples	Prevalence (%)
Cobb	199	45	45/199 (22.6)
Sasso	148	31	31/148 (20.9)
Balady	53	6	6/53 (11.3)
Total	400	82	82/400 (20.5)

The recovery rates of *E. coli* strains in organs of freshly dead, diseased, and apparent healthy chicken's breeds are presented in Table 3. The highest recovery rates of *E. coli* were found in all organs of dead Cobb, Sasso, and Balady breeds when compared to diseased and apparent healthy chickens. In dead chickens, strains of *E. coli* were recovered in rates of 11.11%, 31.11%, 22.22%, 28.89%, and 6.67%; 12.90%, 29.03%, 22.58%, 25.80%, and 9.67%; and 0%, 33.33%, 16.67%, 33.33%, and 16.67% form liver, lung, heart, spleen, and intestine of Cobb, Sasso, and Balady breeds, respectively. It is known that some APEC strains could be detected in the intestinal content of chickens (Dawadi *et al.*, 2021). Nevertheless, strains causing avian colibacillosis are characterized by infection of multiple organs including the liver, spleen, heart, kidneys, and lung (Ievy *et al.*, 2020). The higher isolation rates of *E. coli* isolation from the visceral organs rather than the intestine assume the extra-intestinal invasion of the bacterium (Eid and Erfan, 2013; Awad *et al.*, 2020). So, *E. coli* strains have been obtained also from both the diseased and the apparent healthy chicken's flocks.

Table 3. The recovery rates of *E. coli* strains in organs of different chicken's breeds

Type of sample	Status of chickens					
	Freshly dead		Diseased		Apparent healthy	
	No.	%	No.	%	No.	%
Cobb (n=45)						
Liver	5	11.11	4	8.89	1	2.22
Lung	14	31.11	11	24.44	3	6.67
Heart	10	22.22	7	15.56	3	6.67
Spleen	13	28.89	11	24.44	2	4.44
Intestine	3	6.67	2	4.44	1	2.22
Sasso (n=31)						
Liver	4	12.9	4	12.9	0	0
Lung	9	29.03	7	22.58	2	6.45
Heart	7	22.58	8	25.8	2	6.45
Spleen	8	25.8	5	16.12	3	9.67
Intestine	3	9.67	2	6.45	1	3.22
Balady (n=6)						
Liver	0	0	0	0	0	0
Lung	2	33.33	2	33.33	0	0
Heart	1	16.67	1	16.67	0	0
Spleen	2	33.33	1	16.67	1	16.67
Intestine	1	16.67	0	0	1	16.67

The serological characterization of the isolated strains showed that 2 of the isolated *E. coli* strains were positive and represented as O86:K61 and O26:K60, where serotype O86:K61 (polyvalent 2) was isolated from Sasso breeds and O26:K60 serotype (polyvalent 1) was recovered from Cobb breeds of chicken. The other common serotypes of APEC were not detected maybe owing to the use of vaccines containing these serotype (Schouler *et al.*, 2012). The results obtained by Mohamed *et al.* (2014) demonstrated presence of O86 and other serotypes in chickens of Assiut governorate of Egypt. The involvement of a particular O serotype in the

infection process appeared to vary according to the geographical region (Ali *et al*., 2019). Despite serotyping remains the most commonly used diagnostic laboratory method, it only allows the identification of a limited number of APEC strains (Schouler *et al*., 2012). Thus, molecular identification techniques are more accurate and faster than the traditional bacteriological characterization methods (Eid *et al*., 2016; Awad *et al*., 2020; Hamed *et al*., 2023).

The Commission Implementing Decision 2020/1729 of 17 November 2020 revealed that to monitor and report the antimicrobial resistance, Enterococcus faecalis, Enterococcus faecium, and *E. coli*, as well as food-producing animals including broilers should be considered (European Commission, 2020). The widespread use of antimicrobials in poultry industry, often without professional consultation or supervision, is problematic (Aarestrup, 2005). The therapeutic application of antimicrobials to control *E. coli* losses has led to the emergence of multidrug-resistant bacteria, changes in the gut microbiome, presence of tissues residues, and adverse environmental effects (Subedi *et al*., 2018). Here, the results of the in-vitro antimicrobial susceptibility test of 82 *E. coli* strains showed a high degree of resistance to CE (79; 96.34%), AMC (78; 95.12%), SXT (77; 93.90%), DO (74; 90.24%), E (70; 85.36%), CT (66; 80.48%), CTX (60; 73.17%), S (50; 60.97%), CIP (33; 40.24%), and CN (15; 18.29%) (Table 4). Both commensal and pathogenic *E. coli* strains in broiler chickens showed resistance to various antimicrobials such as β-lactams (Borges *et al*., 2019), sulfamethoxazole-trimethoprim, and colistin (Trobos *et al*., 2008; Messaili *et al*., 2019). Antibiotics produce β-lactam are regarded as the most widely used drugs which induce development of antibiotic-resistant strains of bacteria because of increased selective pressure (Beceiro *et al*., 2013). Some strains of *E. coli* may produce extended-spectrum β-lactamase (ESBL) enzymes which hydrolyze the β-lactam ring, thus inactivating the drug (Castanheira *et al*., 2021). These enzymes can degrade extended-spectrum β-lactam antibiotics such as the third generation cephalosporins and monobactams which are commonly used to control many serious infections (Pitout and Laupland, 2008). The ESBL-producing *E. coli* strains are considered as a global indicator to monitor the success in limitation of the emergence and spread of antimicrobial resistant bacteria in poultry chain (Apostolakos *et al*., 2019; Wibisono *et al*., 2020; Hashim *et al*., 2022). The achievement of ESBL-producing *E. coli* genes plays a significant role in the spread of multidrug-resistant bacteria among humans, animals, poultry, and the environment via the food chain (Day *et al*., 2016; Van Hoek *et al*., 2018; Subramanya *et al*., 2021; Awawdeh *et al*., 2022). The phenotypes and genotypes of ESBL-producing *E. coli* from chickens and human samples in Egypt showed degrees of similarity that suggest potential zoonotic transmission (Badr *et al*., 2022). The spread of ESBL-producing *E. coli* strains from non-symptomatic broiler chickens reveals that the normal inhabitant commensal bacteria can carry a resistance gene reservoir and may act as a potential risk of transfer to humans (Li *et al*., 2016; Gundran *et al*., 2019). Human's consumption of chicken meat containing ESBL-producing *E. coli* represents an important food safety hazards and public health threats (Ramos *et al*., 2020). The results of Päivärinta *et al*. (2020) showed that 18% of cloacal swabs of broiler chickens and 32% of retail chicken meat samples presented ESBL-producing *E. coli* in Finland where there is no use of antibiotics. Similar results were also reported by Mollenkopf *et al*. (2014) and Musa *et al*. (2021) who found that chicken meat and chicken samples from organic farms have resistant *E. coli* strains. The impact of infections caused by ESBL-producing *E. coli* in farm animals is still erratic. Yet, to control this threat, the potential reservoirs for these bacteria need to be assessed from a One Health Perspective (Mughini-Gras *et al*., 2019).

The extra-intestinal APEC strains have virulence genes that allow their accommodation in different organs other than the intestine (Solà-Ginés *et al*., 2015). Additionally, some APEC strains are zoonotic which allows them a widespread (Akya *et al*., 2019). Isolates of APEC carry a wide range of virulence genes, such as adhesions, toxins, siderophores, iron transport systems, and invasions that enhance the pathogenicity of colibacillosis (Amer *et al*., 2018; Borzi *et al*., 2018). Not all the *E. coli* virulence-associated genes are rarely present in the same strain, and they can occur either individually or polygenically with varying rates in the clinical strains (Vandekerchove *et al*., 2005; Wang *et al*., 2015). It is important to mention that the pathogenicity of the bacterium relies mainly on the detection of the virulence genes. In this work, the PCR assay of 15 *E. coli* strains for the detection of *phoA* virulence gene revealed that all strains (100%) were positive at 720 bp band (Figure 2). Regarding *iss* gene, 5 out 15 (33.33%) of *E. coli* strains were positive at 266 bp (Figure 3), while only 2 out of 15 (13.33%) were positive for *tsh* gene at 620 bp (Figure 4). The *phoA*, *tsh*, and *iss* virulence genes were selected due to their common prevalence in APEC isolated from broiler samples (Mbanga and Nyararai, 2015; Paixao *et al*., 2016; Subedi *et al*., 2018).

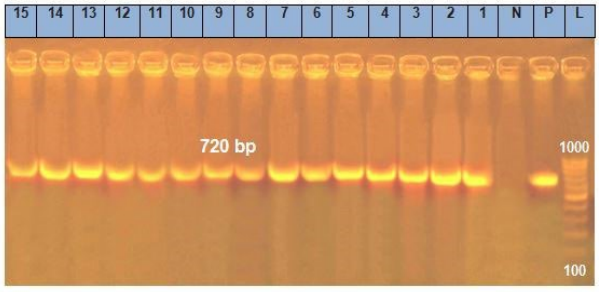

Figure 2. Positive *phoA E. coli* strains produce band at 720 bp using PCR.

Alkaline phosphatase encoding gene (*phoA*) is responsible for removal of phosphate groups from the molecule is common and specific in *E. coli* strains (Yu and Thong, 2009; Hu *et al*., 2011). This gene is resent in most of *E. coli* strains as it is regarded as a housekeeping gene (Chang *et al*., 1986). The presence of *iss* gene in APEC is of high significance and

Table 4. The antimicrobial susceptibility test of the isolated *E. coli* strains.

Antibiotic disc (Code)	Disc content/ µg	Antimicrobial efficacy (%) (n = 82)		
		Susceptible	Intermediate susceptibility	Resistant
Amoxicillin/Clavulanic acid (AMC)	30	0 (0%)	4 (4.87%)	78 (95.12%)
Cefotaxime (CTX)	30	8 (9.75%)	14 (17.07%)	60 (73.17%)
Cephradin (CE)	30	0 (0%)	3 (3.65%)	79 (96.34%)
Ciprofloxacin (CIP)	5	10 (12.19%)	39 (47.56%)	33 (40.24%)
Gentamycin (CN)	10	32 (39.02%)	35 (42.68%)	15 (18.29%)
Colistin sulphate (CT)	10	2 (2.43%)	14 (17.07%)	66 (80.48%)
Streptomycin (S)	10	16 (19.51%)	16 (19.51%)	50 (60.97%)
Doxycycline (DO)	30	1 (1.21%)	7 (8.53%)	74 (90.24%)
Erythromycin (E)	15	0 (0%)	12 (14.63%)	70 (85.36%)
Sulfamethoxazole-trimethoprim (SXT)	25	1 (1.21%)	4 (4.87%)	77 (93.90%)

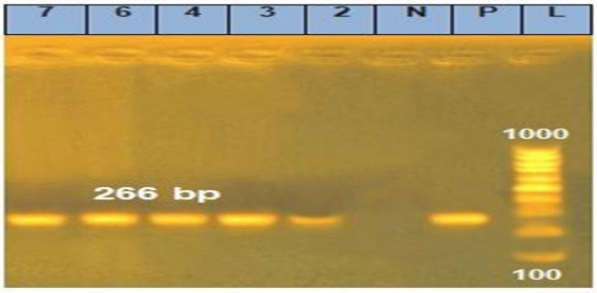

Figure 3. Detection of *iss* gene in samples; positive samples produce band 266 bp.

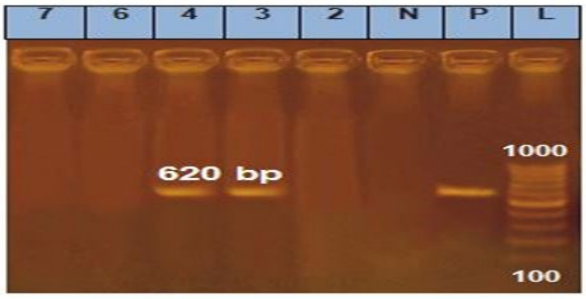

Figure 4. Detection of *tsh* gene in samples. Positive samples produce band 620 bp

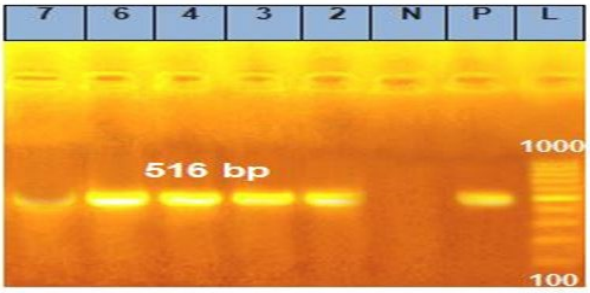

Figure 5. Detection of bla_{TEM} gene in samples; positive samples produce band 516 bp

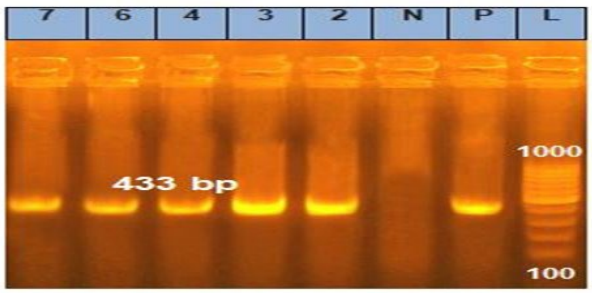

Figure 6. Detection of *sul*1 gene in samples; positive samples produce band 433 bp

it presents in a high incidence among APEC strains (Pfaff-McDonough *et al*., 2000; Oliveira *et al*., 2019; Hassan *et al*., 2020). Ahmed *et al*. (2013), Hussein *et al*. (2013), Mohamed *et al*. (2014), Radwan *et al*. (2014), Hassan (2017), and Abd El-Tawab *et al*. (2018) detected *iss* gene in broilers in Ismailia, Damietta, Assuit, Beni-Suef, El-Fayoum, and El-Gharbia governorates, Egypt, respectively. Increasing the prevalence of *iss* gene (93.3%; Awad *et al*., 2020), (81.5%; Rodriguez-Siek *et al*., 2005), and (73.8%; Rocha *et al*., 2008) has been also reported. This gene is located on conjugated high molecular weight plasmids, encodes to the outer membrane protein, and plays a key role in serum resistance and protection from the complement action (Nolan *et al*., 2003; McPeake *et al*., 2005). It is surprising to found that *iss* gene was more predominant in *E. coli* strains of healthy broilers (97%) than diseased ones (16%) (Johar *et al*., 2021). The high incidence of this gene allows the bacterium to escape the host defenses and multiply and disseminate, thus enhances the development of the disease (López *et al*., 2017). Moreover, *iss* gene on episomes can control expression of protectins/serum resistance genes to enhance the ability of bacteria to survive in the host serum (Sarowska *et al*., 2019). The temperature-sensitive hemagglutinin (*tsh*) gene is regarded as an auto transporter protein with proteolytic and adhesive functions. During the early stages of the infection, *tsh* in the outer membrane aids in process of adhesion. Moreover, it has been detected at a greater frequency on ColV plasmids among APEC strains (Nakazato *et al*., 2009). It has been reported that *tsh* gene was a virulence marker of APEC strains that have a strong association with internal organs colonization and septicemia in a-day-old chickens (Ngeleka *et al*., 2003; Ali *et al*., 2019). *tsh* gene has been detected in chicken farms in Assiut and Qena governorates, Egypt (Mohamed *et al*., 2014; Ahmed *et al*., 2017).

Strains of *E. coli* act as reservoirs of antibiotic-resistant genes that capable of causing diseases in both humans and animals (Roth *et al*., 2019). Strains of APEC can be transmitted among animals and humans via the direct contact, contact with animal excretions, or through the food chain and this considers a major and life-threatening concern (Poirel *et al*., 2018; Pormohammad *et al*., 2019). Strains of *E. coli* may act as donors or recipients of antimicrobial resistance genes that could be transferred horizontally (Poirel *et al*., 2018). The results of the molecular detection of the antibiotic resistance genes including bla_{TEM}, *sul*1, and *mcr*1 are illustrated in figures 5, 6, and 7, respectively. The results reveled presence of bla_{TEM}, *sul*1, and *mcr*1 in 5 out of 15 (33.33%) of *E. coli* strains at 516 bp, 433 bp, and 308 bp, respectively. It has been reported that APEC strains of broiler origin showed resistant to cephalosporins, TEM β-lactamases which are encoded by the bla_{TEM} (Gundran *et al*., 2019). The TEM is large and widespread group that varies from its parental enzymes by one or two amino acids (Smet *et al*., 2010). This minor variation in amino acid sequence is appropriate to extend the spectrum of TEM enzymatic activity and allows it to hydrolyze cephalosporins (Rawat and Nair, 2010). bla_{TEM} gene in *E. coli* has been detected in broiler chickens with septicaemia (Ahmed *et al*., 2013; Khoshbakht and Raeisi, 2016; Lemlem *et al*., 2023). Both Al Azad *et al*. (2019) and Sarker *et al*. (2019) revealed a high prevalence of bla_{TEM} gene in *E. coli* isolated from cloacal swabs of broilers. In the recent study of Abdel-Rahman *et al*. (2023), the bla_{TEM} resistance gene was detected in 93% of *E. coli* strains, which is lower than the study of Moawad *et al*. (2018) who detected this gene in *E. coli* isolates from healthy broilers chickens (20.6%). Moreover, sulfamethoxazole-trimethoprim resistance gene (*sul*1) of *E. coli* is one of the most prevalent genes which detected in strains from live broiler chickens and meat (Trobos *et al*., 2008; Amador *et al*., 2019). Besides, colistin has been extensively used in animal production for the treatment of many bacterial infections, especially β-lactamase-resistant Enterobacteriaceae (Liu *et al*., 2016; Aklilu and Raman, 2020). However, the wide usage of colistin contributes to the appearance of *E. coli* resistant strains, which is usually accompanied by the emergence of the plasmid-mediated colistin resistance determinants, *mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4, and *mcr*-5 (Rebelo *et al*., 2018). The resistance to colistin is usually encoded by the *mcr*-1 gene that has been found in Enterobacteriaceae isolated from humans, food, and livestock (Poirel *et al*., 2017; Li *et al*., 2018). This resistance develops due to the mutations in the lipid synthesis enzymes of the bacterial outer membrane (Gao *et al*., 2016; Hinchliffe *et al*., 2017; Yang *et al*., 2017). Moreover, the coexistence of *mcr*-1 gene with bla_{TEM} in *E. coli* isolates of chicken origin has been reported (Wu *et al*., 2018; Joshi *et al*., 2019; Aklilu *et al*., 2022; Lemlem *et al*., 2023). Further, β-lactamase-encoding genes and *mcr*-1 mediated colistin resistance in multidrug-resistant *E. coli* have been detected in broiler chickens and meat (Aliyu *et al*., 2016; Moawad *et al*., 2018; Aklilu and Raman, 2020; Dhaouadi *et al*., 2020).

Conclusion

This study indicated presence of circulating APEC strains in the Egyptian chicken's farms that cause serious economic losses and show multiple resistance to the commonly used antimicrobials. Therefore, proper cleaning and disinfection practices as well as strict biosecurity measures are critical for the control of such zoonotic disease in poultry production facilities. Additionally, enhancing awareness, monitoring, and surveillance

programs are the must. The development of recent techniques for identification of APEC in the top layer of the production pyramid could be regarded as an initial step for combating the transmission of infection in broiler production system. To better understanding the probability of antimicrobial resistance transmission, more research work regarding veterinary and human epidemiology are required. It is important to restrict the use of antimicrobials for humans in food animals and discover natural and safe alternatives for animal production.

Conflict of interest

The authors declare that they have no conflict of interest.

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