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Investigation of Antibiotic Resistance Pattern and Virulence Determinants in Avian Pathogenic *Escherichia coli* Isolated from Broiler Chickens in Egypt

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ABSTRACT

Besides its zoonotic importance, avian pathogenic *Escherichia coli* (APEC) causes substantial financial losses in the poultry industry globally. The progress of antimicrobial resistance in APEC is mainly associated with excessive antimicrobial use and improper sanitation. Since its beginning in the 1970s, the VITEK system has developed into the VITEK 2 system, which has used an automated system to perform all the steps required for microbial identification and antibiotic susceptibility rapidly and accurately. The present study aimed to update the available circulating data about APEC isolates by phenotypic identification, sero-grouping of APEC from broilers chickens and breeders in five governates of Egypt, investigation of their antibiotic resistance pattern by VITEK 2 system, and molecular identification of their virulence determinants. The prevalence of APEC isolated from the different internal organs (liver, lung, heart, heart blood, and spleen) was 67.5%. The most prevalent serotypes were O125, 0114, O44, O127, O142, and O78. Virulence-associated genes (*iutA, fimC, and papC*) were detected at rates of 84.4%, 74%, and 54.8%, respectively. The highest resistance was found against ampicillin (100%), trimethoprim-sulfamethoxazole (80%), and ampicillin-sulbactam (78.5%), which indicates that the poultry farms need a surveillance and intervention system with proper accuracy and rapidity to prevent the misuse of antibiotics and APEC outbreaks.

Keywords: *Escherichia coli*, Colibacillosis, PCR, VITEK, Virulence genes

INTRODUCTION

Escherichia coli is considered one of the normal inhabitants of the digestive tract of both humans and animals (Sarowska et al., 2019). It is considered a normal element of intestinal flora in poultry (De Carli et al., 2015). The avian pathogenic *Escherichia coli* (*E. coli*), is a subclass of extraintestinal pathogenic *E. coli*, which has many routes for entry, including the vaginal and respiratory systems, leading to various extraintestinal infections in poultry, known as colibacillosis (Matthijs et al., 2009). Perihepatitis, egg peritonitis, airsacculitis, pericarditis, omphalitis, cellulitis, and osteomyelitis/arthritis are the most major symptoms of colibacillosis in poultry (Dziva and Stevens, 2008). It is one of the leading causes of mortality (up to 20%) and morbidity in poultry, as well as a significant reduction in meat (2% decrease in live weight), 2.7% rapid decline in feed conversion ratio, and egg production (up to 20%), decreased hatching rates, and increased carcass condemnation (up to 43%) at slaughter (Guabiraba and Schouler, 2015). Avian colibacillosis triggered by APEC strains is mostly associated with various virulence genes and serogroups (Wang et al., 2010). The virulence of *E. coli* is assisted by many virulence factors expressed by virulence-associated genes, including *iutA, iss, papC, iucD, tsh,ompT, hlyF, iron, and astA* (De Carli et al., 2015).

According to reports, antibiotics are utilized massively in disease prevention strategies and commonly as a growth stimulant in broilers (Osti et al., 2017). However, the therapeutic use of antibiotics in chicken has led to the emergence of multidrug-resistant bacteria, gut microflora changes, antibiotic residue in meat, and environmental effects (Subedi et al., 2018). Since these *E. coli* strains could be transmitted to people through the food chain or direct contact with infected broilers, they pose a major threat to public health. Additionally, Guerra et al. (2018) speculated that resistant *E. coli* might be a carrier of genes that makes other microorganisms resistant to antibiotics.

Avian fecal *E. coli* (AFEC), which have recently been isolated from normal broiler chicken, may also hold several virulence components that indicate their capability for virulence (Hiki et al., 2014). The putative avian hemolysin (*hly*F), the episomal increased serum survival (*iss*), the salmochelin siderophore receptor (*iro*N), the episomal outer membrane protease T (*omp*T), and the ferric aerobactin receptor (*iut*A), which are all located on the large virulence plasmid ColV, have all been linked to highly pathogenic APEC strains and are more frequently isolated from them De Oliveira et al. (2015). Type 1 fimbriae C (*FimC*) is an additional virulence factor that is necessary for fimbrial assembly and anchoring as well as for *E. coli* adhesion to host respiratory epithelial cells for colonization (Jeong et al., 2012).

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In comparison to manual biochemical testing, automation of biochemical assays has reduced the identification time to 2-10 hours (3 hours for Gram-negative rods), enhanced reliability, and increased efficiency with little manual sample preparation (Funke et al., 1998). One of the most popular integrated and automated systems for identifying bacteria based on the biochemical profiles of tested strains is the VITEK 2 system. Bacterial identification using this fluorescence and/or colorimetry-based technique is quick when used in conjunction with modest amounts of selective or differentiated media or reagents contained on compact plastic cards (Barry et al., 2003).

Through accurate microbiological identification and antibiotic susceptibility testing, the effectiveness of the VITEK 2 system equipment and VITEK® 2 PC software offers the potential to increase therapeutic success and result outcomes, according to Barenfanger et al. (1999) with a cost-effective, space-saving design, it also increases laboratory efficiency by reducing work labor and enabling quick reporting (Sanders et al., 2001).

The management of APEC infections in poultry relies on antibiotic prescription and immunization instead of reducing the environmental stressors, employing biosecurity procedures, and vaccination programs for the broiler chicken against the majority of virus-related and immunosuppressor diseases. Therefore, the current study was conducted to isolate and detect the antibiotic resistance pattern of APEC from different farms in Egypt using the VITEK 2 system, determine their serotype, and detect some virulence-related genes to improve applicable preventative measures to control colibacillosis in broilers.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Institutional Animal Care and Use Committee at the Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, with certificate reference Vet CU 01122022561.

Samples collection

In this study, 370 clinical cases of broiler chicken (7-21 days old) and broiler breeders (25-30 weeks old) in different breeds were examined for gross lesions of colibacillosis by postmortem examination in the Microbiology Department, Faculty of Veterinary medicine, Cairo University, Giza, Egypt during the period from January 2020 to January 2021. Samples were collected from five Egyptian governorates (Sharkia, Kafr-el-sheikh, Fayoum, Giza, and Qalubia). All investigated farms suffered clinical signs of colibacillosis, including high mortalities, respiratory signs, reduced appetite, and declined growth rate. The samples were collected from different organs (liver, lung, heart, heart blood, and spleen) aseptically and kept individually in sterile, zipped plastic bags for bacterial isolation.

Bacteriological identification

The collected organs were minced and inoculated into the nutrient broth (Oxoid®) aerobically at 37°C for 18-24 hours, before being sub-cultured on MacConkey agar (Oxoid®) and Eosin methylene blue agar (EMB, Oxoid®) aerobically at 37°C for 18-24 hours (Collee et al., 1996). All the recovered isolates were identified morphologically and biochemically as *E. coli* by observing their culture characteristics, morphology by Gram's stain, oxidase test, biochemical reactions using indole, methyl-red, Voges-Preuskuar, citrate tests (IMViC), and TSI as described by Quinn et al. (2002). The suspected isolates were maintained in cryostat tubes containing 20% glycerol with LB Luria Bertani broth at -70°C for further identification.

Identification of isolates using the VITEK 2 system

Each isolate was identified by the Gram-negative test kit VITEK 2 system (bioMérieux, France) according to the manufacturer's instructions. Using VITEK 2 DensiChek equipment (bioMe'rieux, France), a bacterial suspension was adjusted to the McFarland standard of 0.5 in 2.5 ml of a 0.45% sodium chloride solution. There were never more than 30 minutes between making the inoculum and filling out the card. The card was placed on the cassette made specifically for the VITEK 2 device, put inside, automatically filled with vacuum, sealed, incubated at 35.5°C, and subjected to an automatic colorimetric measurement using a new optical reading head every 15 minutes for a maximum incubation period of 10 hours. Version 9.02 of the VITEK 2 database was used to examine the data. The 64-well plastic GN card contains 41 tests, including 18 tests for sugar incorporation, 18 tests for sugar fermentation, 2 tests for decarboxylase, and 3 tests for miscellaneous (Urease, tryptophan deaminase, and utilization of malonate).

Serotyping of *Escherichia coli* isolates

The identified *E. coli* isolates were subjected to serotyping as described by Edward (1972). Polyvalent and monovalent diagnostic *E. coli* antisera were used for the sero-grouping of *E. coli* isolates according to somatic (O) and capsular (K) antigens. Suspected isolates of *E. coli* were subcultured on semisolid or slop agar and incubated for 24 hours at 37°C, then subcultured on MacConkey agar medium and incubated for 24 hours at 37°C. Three to five colonies were suspended in 3 ml saline and kept in the water bath at 100°C for one hour, then centrifuged at 2000 rpm for 10 minutes. The supernatant was discarded, and the precipitate was maintained, to which 0.5 ml saline was added. A drop

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from this suspension was placed on a glass slide and mixed with one drop from the O polyvalent anti-serum using a wooden strike.

Antimicrobial susceptibility testing using VITEK 2 system

Escherichia coli isolates were subjected to antimicrobial susceptibility testing using AST-GN73 TEST KIT (bioMérieux, Incorporation., Durham, NC) using VITEK 2 system version 9.02 software version according to manufacturer's instructions. Minimal Inhibitory Concentration (MIC) interpretation guideline was done according to (CLSI, 2020). The AST-GN73 card contains multiple cards of antimicrobial agents as dehydrated substances at the indicated concentrations. The cards overflowed with an inoculum ready by transferring 200 µL of culture suspension from the 0.5 McFarland culture suspension. The VITEK-2 system automatically processes the antimicrobial susceptibility cards until MICs are obtained (Joyanes et al., 2001).

Molecular identification of *Escherichia coli 16sr RNA* and their virulence determinants

Genomic DNA extraction was accomplished as described by Blanco et al. (2004). Bacterial isolates were first subcultured overnight at 37°C in Trypticase Soya broth before being suspended in 200 µL of sterile water. Bacteria were boiled for 10 minutes to disrupt the cells to release the DNA, followed by centrifugation at 10,000 rpm for five minutes. The supernatant containing DNA was stored at -20°C until used for polymerase chain reaction (PCR).

Molecular identification was made for the analysis of *16sr*RNA, *pap*C, *fim*C, and *iut*A genes in *E. coli* isolates by simple PCR using 200 ng of DNA, 12.5 µL of DreamTaq PCR Master Mix (2X, Thermo Scientific™), 10.5 µL nuclease-free water (Thermo Scientific™) and 0.5 µL of each primer (10 µM, Table 1). The cycling conditions consisted of five minutes activation step at 95°C, followed by 35 cycles of 95°C for 30 seconds, annealing temperature for 30 seconds, 72°C for one minute, and followed by the final extension step at 72°C for ten minutes. Amplicons were separated by 1% ethidium bromide-stained agarose gel electrophoresis along with a 100-bp ladder (BIOWEST, Hong Kong, China) and visualized under UV light (Sambrook et al., 1989).

Table 1. The sequence of used primers and the used annealing temperature in PCR for the different used genes

Target gene		Primers' sequences (5'- 3')	Product size	Annealing Temperature	Reference
16srRNA	Eco-1	GACCTCGGTTTAGTTCACAGA	585 bp	50°C	(Candrian et al., 1991)
	Eco-2	CACACGCTGACGCTGACCA			
fimC	F	GGAAATAACATTCTGCTTGC		51°C	(Jeong et al., 2012)
	R	TTTGTTGCATCAAGAATACG	288 bp		
papC	F	TGATATCACGCAGTCAGTAGC	501 bp		(Ewers et al., 2005)
	R	CCGGCCATATTCACATAA		59.2°C	
iutA	F	ATGAGCATATCTCCGGACG	587 bp	55°C	(Moulin et al., 2006)
	R	CAGGTCGAAGAACATCTGG			

RESULTS

Visceral organs were collected aseptically for bacteriological investigation. According to the findings of the bacteriological study, *E. coli* was detected and confirmed in 251 (67.8 %) out of 370 samples by standard biochemical tests and VITEK 2 system Id cards from diseased and freshly dead broiler chickens. They showed distinctive green metallic sheen with a black center colony on EMB agar, while they indicated medium-sized rounded pink colonies on MacConkey's agar media. The prevalence of *E. coli* isolates varied among the internal organs as the liver samples were the highest (78.6%), followed by spleen samples (67%), heart and heart blood samples (60%), and lung samples (47%, Table 2).

The serological examination of 135 *E. coli* isolates resulted in the detection of different serogroups using specific eight polyvalent, then 43 monovalent group O somatic antisera. Different serogroups were detected contained O142 K 86, O91 K -, O125 K 70, 0114 k 90, O44 k 74, O127 K 63, O1 K -, O166 K -, O158 K -, O144 K -, O103 K -, O86 K 64, O27 K -, O103 K -, O151 K -, O78 K -. O55 K 59 while 19 strains were un-typed (Table 3).

The results of the antibiotic resistance pattern of *E. coli* isolates are presented in Table 4. All *E. coli* isolates showed a high rate of resistance against ampicillin (100%), followed by trimethoprim-sulfamethoxazole (78.5%), ampicillin-sulbactam (78.4%), Ceftazidime (75.5%), cefepime (74%), ceftriaxone (65.3%), ciprofloxacin (47.8%), gentamicin (29%), and levofloxacin (28.8%). Low levels of resistance were indicated against Cefazolin 17.5%, Meropenem 9.5%, Tobramycin 9%, and Amikacin 5%, respectively.

The molecular identification of *E. coli* isolates by PCR revealed that all isolates were confirmed as *E. coli* by the universal primer for *16srRNA* with 585 bp band size. *Escherichia coli* ATCC 25922 was used as a positive control, as

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demonstrated in figures 1- 4. In this study, the prevalence of three virulence-associated genes was investigated. Out of 135 APEC isolates, 114/135 (84.4%) were positive for the *iutA* gene, 100/ 135 (74%) for the *fimC* gene, and 74 /135 (54.8%) for the *papC* gene.

Table 2. Prevalence of *Escherichia coli* isolated from internal organs of broiler chickens in five Egyptian governorates

Examined organs	Number of examined organs	Positive samples	Percentage
Liver	140	110	78.6
Spleen	97	65	67
Heart and heart blood	84	47	60
Lung	49	29	59.1
Total	370	251	67.8

Table 3. Serogrouping of *Escherichia coli* isolates from broiler chickens of five different Egyptian governorates from January 2020 to January 2021

Isolated *E. coli** serotypes	Number of positive samples	Percentage
O125 K 70	27	20
0114 k 90	16	12
O44 k 74	10	7.4
O127 K 63	10	7.4
O142 K 86	9	6.7
O78 K -	8	6
O91 K -	7	5.2
O1 K -	6	4.4
O166 K -	2	1.4
O158 K -	4	3
O144 K -	4	3
O103 K -	3	2.2
O86 K 64	3	2.2
O27 K -	2	1.4
O103 K -	2	1.5
O151 K -	2	1.5
O55 K 59	1	0.7
Untyped	19	14
Total	135	100

**E. coli*: *Escherichia coli*

Table 4. Antibiotic resistance patterns of *Escherichia coli* isolates from broiler chickens of five different Egyptian governorates from January 2020 to January 2021.

Classes of antibiotics		N	S (%)	N	I (%)	N	R (%)
ß-lactam	1. ESBL producers	63	25	0	0	188	74.9
	2. Ampicillin	0	0	0	0	251	100
	3. Ampicillin-Sulbactam	0	0	54	21.5	197	78.4
	4. Piperacillin-Tazobactam	251	100	0	0	0	0
	5. Meropenem	217	86.5	10	4	24	9.5
Cephalosporins	6. Cefazolin	188	75	48	19	44	17.5
	7. Cefoxitin	198	78.8	53	21	0	0
	8. Ceftazidime	49	19.5	13	5	189	75.5
	9. Ceftriaxone	77	31	10	4	164	65.3
	10. Cefepime	60	24	6	2.4	185	74
Aminoglycosides	11. Amikacin	217	86.5	21	8.3	13	5
	12. Gentamicin	128	51	50	20	73	29
	13. Tobramycin	176	70	52	20.7	23	9
Fluoroquinolones	14. Ciprofloxacin	131	52	0	0	120	47.8
	15. Levofloxacin	122	48.6	57	23	72	28.7
Nitrofurans	16. Nitrofurantoin	234	93.2	17	6.7	0	0
Sulfonamides and potentiated sulfonamides	17. Trimethoprim-Sulfamethoxazole	54	21.5	0	0	197	78.5

N: Number of the isolates, S: Susceptible, I: Intermediate, and R: Resistant to antimicrobial agents by VITEK GN AST37 card

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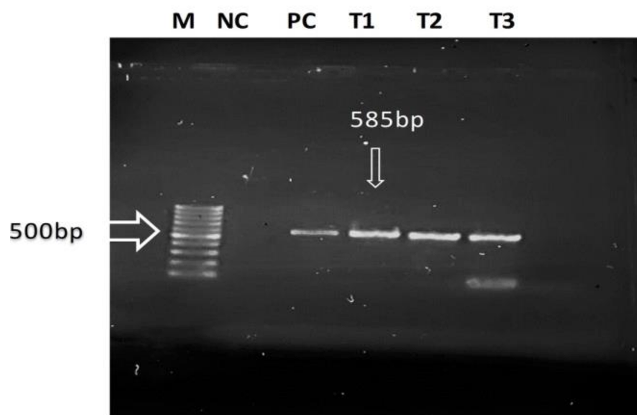

Figure 1. Agarose Gel electrophoresis of the amplified 16sRNA gene of *Escherichia coli* isolates. Lane M: 100 bp ladder; Lane NC: Negative control; Lane PC: Positive control *Escherichia coli* ATCC 25922; Lane T1-T3: Tested samples showing a positive result for 16srRNA gene with band size 585 bp

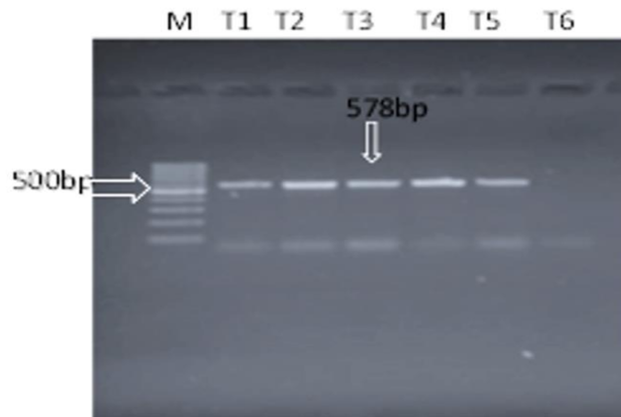

Figure 2. Agarose Gel electrophoresis of the amplified *iut*A gene of *Escherichia coli* isolates. Lane M: 100 bp ladder; Lane T1-T5: Tested samples showing a positive result for *iut*A gene with band size 578 bp; Lane T6 indicating a negative result.

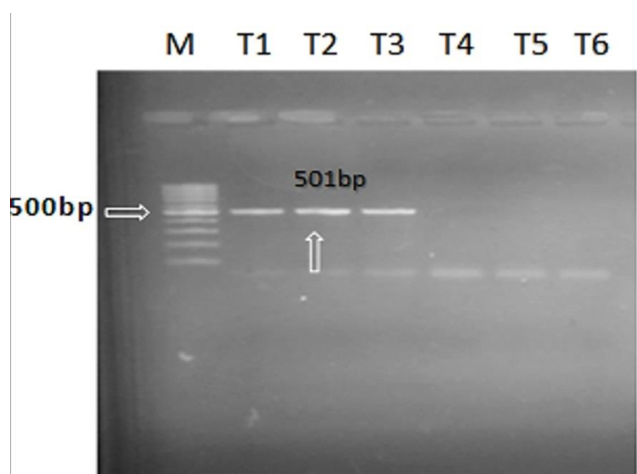

Figure 3. Agarose Gel electrophoresis of the amplified *pap*C gene of *Escherichia coli* isolates. Lane M: 100 bp ladder; Lane T1-T3: Tested samples showing a positive result for *pap*C gene with band size 501bp; Lane T4-T6 showing a negative result.

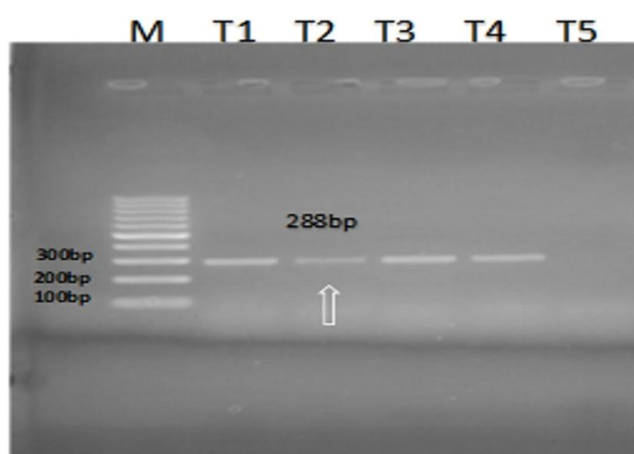

Figure 4. Agarose Gel electrophoresis of the amplified *fim*C gene of *Escherichia coli* isolates. Lane M: 100-bp ladder; Lane T1-T4: Tested samples showing a positive result for *fim*C gene with band size 288bp; Lane T5 indicating a negative result.

DISCUSSION

Avian pathogenic *Escherichia coli* (APEC) is a widespread and significant cause of economic loss in the poultry industry owing to morbidity, mortality, and loss of production (Barnes et al., 2008). Additionally, it is reported as a danger to the most affordable sources of high-quality protein in the world (Hussein et al., 2013). Strains of APEC are categorized as the extraintestinal pathogen, which is characterized by the presence of virulence genes that permit their accommodation in various organs other than the intestine (Johnson et al., 2006). Besides, evidence suggests that some APEC strains are zoonotic, allowing them to spread widely (Tivendale et al., 2010).

In the present study, broiler chickens and breeders were examined for gross lesions of colibacillosis from five different Egyptian governorates. The prevalence of *E. coli* isolates among the internal organs varies, but the highest was in liver samples, followed by spleen, heart, heart blood, and lung samples. Similar results were obtained by Abd El Tawab et al. (2016), who recorded a high isolation rate from the liver followed by heart, blood, and lung. In contrast, this result agreed with Eid and Erfan (2013), who recorded a high isolation rate from the liver (57.14%), followed by the lung (54.29%) and heart blood (37.14%). In addition, Yousef et al. (2015) reported that out of 95 liver samples, 88 were positive for *E. coli* with a percentage of 92.6%, while this result disagreed with Ola (2017), who found that 14% of *E. coli* isolates (28/200) were isolated from liver. The higher rates of *E. coli* isolation from the liver and lung assume the extraintestinal *E. coli* invasion into other organs and tissues, including lung, heart, and liver tissues (Awad et al., 2020).

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The serological examination revealed the detection of different serogroups including O142 K 86, O91 K -, O125 K 70, 0114 k 90, O44 k 74, O127 K 63, O1 K -, O166 K -, O158 K -, O144 K -, O103 K -, O86 K 64, O27 K -, O103 K -, O151 K -, O78 K -. O55 K 59 while 19 strains were untyped. Early studies on avian *E. coli* strains showed that O1, O2 O15, O35, and O78 serotypes, were mostly associated with colibacillosis outbreaks (Dziva and Stevens, 2008). A later study by Nolan et al. (2013) revealed the presence of O18, O81, O115, O116, and O132, serotypes, was a signal for the emergence of new pathogenic serotypes. Recently El-Sawah et al. (2018) showed that a wide antigenic diversity is existed among avian pathogenic *E. coli* strains in Egypt, and worldwide. Thus, the involvement of a particular O serotype in the infection process appeared to vary according to the geographical region. Some of the studies conducted in Egypt, nearly isolated the same serotypes with a predominance of the O78 serotype (Awawdeh et al., 2019; Ramadan et al., 2016).

In the present study, the highest rate of antibiotic resistance was shown against ampicillin and trimethoprim-sulfamethoxazole, while low levels of resistance were against cefazolin, meropenem, tobramycin, amikacin, and cefoxitin. These antibiotic resistivity patterns of *E. coli* strains were in agreement with other previous studies (Matin et al., 2017).

The resistance of APEC isolates to the cephalosporins, ceftazidime, ceftriaxone, and cefepime was the lowest resistance level among the tested panel of antimicrobials because they are not used in the poultry industry (Johar et al., 2021). Antimicrobials used in broiler chicken feed, water, and as growth promoters in suboptimal levels need to be monitored. The widespread usage of these antibiotic families in poultry is reflected in the significant levels of resistance that have been found (Ibrahim et al., 2019) The widespread use of these antibiotics for treatment and prevention of the disease without veterinary advice in Egypt is responsible for the high frequencies of antimicrobial resistance of *E. coli* isolates in broiler chickens (Ibrahim et al., 2022). The tremendous rise of multi-drug-resistant bacteria still poses a serious concern despite the effectiveness of modern antibacterial medications, necessitating the introduction of certain alternatives like nanoparticles, herbal extracts, and probiotics (Khalil et al., 2020; Ragab et al., 2020; Syed et al., 2020; Hassanen and Ragab., 2021; Prentza et al., 2022). Virulence genes accompanied by antimicrobial resistance are considered the main factors that increase the pathogenicity of bacteria and lead to an increase in infection severity leading to a therapeutic failure (Abd El-Baky et al., 2020). Although many techniques can be used to identify virulence factors (some phenotypic characters on chromogenic medium), PCR is still a powerful technique for detecting pathogens because of its rapidity, specificity, and sensitivity. It is an effective procedure for generating large quantities of a specific DNA sequence *in vitro* (Holland et al., 2000)

It was stated that the main virulence markers for APEC are *iro*N, *omp*T, *hly*F, iss, and *iut*A genes (Johnson et al., 2006). These genes were verified to be essentially present in APEC. In addition to that, it was suggested that the presence of two of these genes in an *E. coli* avian isolate could mean that this isolate is an APEC, and the absence or presence of a gene could reveal non-pathogenic *E. coli* (Schouler et al., 2012). The *iut*A gene encodes an outer membrane protein implicated in the high-affinity binding of Fe^{+3} aerobactin and can be plasmid-located (De Carli et al., 2015) or encoded on chromosomes in some *E. coli* strains (Unno et al., 2011).

The present study found that the prevalence rate of the *iut*A gene was 84.4% of the tested *E. coli* isolates. This finding is in accordance with a study that reported a high prevalence of about 80% (Eftekharian et al., 2016). However, this result disagrees with previous studies showing low levels of prevalences of 64% and 70% (Kwon et al., 2008; Subedi et al., 2018)

Adherence of bacteria to tissue surfaces is an important initial step in bacterial infections. In *E. coli*, P-fimbriae, which mediates bacterial colonization in the respiratory epithelium, is coded by the pyelonephritis-associated pili (*pap*C) gene. In addition to tissue adhesion, P-fimbriae protects *E. coli* from the antibacterial activity of neutrophils (Varga et al., 2018). The obtained results revealed that the prevalence rate of *pap*C gene in *E. coli* isolates was 54.8%. The same result was detected by Subed et al. (2018) with a rate of 55.6%, while lower rates were obtained by Kown et al. (2008), Varga et al. 2018), and Tidiane et al. (2019) who reported papC gene prevalence 11%, 10.27% and 12.9% in *E. coli* isolates, respectively. In the same line, a low prevalence rate was recorded by Oliveira et al. (2019), who detected papC gene in 1.5% of *E. coli* isolates.

Fimbrial adhesins are hypothesized to start host tissue colonization. The most typical type of fimbria found in APEC is type 1. According to Huja et al. (2015), fimbriae type 1 bind to mannose-containing glycoproteins on epithelial host cells. The fim cluster gene, which includes nine related genes (*A*, *B*, *C*, *D*, *E*, *F*, *G*, *H*, and *I*) necessary for its production, encodes type 1 fimbriae (Pusz et al., 2014). A chaperone protein that aids in the translocation of fimbria proteins through the periplasm is often produced by the type 1 fimbriae C (*fim*C) gene (Kostakioti et al., 2004). The present study found that 74% of the tested isolates were positive for *fim*C, which is comparable to the research done by Dou et al. (2016), who found that APEC isolates had a high prevalence of this gene, 95.88%.

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CONCLUSION

The spread of APEC is global, which emphasizes the importance of studying APEC from diverse geographical angles. The results of the present study revealed that *E. coli* is one of the main factors contributing to various disease conditions in chickens that create financial losses for the poultry business. Almost all isolated *E. coli* serotypes have been determined to be harmful to all broilers breed; however, no single illness condition or age group could be associated with a specific serotype. The present study revealed a significant prevalence of multidrug-resistant *E. coli* strains accompanied by a high frequency of virulence genes. Implementing an intervention program to reduce the risk of colibacillosis requires regular screening and monitoring of the virulence genes linked to the antibiotic-resistant APEC strains. Future studies must be established to monitor the expression of virulence genes and find suitable antibiotic alternatives.

DECLARATIONS

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Authors' contribution

Mona I. Elenbaawy, Eman Ragab, and Basma M. Hamed hypothesized the idea and conducted laboratory work. Both authors (Hossam Mahmoud and Eman Ragab) contributed to the drafting, editing, and production of the final draft. Eman Ragab is the corresponding author. All authors confirmed and consented to the final submission.

Competing interests

The authors declare that they have no competing interests.

Ethical consideration

All authors have confirmed ethical issues, including plagiarism, double publication and/or submission, and redundancy, data fabrication and/or falsification, consent to publish.

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