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Antimicrobial resistance and virulence factors in chicken-derived *E. coli* isolates

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ABSTRACT

A total of 180 samples were taken from diseased and freshly dead broiler chickens of various ages from various farms in El-Minya and Beni-Suef governorates and transferred to the lab for bacterial isolation and further molecular examination targeting *E. coli*. The results showed that 91 of 180 samples (50.6%) tested positive for *E. coli*. The prevalence of some in vitro virulence markers of avian pathogenic *E. coli* was Congo red binding (CRB) (95.6% positivity) and moderate to strong biofilm production (92%). On 5% sheep blood agar, (67%) of examined isolates showed alpha hemolysis, while (27%) showed gamma hemolysis and (5.5%) showed beta hemolysis. All the tested isolates exhibited a multidrug resistance (MDR) pattern. *E. coli* isolates demonstrated various degrees of resistance against, amoxicillin–sulbactam (82%) followed by streptomycin (76%), cefotaxime and trimethoprim/sulphamethoxazole (73%), tetracycline and ciprofloxacin (68%), chloramphenicol (66%), cefaclor (65%), cefixime (62%) respectively. Five MDR selected isolates were examined using PCR. The intended virulence genes were *iss*, *tsh*, *fimH*, and *iroN* genes. The genes *iss* and *fimH* were detected in all of the isolates investigated, whereas *iroN* was present in four isolates but *tsh* was found in only one isolate.

Introduction

Avian colibacillosis, caused by *Escherichia coli* strains known as avian pathogenic *E. coli* (APEC), is an acute and predominantly systemic disease that causes significant economic losses in the global poultry industry due to increased mortality, treatment costs, and carcass condemnation. APEC is an *E. coli* pathotype comprising uropathogenic, neonatal meningitis, and septicemic *E. coli* (Kobayashi *et al*., 2011). APEC is one of the most common infections in poultry, and it causes large losses in terms of mortality, productivity losses, and condemnations (Vandemaele *et al*., 2002). *E. coli* that causes systemic or localized infections outside of the avian gut is known as extra-intestinal pathogenic *E. coli* (EXPEC) which is also known as avian pathogenic *E. coli*. Colibacillosis, an infectious illness caused by EXPEC, affects broiler chickens aged 4-6 weeks causing acute lethal septicemia or sub-acute fibrinous pericarditis, airsacculitis, salpingitis, and peritonitis (Alexander and Senne, 2008).

The virulence of *E. coli* is influenced by a number of phenotypic factors, including hemolysis, serum resistance, Congo red binding (CRB), and toxin production (Osman *et al*., 2018). Hemolysin production has been noted as an emerging and significant virulence factor in APEC (Fatima *et al*., 2012). The APEC strains were characterized using the CRB assay as an epidemiological indicator of virulence (Amer *et al*., 2015; Zahid *et al*., 2016). The CRB is associated with the production of various antibiotic resistance genes as well as virulence genes like *iss* and *fimH* (Zahid *et al*., 2016). On biological or inanimate surfaces, biofilms are densely populated microbial populations that are encased in secreted polymers (Sawhney and Berry, 2009 and Romling and Balsalobre, 2012). Additionally, in order to evade immune clearance and live, *E. coli* forms biofilm, which leads to resistance to various therapies (Hoffman *et al*., 2005). Some *E. coli* infections are linked to biofilm formation, which makes it difficult to treat the infection because of its innate ability to withstand high doses of antibiotics (Chen *et al*., 2010).

Antibiotic therapy is one of the most effective methods for reducing avian colibacillosis incidence and subsequent mortalities. The prolonged use of antibiotics for the treatment and growth improvement in chickens led to the emergence of bacteria that are resistant to multiple antibiotics (Singer and Hofacre, 2006; Founou *et al*., 2016). Among these bacteria, *E. coli* strains pose a significant concern to public health because they can be transferred to humans through the food chain or through direct contact of poultry handlers with diseased birds. Furthermore, resistant *E. coli* may pass antibiotic resistance genes to other organisms (Akond *et al*., 2009). The most serious threat to the chicken industry related food safety is antimicrobial-resistance and the spread of multidrug resistant (MDR) bacteria. Bacterial antimicrobial resistance evolves over time; the astonishing rise in antimicrobial resistant organisms is linked to the extensive use of antimicrobial drugs in human and animal medicine for disease treatment and prevention (Levy, 2014). The regular use of antimicrobial drugs causes selection pressure, which results in antimicrobial resistance among APEC (Zakeri and Kashefi, 2012b).

The genotyping approaches enable more precise identification of APEC isolates as compared to normal serotyping protocols used in veterinary laboratories (Schouler *et al*., 2012). The PCR approach can detect the most highly pathogenic *E. coli* isolates in a flock (JanBen *et al*., 2001). Based on the fact that virulence varies not only between species but also between strains of the same species; numerous studies have been conducted to determine the virulence factors of isolated pathogenic *E. coli*

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strains. (Kaipainen *et al*., 2002; Zaki *et al*., 2004; Ewers *et al*., 2009). Despite the fact that *E. coli* infections have proved costly for the poultry industry, the precise virulence mechanisms used by these organisms to infect birds with disease remain a fascinating focus of research. The presence of many virulence genes has been discovered to be significantly related to the pathogenicity of APEC strains (Ewers *et al*. 2005). APEC has been linked to several virulence genes, including adhesin factors like *fimH*, which are involved in adhesion to the avian upper respiratory tract (Wooley *et al*., 2000). However, the pathophysiology and function of virulence factors in avian pathogenic strains are not fully known, making therapy more difficult. There are several methods for identifying different genomic segments of pathogenic *E. coli* (Barbieri *et al*., 2017). *tsh* gene exhibits haemagglutinating activity, but the *iroN* gene assists *E. coli* in surviving in their host aquatic habitat in APEC. (Khafagy *et al*., 2019). The *iss* gene contributes to increased serum survival in chickens, which has been identified as a crucial characteristic of virulent *E. coli* (Ellis *et al*., 1988). *Iss* and the protein encoded by *iss* were efficient targets for colibacillosis (Pfaff-McDonough *et al*., 2000; Ewers *et al*., 2005). Therefore, this study aimed to investigate antimicrobial resistance and virulence factors in chicken-derived *E. coli* isolates.

Materials and methods

Chicken samples

One hundred and eighty samples from broiler chickens of various ages were collected from farms in El-Minya and Beni-Suef provinces during the period from March to September 2021. Before being sent to the lab for *E. coli* recovery from internal organs and the gut, all samples were assigned serial numbers and detailed information about age, clinical signs and postmortem lesions.

Isolation and identification of suspected E. coli colonies (Quinn et al., 2002)

The collected samples were individually inoculated into tryptone soya broth (TSB) under aseptic conditions and incubated at 37°C for 18-24 hours. A loop of broth was streaked onto MacConkey agar medium and then incubated aerobically for 24 hours at 37°C. On eosin methylene blue (EMB) agar medium, one distinct colony was selected, and it was cultured aerobically for 24 hours at 37°C.

Microscopic examination

Gram's stain was prepared and used as described by Cruickshank *et al*. (1975) for morphological study.

Biochemical identification (Quinn et al. 2002)

Among the tests done are the Indole reaction, Methyl red test, Voges Proskauer test, Citrate utilization test, Catalase test, Sugar fermentation test, Oxidase test, Triple sugar *iroN* test, and Christener's urea agar test.

Detection of some in vitro phenotypic virulence properties of avian pathogenic E. coli isolates.

Congo red binding assay

It was performed according to Saha *et al*. (2020). Bacterial suspension was streaked onto the agar plates supplemented with 0.003% CR dye and 0.15% bile salt, and the plates were incubated at 37°C for 24 h. The strong Cong red binder isolates were visualized as deep brick red-colored colonies on the agar plates.

Biofilm formation on YESCA CR agar

It was performed according to Zhou *et al*. (2013). Pure colonies of *E. coli* isolates were streaked out onto LB agar plate, incubated at 37°C for 24 h. Single colonies were picked up and streaked out on a YESCA CR agar plate, incubated at 26°C for 48 h. Biofilm producing *E. coli* isolates stained red or dark red on YESCA CR agar, whereas non-producing isolates grown as pink or white colonies.

Haemolysin production

It was performed according to Siegfried *et al*. (1994). Plate hemolysis test was done by using 5% sheep blood agar to detect haemolysin produced by *E. coli*. Tested organism was inoculated on sheep blood agar and incubated over night at 35°C. Haemolysin production was detected by the presence of a zone of complete lysis of erythrocytes around the colony and clearing of the medium

Table 1. Oligonucleotide primers sequences.

Gene	Primer sequence (5'-3')	Length of amplified product	Length of amplified product	Reference
iss	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATACCC	266 bp	266 bp	Yaguchi *et al*. (2007)
fimH	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508 bp	508 bp	Ghanbarpour and Salehi (2010)
Tsh	GGT GGT GCA CTG GAG TGG AGT CCA GCG TGA TAG TGG	620 bp	620 bp	Delicato *et al*. (2003)
iroN	ATC CTC TGG TCG CTA ACT G CTG CAC TGG AAG AAC TGT TCT	847 bp	847 bp	Ewers *et al.* (2007)

Table 2. Thermal profile followed during PCR detection of E.coli virulence genes.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
Iss	94˚C 5 min.	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
fimH	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	35	72˚C 7 min.
Tsh	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
iroN	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	35	72˚C 10 min.

Antimicrobial susceptibility patterns of E. coli isolates

The disk diffusion technique and interpretation were carried out according to CLSI (2020). Sixteen types of antibiotics from various antibiotic classes were used; piperacillin/tazobactam (100/10 µg), tetracycline (30µg), streptomycin (10µg), ciprofloxacin (5µg), chloramphincol (30µg), cefaclor (30µg), amikacin (30µg), cefotaxime (30µg), ofloxacin (5µg), ampicillin/sulbactam (10/10µg), trimethoprim/sulphamethoxazole (1.25/23.75µg), doxycycline (30µg), gentamicin (10µg), cefixime (5µg), norfloxacin (10µg) and colistin sulphate (10µg).

E. coli virulence genes detection by PCR

Four sets of primers were used in conventional uniplex PCR to detect four virulence genes that could contribute to APEC pathogenicity: *iss* (increase serum survival), *tsh* (temperature sensitive hemagglutinin), *fimH* (Fimbrin D-mannose specific adhesin), and *iroN* (catecholate siderophore receptor).The primers used for the amplification are listed with their sequences and references in Table 1.

DNA extraction and PCR were applied on five *E. coli* strain following the instructions of the QIAamp DNA mini kit (Catalogue No. 51304) Primers were utilized in a 25 µL reaction tube containing 12.5 µL of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µL of each primer of 20 pmol concentrations, 6 µL of DNA template, and 4.5 µL of nuclease free water. The reactions were performed in an Applied Biosystem 2720 thermal cycler. Agar gel electrophoresis was then applied (Sambrook *et al*., 1989).

Data analysis

Data was analyzed using alpha Innotech and protein simple software, Biometra, Germany

Results

The biochemical testing of suspected E. coli

When grown on MacConkey agar media, *E. coli* isolates had pink colonies that were Gram-negative rods, and green metallic colonies on EMB medium. All of the putative *E. coli* isolates had positive indole, methyl red, and catalase biochemical tests, as well as lactose-fermenting colonies. Oxidase, urea hydrolysis, citrate utilization, Voges Proskauer, and H_2S generation were all negative in all samples. *E. coli* was suspected in 50.6% of the isolates.

Recovery of E. coli from internal organs

The overall isolation rate of *E. coli* was 50.6 % according to the total number of examined samples (91/180). Regarding to the site of isolation, avian pathogenic *E. coli* isolates were obtained from intestine, cloacal swabs, yolk sac, heart blood, liver, lung and spleen with prevalence of 56%, 44%, 80%, 50%, 55%, 40% and 30%; respectively. The result in Table 3 reveals the overall isolation rate of *E. coli* from different sites of diseased birds.

Some in-vitro virulence associated properties

CR-binding assay

87 out of 91 examined isolates (95.6%) were positive. Biofilm formation ability: 84 out of 91 examined isolates (92%) were able to form a moderate or strong biofilm.

Haemolysis

67% of examined isolates showed alpha hemolysis, while (27%) showed gamma hemolysis and (5.5%) showed beta hemolysis on 5% sheep blood agar.

Antimicrobial susceptibility patterns of avian pathogenic E. coli isolated from broiler chickens

All the examined isolates exhibited multidrug resistance (MDR). The resistance of *E. coli* isolates varied; amoxicillin–sulbactam (82%), streptomycin (76%), cefotaxime and trimethoprim/sulphamethoxazole (73%), tetracycline and ciprofloxacin (68%), chloramphenicol (66%), cefaclor (65%) and finally cefixime (62%).

Occurrence of targeted virulence genes among E. coli isolates

Five MDR selected isolates were examined using PCR. The intended virulence genes were *iss*, *tsh*, *fimH*, and *iroN* genes. *iss* and *fimH* genes were detected in all the studied isolates, while *iroN* was detected in four isolates but *tsh* gene was found in one isolate only.

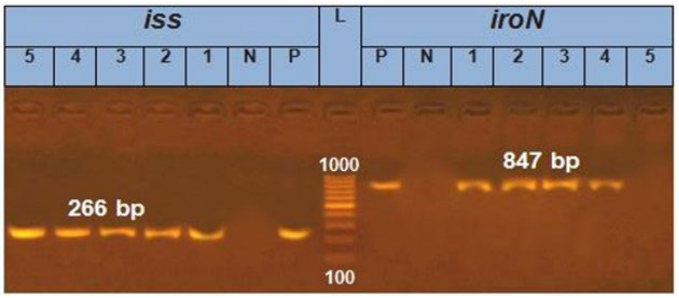

Fig. 1. Representative Agar gel electrophoresis showing positive amplification for *iss* gene at 266 bp and *iroN* gene at 847 bp of *E. coli* by using the specific primers.

Table 3. Prevalence of *E. coli* isolated from broiler chickens and their phenotypic markers of virulence.

Sample source	Number of samples	positive isolates No (%)	Phenotypic virulence markers				
			CR-binding	Biofilm formation	Alpha hemolysis	Beta hemolysis	Gamma hemolysis
Intestine	50	28 (56%)	26	25	13	2	13
Cloacal swabs	50	22 (44%)	20	18	12	1	9
Yolk sac	10	8 (80%)	8	8	7	0	1
Heart blood	30	15 (50%)	15	15	12	2	1
Liver	20	11 (55%)	11	11	10	0	1
Lung	10	4 (40%)	4	4	4	0	0
Spleen	10	3 (30%)	3	3	3	0	0
Total	180	91 (50.6%)	87	84	61	5	25

Table 4. Antimicrobial susceptibility patterns of members of avian pathogenic *E. coli* recovered from broiler chickens.

Chemotherapeutic disks	Disk content (µg)	APAC						Total
		R		I		S		
		NO.	%	NO.	%	NO.	%	
Amoxicillin–Sulbactam (SAM)	10/10	75	82	11	12	5	5	91
Piperacillin-Tazobactam (TZP)	100/10	9	9.8	36	40	46	51	
Amikacin (AK)	30	7	8	16	18	68	75	
Gentamycin (CN)	10	20	22	19	21	52	57	
Streptomycin(S)	10	69	76	8	9	14	15	
Cefaclor (CEC)	30	59	65	10	11	22	24	
Cefixime (CFM)	5	56	62	7	8	28	31	
Cefotaxime (CTX)	30	66	73	16	18	9	10	
Doxycycline (DO)	30	26	29	17	19	48	53	
Tetracycline (TE)	30	62	68	11	12	18	20	
Chloramphenicol(C)	30	60	66	3	3	28	31	
Colistin sulphate (Cl)	10	30	33	27	30	34	37	
Ciprofloxacin (CIP)	5	62	68	9	10	20	22	
Norfloxacin (NX)	10	31	34	14	15	47	52	
Ofloxacin (OFX)	5	35	38	8	9	48	53	
Trimethoprim/Sulphamethoxazole (SXT)	1.25/23.75	66	73	3	3	22	24	

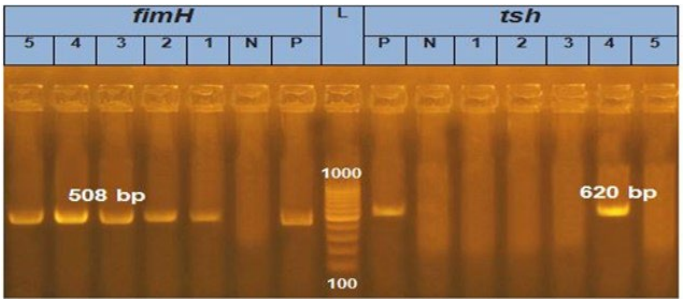

Fig. 2. Representative Agar gel electrophoresis showing positive amplification for *fimH* gene at 508 bp and *tsh* gene at 620 bp of *E. coli* by using the specific primers.

Discussion

Even though *E. coli* is a component of the healthy microbiota, in chicken intestines, some strains, such as avian pathogenic *E. coli* (APEC), can spread to various internal organs and cause colibacillosis, a fatal disease with a recurring pattern (Someya *et al*., 2007). Extra intestinal pathogenic *E. coli* (EPEC) strains, which include avian pathogenic *E. coli* (APEC) strains, are differentiated by the presence of virulence traits that allow them to spread extra intestinally (systemically) (Johnson *et al*., 2006). To type isolated bacteria, such as *E. coli*, phenotypic and/or genotypic approaches could be utilized. Two ways of identifying *E. coli* using phenotypic characteristics are the morphological and biochemical tests. Gram-negative rods with pink colonies when grown on MacConkey agar media and green metallic colonies when grown on EMB medium are used to identify *E. coli* isolates. The results were fairly comparable as noticed by Kumar *et al*. (1996) and Hogan and Larry (2003).

One hundred and eighty, organ samples from diseased and freshly dead broiler chickens were obtained at random from farms in El-Minya and Beni-Suef governorates, including the intestine, cloacal swabs, heart blood, liver, yolk sac, lung, and spleen. APEC isolates were recovered from the intestine, cloacal swabs, yolk sac, heart blood, liver, lung, and spleen with a prevalence of 56%, 44%, 80%, 50%, 55%, 40%, and 30%; respectively, with an overall isolation rate of 50.6% (91 out of 180). These findings were comparable to those obtained by Awad *et al*., (2020) who discovered that 51.85% (28/54) of the broiler chick flocks tested positive for *E. coli*. While Radwan *et al*., (2016) (56%) and Akond *et al*., (2009) (58%). Interestingly, Eid *et al*. (2016) discovered greater rates of *E. coli* isolation in chicken (60%). Far higher prevalences, such as those reported by AbdElatif (2004); 78.7%, El-Sukhon *et al*. (2002); 88.2% and Abd El-Aziz *et al*. (2007); 90%, were recorded, however, Abd El Tawab *et al*. (2014) reported a prevalence of 24.7% in diseased chickens and El-Seedy *et al*. (2019) discovered that prevalence rate was 23%. Ahmed *et al*. (2017) reported much lower percentage of prevalence as the number of the positive samples with *E. coli* isolation was 50 out of 600 isolates (8.3%). These variations in the *E. coli* prevalence in chicken may be attributed to the difference in strains pathogenicity and virulence beside the severity of the cases as well as the immunological status of the host (Heba *et al*., 2012).

According to the findings in Table 3, the bacteriological examination revealed that higher rates of *E. coli* isolation was from yolk sac (80%), followed by the intestine (56%), liver (55%), heart blood (50%), cloacal swaps (44%), lung (40%), and finally spleen (30%). Nearly the same results were obtained by Tapan *et al*. (2012) isolated *E. coli* from different farms and found the highest isolation rate from yolk sac (52.6%) and heart blood (38.4) in one day old -4week,and the highest percentage of *E. coli* isolation was from pericardial fluid (35.8%) followed by heart blood (33.4%) in older age (4-7 week). Akond *et al*. (2009) reported other results, while isolated the greatest percentage from cloacal swabs and intestinal fluid (66% and 54%) respectively. Also, Younis *et al*. (2017) revealed the recovery rate of *E. coli* from different chicken samples was 28.76%, 27.39%, 23.28%, 15.06%, and 5.46% form lungs, spleen, heart, liver, and intestinal contents, respectively.

The phenotypic in-vitro pathogenicity of all recovered isolates (91 isolates) was assessed using the Congo Red Binding Assay, Assessment of biofilm forming capacity, and hemolysis assay. Curli are proteinaceous amyloid fibres produced on the cell surfaces of many pathogenic bacteria, including *E. coli*, that mediate bacterial cell–cell contacts, host–pathogen interactions, and adhesion to biotic and abiotic surfaces (Zogaj *et al*., 2003; Kikuchi *et al*., 2005). Curli and cellulose produce extracellular matrixes that promote bacterial resilience to environmental stressors and antimicrobial treatments (Uhlich *et al*., 2006). As amyloid, curli fibers are protease resistant and bind to CR and other amyloid dyes. In this study CR-binding assay indicated that 87/91 (95.6%) *E. coli* isolates were positive. Previous reports resulted by Amer *et al*. (2015) and Saha *et al*. (2020) investigated (28.6% and 81.7%) of APEC strains were CR positive; respectively. Also, Khafagy *et al*. (2019) in their study confirmed that (80%) of isolates from fecal swabs were Congo red positive. These variations in prevalence of *E. coli* ability to absorb (CR) dye could be attributed to differences in strain pathogenicity and virulence, according to Berkhoff and Vinal (1986) demonstrated a substantial link between virulence in avian *E. coli* and expression of CR.

In this work, an experimental in vitro methods described by Zhou *et al*., (2013) was followed to distinguish the biogenesis and assembly of curli in *E. coli* isolates by exploiting their amyloid properties and to detect curli-mediated biofilm formation. The result revealed that 84 out of 91 (92%) of the isolates were able to generate a biofilm (Table 2). Skyberg *et al*. (2007) reported the abilities of 10^3 AFEC (75.7%) and 105 APEC (55.2%) to form a moderate or strong biofilm. Nascimento *et al*. (2014) informed the biofilm formation ability of 37 (29%) atypical enteropathogenic *E. coli* strains.

Haemolysis of erythrocytes is considered an important virulence factor for some strains of *E. coli* to overcome host defense mechanism through haemolysis production, which leads to the release of *iroN* into the bacterial environment and cytotoxic effect on neutrophils (Cavalieri *et al*., 1984). Previous studies have demonstrated *E. coli*'s strong hemolysin

production capacity from different sources. (Sharma *et al*., 2007; Fakruddin *et al*., 2013). *E. coli* strains mostly produce α-haemolysin (Shetty *et al*., 2014; Bhrugubalda *et al*., 2016), in this study 67% of isolates showed α-haemolysis on sheep blood agar 5%. While Shetty *et al*., (2014) found that only 28.66% of extra-intestinal and 15% of intestinal commensal *E. coli* isolates are able to produce hemolysin on 5% sheep blood. Haemolytic activity is often associated with the pathogenicity of the *E. coli* strains which possibly contributes to their virulence (Elliott *et al*., 1998).

Antimicrobial therapy is one of the key controls for reducing both the incidence and mortality associated with avian colibacillosis, thereby minimizing the poultry industry's massive losses (Blanco *et al*., 1997). Control of APEC infections in the poultry sector has become difficult due to the advent of multidrug resistant *E. coli* strains and the transmission of resistance genes (Subedi *et al*., 2018),. This is caused by the incorrect use of antimicrobial drugs, which resulted in the formation of resistant bacteria, which eventually work as a source of resistance genes for pathogenic species (Founou *et al*., 2016). In the present work, all recovered isolates (n=91) were subjected to in-vitro antimicrobial sensitivity testing against 16 different antimicrobial medications in order to discover MDR isolates for further analysis. The antibiogram results of isolates revealed high resistance to the majority of antimicrobials used, particularly amoxicillin-sulbactam (82%), streptomycin (76%), cefotaxime (73%), trimethoprim/ sulphamethoxazole (73%), tetracycline (68%), ciprofloxacin (68%), chloramphenicol (66%), cefaclor (65%), and cefixime (62%). These findings are consistent with prior research (Sharada *et al*. (2001), Schroeder *et al*., (2002), Radwan *et al*., (2014). Jesus *et al*. (1997) found a higher degree of tetracycline resistance (94%) than we did, while Adam *et al*. (2021) found a lower level of resistance (20%).

Furthermore, Radwan *et al*., (2021) found that *E. coli* was resistant to amoxicillin (97%), ciprofloxacin (92.5%), streptomycin (90%), and sulphamethoxazole-trimethoprim (77.5%). Amer *et al*. (2018) recorded nearly the same resistance to chloramphenicol (65%). In the present study multidrug resistance was detected in all isolates (100%) this was in accordance with Radwan *et al*., (2021) observation that 100% of the isolates tested positive for MDR. These findings were consistent with previous reports from Egypt and other nations. Within Egypt, Amer *et al*. (2018) and Qurani (2019) recorded that all isolates were MDR. In the meantime, Radwan *et al*. (2014) discovered 90.4% of the isolates were MDR. In addition, Chandran *et al*. (2008) showed that all *E. coli* isolates were MDR in India, Bashar *et al*. (2011) in Bangladesh, Saidi *et al*. (2012) in Zimbabwe, Zakeri and Kashefi (2012a) in Iran, Messai *et al*. (2013) in Algeria, and Jahantigh and Dizaji (2015) in Pakistan. Lower levels of MDR recorded all over the world by Dou *et al*. (2016) in China; 80.3%, Rahimi (2013) in Iran, 63.3%, Rahman *et al*. (2017) in Bangladesh, 76%, Aggad *et al*. (2010) in Algeria, 72% . Furthermore, as previously shown by Yang *et al*. (2004), Zhao *et al*. (2005), and Ozawa *et al*. (2008), approximately (54.6%) displayed resistance to four or more antibiotics. Furthermore, Chen and Wang (1997) and Hammoudi and Aggad (2008) discovered high levels of antibacterial drug resistance in pathogenic strains of *E. coli* recovered from hens, indicating that multiple drug resistance was frequent. For the past few years, the selection pressure exerted on avian *E. coli* by antimicrobials present in broiler feed has been related to the spread of multidrug resistance in this species of *E. coli* (Singer and Hofacre, 2006). Also, Sharada *et al*. (2001) discovered that no one antimicrobial treatment was 100% effective against *E. coli* isolates, which could be attributable to the development of resistance due to indiscriminate antibiotic use. The data from additional regional research (Ahmed *et al*., 2013) as well as international studies (Zhao *et al*., 2005; Jiang *et al*., 2011; Saidi *et al*., 2013) are comparable to the current resistance phenotypes with MDR. Antibiotic-resistant *E. coli* strains isolated from chickens have been found to be increasingly resistant to numerous antibiotics commonly used in the poultry industry. On the contrary, sensitivity to Amikacin (75%) was seen; this finding was consistent with Radwan *et al*., (2016), who showed higher sensitivity to Amikacin (97.6%). This high sensitivity against Amikacin was due to it less frequently applied in case of APEC infection due to its nephrotoxicity. The APEC revealed moderate sensitivity to gentamicin (57%), doxycycline (53%), ofloxacin (53%) and norfloxacin (52%) as Chansiripornchai *et al*., (2011) revealed nearly the same sensitivity to doxycycline (52%) but higher sensitivity to gentamicin and norfloxacin (64%) and (70%) respectively than our result, while Langata *et al*.,(2019) observed higher sensitivity to gentamicin (98%). The unusual usage of gentamicin and norfloxacin in the chicken sector may be the cause of this high sensitivity. The results of this study's antibiotic susceptibility testing are inconsistent with certain studies but consistent with others, demonstrating that antibiotic susceptibility patterns vary with various isolates, time, and the development of multiple drug resistance *E. coli* (Eid and Erfan, 2013).

Alternative virulence genes may participate in the pathogenicity mechanism of avian pathogenic *E. coli* at various stages of infection, including colonization (*fimH*, f*imC, papC, papEF*, and *tsh*), invasion (*ibeA*, *vat*), *iroN* acquisition (*iutA, iroN, IreA, feoB*), serum complement resistance (*iss*), and putative *iroN* transport (*sitA*) (Awawdeh, 2022). The PCR results in this investigation revealed that all of the tested isolates (n=5) carried *fimH* and *iss* (100% for each), as well as *iroN* (80%) and *tsh* (20%) genes (Figures 1 and 2). As a result, the current findings extend and corroborate the involvement of many putative virulence genes in the pathogenesis of avian pathogenic *E. coli*. However, the frequency with which some of those genes were found in various reports vary significantly. The obtained result in this study of detection of *fimH* gene in 100 % of the isolates were reinforced by previous studies conducted by Lopez *et al*., (2017) who found Similar prevalence of *fimH* gene in Mexico (95%) and Ibrahim *et al*., (2019) detected *fimH* in (92.7%) of (APEC) isolates. Nonetheless Mbanga and Nyararai (2015) reported a low prevalence (33.3%) of the *fimH* gene in *E. coli* isolates from collibacillosis cases.

In 1979, the increased serum survival gene (*iss*) was discovered. It is coupled with a ColV plasmid and has a function in complement resistance. The *iss* represents a lipoprotein revealed on the outer membranes of *Escherichia coli* and is encoded by the *iss* gene. Usually it is present on avian pathogenic *E. coli* and more rarely encountered in commensal strains (Nolan *et al*., 2013). In the current study; the prevalence of the *iss* gene in *E. coli* isolates was 100%, which had a vital role in *E. coli* pathogenicity and could be a potential target for developing novel therapeutics and prevention strategies. Kwon *et al*. (2008) also detected 100% prevalence of the *iss* gene in layers in Korea, in Japan (100% in layers) and Germany (82.7% in layers and broilers) (Ewers *et al*., 2004; Someya *et al*., 2007). A lower prevalence was found in Brazil (51.4% in broilers, breeders and turkeys) by De Carli *et al*. (2015). Van der Westhuizen and Bragg (2012) stated that 31.4% of the *iss* gene among APEC isolates from poultry with colibacillosis in South Africa and Zimbabwe.

Other adhesins were found, including temperature-sensitive hemagglutinin (*tsh*), an autotransporter that leads to the formation of lesions and fibrin buildup in avian air sacs. It agglutinates erythrocytes, cleaves casein, and has mucolytic action. The *tsh* gene was a major APEC virulence marker with a substantial correlation with internal organ colonization, septicemia, and fatality in one-day-old hens (Ngeleka *et al*., 2002), which made it a useful target for pathotyping of APEC. The current study reported a low prevalence of detection of *tsh* gene which was detected in only in 20 % of the tested isolates. Nearly identical result obtained by Delicato *et al*. (2003) detected *tsh* gene in APEC isolates and fecal isolates with prevalence (39.5% and 4%) respectively. Conversely, a high incidence was recorded by Kwon *et al*. (2008); Subedi *et al*. (2018) and Ibrahim *et al*. (2019) they recorded the frequency of *tsh* virulence gene in avian pathogenic *E. coli* as (94%, 62.2% and 46.5%); respectively.

The prevalence of the *iroN* gene in *E. coli* isolates was 80% in the current investigation, which was close to the percentages reported by Radwan *et al*. (2014) and other regional and worldwide studies (Rodriguez-Siek *et al*., 2005; Johnson *et al*., 2008; Kobayashi *et al*., 2011; Ahmed *et al*., 2013; Hussein *et al*., 2013).

Conclusion

The current study demonstrates conclusively that *E. coli* is a key pathogen responsible for a variety of illnesses in chickens, resulting in economic losses for the poultry industry. Nearly every isolated strain of *E. coli* has been identified as pathogenic. The APEC examined in this study possesses a high rate of antibiotic resistance and genes linked to pathogenesis. Antibiotic overuse has led to the emergence of multidrug-resistant bacteria, which is viewed as a significant problem.

Conflict of interest

The authors declare that they have no conflict of interest.

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