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RESEARCH ARTICLE

Molecular Characterization of *Escherichia coli* Strains Causing Respiratory Signs in Broiler Chickens in Egypt

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Abstract

Colibacillosis is a complicated disease causing severe economic losses and challenging veterinarians and producers. Therefore, this study aimed to characterize avian pathogenic *Escherichia coli* (APEC) strains causing respiratory signs in chickens. Thirty broiler chicken flocks at age of 17-35 days showed respiratory signs and greenish diarrhea during 2013-2016 outbreaks that occurred in Sharkia, Ismailia, Dakahlia and Sinai Governorates. The postmortem findings revealed typical colisepticemia picture including air sacculitis, fibrinous pericarditis and perihepatitis. The percentage of APEC isolation was 100%. Mixed bacterial infections with *Enterobacter aerogenes* or *Providencia rettgeri* (3 flocks, each), *Klebsiella pneumoniae* (2 flocks), *Serratia liquefaciens* or *Enterobacter agglomerans* (1 flock, each) was evidenced. From 284 collected samples (air sacs, heart blood, lungs and liver), *E. coli* was predominantly isolated from air sacs (76.1 %) and lung (73.2 %) followed by heart blood (67.6%) and liver (54.9%). Based on serogrouping, the most common serogroups were O78 and O2 with percentages of 15%, each. Utilizing antimicrobial disc diffusion test, the isolates showed 32.7% resistance to doxycycline and 100% resistance to lincomycin, spiramycin, oxacillin and amoxicillin. Polymerase chain reaction (PCR) analysis for 55 MDR *E. coli* isolates from air sac and heart blood revealed 3 β-lactamase resistance genes; *bla*TEM (87.3 %), *bla*CTX-M (85.5 %) and *bla*OXA (5.5 %) and 6 virulence genes in two multiplex PCR; *iuc*D (96.4%), *Fim* H (92.7%), *iss* (76.4%), *omp*T (58.2%), *tsh* (45.5%) and *cva*C (9.1%). An association of virulence with multidrug resistance genes in *E. coli* was recorded, that hindered the control measures. Therefore, alternative strategies were necessary to minimize the antibiotic use and reduce the virulent strains' occurrence.

Keywords: *Escherichia coli*, Virulence, β-lactamase, Resistance, Broilers

Introduction

Avian colibacillosis is considered the most common bacterial disease influencing poultry production at all ages. It is caused by avian pathogenic *Escherichia coli* (APEC) [1], which are Gram-negative facultative anaerobic bacilli [2]. This disease causes economic losses due to high mortality, delayed growth and condemnation at slaughter houses. In contrast to mammals, it is not only enteric infection but also is mostly associated with extra intestinal infections, principally of the respiratory tract or systemic infections as perihepatitis, pericarditis, peritonitis, omphalitis, cellulitis, air sacculitis, panophthalmitis and peritonitis [1, 3]. Some APEC strains are considered latent zoonotic agents as a positive relation was reported between APEC and extra intestinal pathogenic *E. coli* (ExPEC) infection in human, newborn meningitis-causing *E. coli* (NMEC) and uropathogenic *E. coli* (UPEC) [4]. Virulence factors contributing the bacterial pathogenicity include bacterial toxins, cell surface proteins for adhesion, cell surface carbohydrates and proteins for protection from bactericidal host factors, hydrolytic enzymes [5], iron

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acquisition system and hemolysis [6]**.** Six virulence plasmid mediated genes (*tsh, iss, iuc*D, *cva*C, *fim* H *and omp*T) are among the most genes associated with APEC strains, distinguishing them from avian fecal *E. coli* [4]. In Egypt, different serogroups of APEC were isolated from chicken flocks for example O1, O2, O26, O44, O78, O124, O145 and O158 [7-11] as well as non typeable strains [9].

ß-lactamase production confers resistance to most β-lactams [12], especially in isolates from intensive broiler productions [13]. Ampicillin and amoxicillin are the most commonly used β-lactamse [14]**.** Extended-spectrum cephalosporins, as ceftiofur and cefquinome are approved in China for animal use [15]. TEM, *tet*, *dhfr1,* Sul-*1,* CTX-M, *cat2* and *flo-R* were APEC resistance genes against amoxicillin-clavulanic acid, tetracycline, trimethoprim, sulphonamide, cephotaxime, chloramphenicol and florphenicol in chickens respectively [16]. The aim of this study is to characterize APEC strains causing respiratory troubles in chickens in different localities in Egypt..

Materials and Methods

Sampling, bacterial isolation and identification

A total of 90 live diseased broiler chickens (Sasso, Cobb and Ross) at age of 17-35 days were collected from 30 broiler flocks at different localities (Sharkia, Ismailia, Dakahlia and Sinai Governorates) in Egypt during 2013 to 2016. The selected flocks have a history of respiratory manifestations and PM lesions (pericarditis, perihepatitis and air saculitis). The chicken flocks were reared in open poultry houses of variable stocking densities (1200-12000 birds / house). Based on the presence of PM lesions of suspected *E. coli* infection, a total of 284 samples were collected under aseptic conditions from the diseased birds including air sacs, heart blood, lungs and liver (71/each). A loopful of each sample was inoculated into buffered peptone water and then incubated for 24h at 37°C, followed by culturing onto MacConkey agar media (Oxoid) for 24h at 37°C. Pink colonies were then cultured on Eosine Methylene Blue (EMB) agar (Oxoid) and incubated at 37°C for 24h **[**17]. Biochemical characterization of E. coli isolates was performed using IMViC test (Indole, Methyl red, Voges-Proskauer and citrate utilization), urease and triple sugar iron agar test (TSI) test [18].

Serotyping of E. coli isolates

Serogrouping of 20 representative *E. coli* isolates from heart blood of diseased chickens from different flocks by slide agglutination test was performed using *E. coli* polyvalent and monovalent antisera (Denka Seikenco., Japan) at the serology unit, Faculty of Veterinary Medicine, Benha University, Egypt [19].

Disc diffusion antibiotic sensitivity test

The test was carried out as previously described [20]. In brief, from each *E. coli* isolate, a standardized suspension (adjusted to 0.5 McFarland standard) was evenly streaked on Mueller Hinton agar plate (CM0337, Oxoid, UK). The plates were left to dry at room temperature for 5-15 min. The antibiotic discs (Oxoid, UK) (amoxicillin/clavulanic acid (20/10 µg), amoxicillin (25 µg), oxacillin (1 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cephalexin (30 µg), doxycycline (30 µg), gentamicin (10 µg), enerofloxacin (30 µg), lincomycin (15 µg), spectinomycin (100 µg) spiramycin (100 µg) and ciprofloxacin (5 µg) were then distributed evenly and firmly pressed. After 24h/37°c incubation, the inhibition zone diameter for each antimicrobial was measured and interpreted [21].

PCR detection of virulence and antibiotic resistance genes of E. coli isolates

Bacterial DNA was extracted from 55 *E. coli* isolates (29 from air sac and 26 from heart blood) using boiled lysates at 95°C for 10 min in heat block and then centrifuged at 4°C for 10 min. The DNA concentration in the supernatant was measured using spectrophotometer and then stored at −20°C till use [22].

The PCR was performed using Biometra thermal cycler, UK. A uniplex PCR was used for each of the resistance genes (*blaCTX-M*, *blaTEM* and *bla OXA*). Two multiplex PCR were used for virulence genes; one for *cvaC*, *ompT* and *iss* genes and the other one for *fimH*, *iucD* and *tsh* genes. A total reaction volume of 30 µl consisted of 12.5 µl of iNtRON's PCR

master mix (Cat. No. 25027), 1 µl of each (20 pmol) primer, 3 µl of template DNA and 8.5 µl of nuclease free water was used in multiplex PCR. However, a 25 µl reaction volume (12.5 µl of iNtRON's PCR master mix, 1 µl of each (20 pmol) primer, 3 µl of template DNA and 7.5 µl of nuclease free water) was used in uniplex PCR. DNA isolated from reference *E. coli* strain DH5 α and *E. coli* 15 (kindly supplied by Dr. Lisa Nolan, bacterial pathogenesis laboratory, College of Veterinary Medicine, ISU, USA) were used as negative and positive control, respectively. The primer sequences, thermal cycling conditions as well as the amplified products' size for each PCR assay used were shown in Table 1.

Agarose gel electrophoreses

Ten microliters of each amplicon were loaded in 1.5 % agarose (iNtRON) and allowed to run 2/3 of the gel length before terminating the run. Specific amplicons were photographed under UV transilluminator (Bio-Rad) [22].

Results

Clinical and PM findings of examined birds

The clinical examination of investigated 30 broiler chicken flocks revealed sneezing, coughing, nasal discharge, greenish diarrhea, anorexia, depression as well as variable mortalities. On PM examination; picture of colisepticemia including fibrinous perihepatitis, fibrinous pericarditis and fibrinous airsaculitis, catarrhal enteritis with greenish content and congested lung were recorded.

Isolation rate of different pathogens

E. coli was isolated from all examined flocks (n=30) with variation among different organs. One hundred and ninety three *E. coli* isolates were recovered from 284 samples with percentage of 67.96%. Concurrent infections other than *E. coli* were recorded including *Enterobacter aerogenes* (3 flocks), *Providencia rettgeri* (3 flocks), *Klebsiella pneumoniae* (2 flocks), *Serratia liquefaciens* (1 flock) and *Enterobacter agglomerans* (1 flock).

Prevalence of E. coli in different organs

E. coli was isolated from different organs with higher recovery from air sac 54/71 (76.1 %), followed by lung 52/71 (73.2 %), heart blood 48/71 (67.6 %) and liver 39/71 (54.9 %).

Serotyping of E. coli isolates

Serogrouping of the representative 20 *E. coli* isolates from different diseased flocks showed twelve different serotypes. The most common serotype were O78 and O2 with percentage of 15% for each one, O44, O128 and O158 (10% each), in addition to O145, O124, O113, O163, O121, O26, O1 and a non typeable isolate (5%).

Antimicrobial susceptibility of E. coli isolates

All *E. coli* (n= 55) isolates showed complete resistance to lincomycin, spiramycin, amoxicillin and oxacillin (100%). Followed by ciprofloxacin (90%), enrofluxacin (87.2%), amoxicillin/ clavulinic (83.6%), cephalexin (81.8%) while the least resistance rate was detected to doxycycline (32.7%), followed by gentamicin (36.3%), ceftriaxone (43.6%) and cephotaxime (50.9%).

Multidrug resistance was detected in 10 (18.2%), 9 (16.4%), 7 (12.7%), 5 (9.1%), 3 (5.5%) and 1 (1.8%) of *E. coli* isolates to (11 and 12, each), 8, (9 and 10, each), 13 drugs, (6 and 7, each) and 5 antibiotics (Table 2).

Prevalence of cvaC, ompT, iss, fimH, iucD and *tsh virulence genes among E. coli isolates*

A multiplex PCR for detection of *E. coli* virulence genes succeeded in amplification of *cvaC*, *ompT* and *iss* genes (Figure 1.a) and *fimH*, *iucD* and *tsh* (Figure 1.b). *iucD* gene was the most prevalent virulence gene (96.4%), followed by *fimH* (92.7%), *iss* (76.4%), *ompT* (58.2%) and *tsh* (45.5%) while *cvaC* gene was the least one (9.1%).

Table 1: Oligonucleotide primer sequences and thermal cycling conditions for virulence and antibiotic resistance genes of *E. coli* isolates of broiler chickens

Target gene	Function	Primers (5-3)	Type of PCR	Initial denaturation °c/min	Actual cycles temp / time (seconds) 35-40 cycles			Final Extension °c/min	Size of amplified products	References
					Denaturation	Annealing	Extension			
*bla*TEM	Amoxicillin-clavulanic acid resistance	F: ATAAAATTCTTGAAGACGAAA R: GACAGTTACCAATGCTTAATC	Uniplex pcr	94/4	94/30	45/45	72/80	72/7	1080	[23]
*bla*OXA	Oxacillin resistance	F:TCAACTTTCAAGATCGCA R:GTGTGTTTAGAATGGTGA	Uniplex pcr	94/4	94/30	45/30	72/80	72/7	591	[24]
*bla*CTX-M	Cefotaxime resistance	F:CGCTTTGCGATGTGCAG R:ACCGCGATATCGTTGGT	Uniplex pcr	94/4	94/30	45/30	72/80	72/7	550	[25]
Tsh	Temperature - sensitive hemagglutinin gene	F:GGGAAATGACCTGAATGCTGG R:CCGCTCATCAGTCAGTACCAC		95/4	94/30	56/30	68/180	72/10	400	[26]
fimH	D-mannose – specific adhesion of type 1 fimbriae	F:TGCAGAACGGATAAGCCGTGG R: GCAGTCACCTGCCCTCCGGTA	Multiplex PCR	95/4	94/30	56/30	68/180	72/10	508	[27]
iucD	Aerobactin system involved in iron uptake and transport	F: TACCGGATTGTCATATGCAGACCGT R: AATATCTTCCTCCAGTCCGGAGAAG		95/4	94/30	56/30	68/180	72/10	602	[28]
cvaC	Structural gene of ColV operon	F- CACACACAAACGGGAGCTGTT R- CTTCCGCAGCATAGTTCCAT		95/4	94/30	63/30	68/180	72/10	679	[29]
ompT	Episomal outer membrane protease gene	F- TCATCCCGGAAGCCTCCCTCACTACTAT R- TAGCGTTTGCTGCACTGGCTTCTGATAC	Multiplex PCR	95/4	94/30	63/30	68/160	72/10	496	[30]
Iss	Increased serum survival gene	F: CAGCAACCCGAACCACTTGATG R: AGCATTGCCAGAGCGGCAGAA		95/4	94/30	63/30	68/160	72/10	323	[30]

Table 2: Cumulative data showing positive samples, serogrouping, virulence and antibiotic resistance genes among APEC isolates from different organs of broiler chickens.

Type of samples	No. of positive samples (%)	Serotyping			Molecular identification									
					No. of isolates subjected to Molecular identification	No. of isolates positive for virulence genes						No. of isolates positive for resistance genes		
		No. of sero-grouped isolates	Identified serotypes	No. (%) [a]		*cvaC*	*ompT*	*iss*	*iucD*	*fimH*	*tsh*	*bla*CTX-M	*bla*TEM	*bla*OXA
Air sac (n=71)	54	-	-	-	29	2	16	21	27	26	12	22	23	1
Heart blood (n=71)	48	20	O78 O2:H6 O44 O128 O158 O145 O124 O113 O163 O121 O26 O1 Non typeable *E.coli*	3 (15%) 3 (15%) 2 (10%) 2 (10%) 2 (10%) 1 (5%) 1 (5%) 1 (5%) 1 (5%) 1 (5%) 1 (5%) 1 (5%) 1 (5%)	26	3	16	21	26	25	13	25	25	2
Lung (n=71)	52	-	-	-	-	-	-	-	-	-	-	-	-	-
Liver (n=71)	39	-	-	-	-	-	-	-	-	-	-	-	-	-
Total (n=284)	193	20	-	-	55[b]	5 (9.1%)	32 (58.2%)	42 (76.4%)	53 (96.4%)	51 (92.7%)	25 (45.5%)	47 (85.5%)	48 (87.3 %)	3 (5.5%)

[a] The percentage is calculated in relation to the total number of isolates subjected to seropgrouping (n = 20).

[b] Multidrug resistance was detected in 10 (18.2%), 9 (16.4%), 7 (12.7%), 5 (9.1%), 3 (5.5%) and 1 (1.8%) of *E. coli* isolates to (11 and 12, each), 8, (9 and 10, each), 13 drugs, (6 and 7, each) and 5 antibiotics.

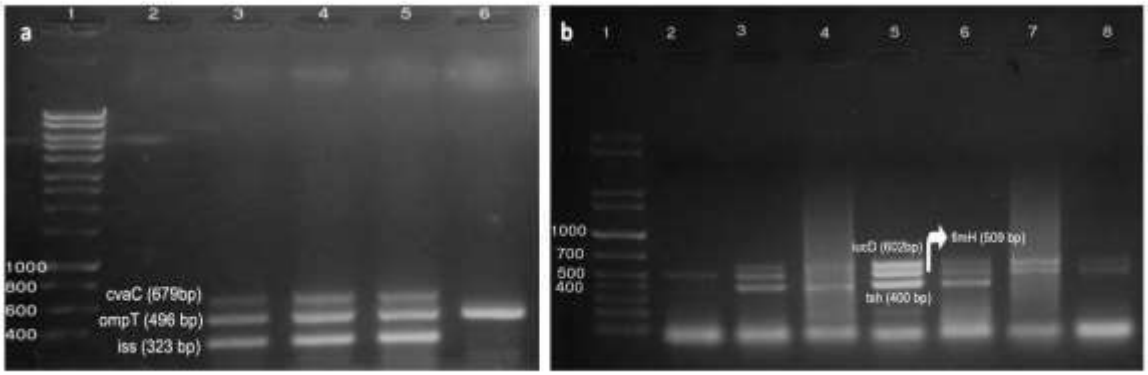

Figure 1: Agarose gel electrophoresis for amplicons produced by multiplex PCR amplification for *E. coli* virulence genes; (a): *cvaC* (679bp), *ompT* (496 bp) and *iss* (323 bp) and (b): *iucD* (602bp), *fimH* (509 bp) and *tsh* (400 bp) genes of broiler chickens. 1.a. lane1, 1kb DNA ladder; lane 2, negative control (Dh5α) showing no specific bands; lanes 3-4, APEC strains positive for *cvaC*, *ompT* and *iss* genes; lane 5, positive control and lane 6, APEC strain positive for *ompT* gene only. 1.b. lane1, 1kb plus DNA ladder; lane2, APEC strain positive for *fim H* gene; lane3, strain positive for *fimH, iucD* and *tsh* genes; lane 4, APEC strain positive for *iucD* and *tsh* genes; lane 5, positive control; lane 6, APEC strain positive for *fimH, iucD* and *tsh* genes; lanes 7 and 8: APEC strains positive for *iucD* and *fimH* genes.

Prevalence of β- lactamase resistance genes in E. coli isolates

Uniplex PCR detected three β-lactamase resistance genes (*bla*CTX-M, *bla*TEM and *bla*OXA) in 55 *E. coli* isolates (Figures 2 and 3). *Bla*TEM was the most prevalent (87.3%) followed by *bla*CTX-M (85.5%) and *bla*OXA gene (5.5%). Cumulative data showing the association of MDR, virulence and *β*-lactamase resistance genes are summarized in Table 2.

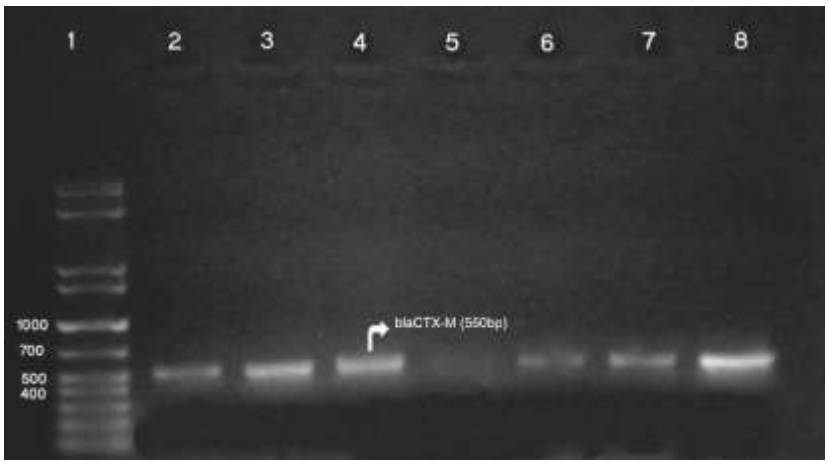

Figure 2: Uniplex PCR amplification of *blaCTX-M* (550bp) gene in *E. coli* isolates of broiler chickens. lane1, 1kb plus DNA ladder; lanes2-7, APEC strains showing amplification products of *bla CTX –M* gene; lane8, positive control.

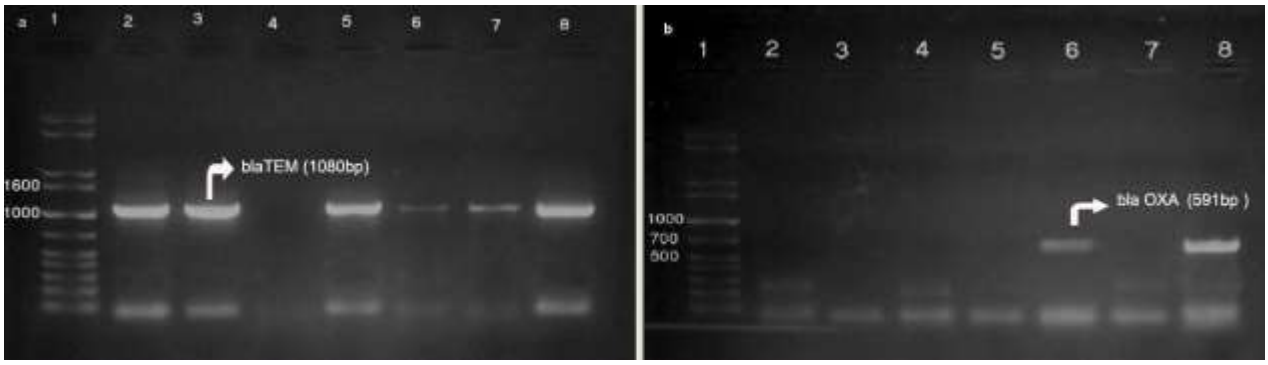

Figure 3: Uniplex PCR amplification of *blaTEM* (1080bp) and *bla OXA* (591bp) genes in *E. coli* isolates of broiler chickens. 3.a. lane1, 1kb plus DNA ladder; lanes 2-7, APEC strains showing amplification products of *bla TEM* gene; lane4 negative; lane8, positive control. 3.b. lane1, 1kb plus DNA ladder; lanes2-5, APEC strains showing -ve *bla OXA* gene; lane 6, strain showing amplification products of *bla OXA* gene; lane7, Dh5α strain, negative control; lane 8, positive control.

Discussion

The present study aimed to characterize APEC strains from broilers with respiratory signs, air sac lesions and variable mortalities in different broiler breeds (17-35 days old) based on serogrouping, virulence and antimicrobial resistance. Similar signs were recorded by many authors with variable mortalities and complications [7, 31-33].

PM examination revealed picture of colisepticemia, fibrinous pericarditis, perihepatitis and air sacculitis beside catarrhal enteritis with greenish contents that were concordant with the previous reports [6, 8, 34, 35].

The isolation rate from examined flocks (n=30) was 100% which may be attributed to environmental stressors, concurrent infections, bad management (ammonia toxicity) and other stress factors [36]. The isolation of APEC from both respiratory and visceral organs in this study and previous ones strengthens the incrimination of *E. coli* as a main cause of septicemia and respiratory troubles [8, 9, 33].

Higher isolation rates from different samples from broiler chickens with percentages of 92% and 94.5% were previously recorded [37, 38], while the lower isolation rate was detected by Sharada *et al*. [39]; Hasan *et al*. [40] and Literak *et al*. [41] who isolated *E. coli* with the percentages of 44.6, 36.2 and 35.7 %, respectively. The variation in the percentage of isolation could be credited to different environmental conditions, system of management and microbial load in each flock.

Mixed bacterial infections with *Enterobacter aerogenes* or *Providencia rettgeri* (3 flocks, each), *Klebsiella pneumoniae* (2 flocks), and *Serratia liquefaciens* or *Enterobacter agglomerans* (1 flock, each) were recorded. The presence of mixed infections with other pathogens might explain the elevated mortalities in some of the examined flocks. Ganapathy *et al.* [42] reported concurrent occurrence of salmonellosis, colibacillosis and histomoniasis in 3-week-old broiler chicken flock. Moreover, Olsen *et al.* [43] identified *E. coli* and *Enterococcus faecalis* as the most significant bacterial pathogens associated with first week mortality in chicks.

In the present study, the most common serogroups were O78 and O2 (15%, each). Several investigators reported both serogroups from outbreaks of colibacillosis [6]. Similarly, in flocks at Dakahlia Governorate the serotypes O2 and O78 percentages were 35.6% and 30.5%, respectively [32]. Also O78 was the most predominant serotypes in Sharkia Governorate with percentages of 20% and 33.33% [7, 10]. In this study, the percentage of O26 was (5%), O145 (5%), O44 (10%) while

Eid and Erfan, [8] isolated O26:K60 (10.7%) and O145, O44 (3.6% each). Awad *et al.* [11] detected that O78 was the most prevalent serotype (27.6%) while O2 (15.5%), O1 (12.1%), O124 (3.4%), O44 (1.7%) and O158 (1.7%) were less prevalent. In some cases, O serotyping was not able to classify around 50% of total tested APEC strains [44, 45].

The resistance to antibiotics is progressive [46]. In this study the resistance to oxacillin (100%) was higher than that recorded by Ahmed *et al*. [47] who detected resistance rate of 78.1% among the recovered *E. coli* from broilers in Egypt. Resistance to ceftriaxone was 43.6% which is higher than recorded by Yaqoob *et al.* [16] who recorded 11 % ceftriaxone resistance. The presence of oxacillin and ceftriaxone resistance could be attributed to the transfer of antibiotic resistance from other host to poultry due to lack of biosecurity. All tested APEC were resistant to amoxicillin (100%), similar results were reported [16, 48]. Amoxicillin-clavulanic acid resistance was 83.6% which is lower than other researcher [33] who reported 100% resistance. Others reported very low percentage of amoxicillin- clavulanic acid resistance (2 – 4.6 %) [41, 49]. Cefotaxime resistance rate in our findings was 50.9% which is similar to other records [11, 50]. The explanation of this high resistance rate to amoxicillin, amoxicillin- clavulanic acid and cefotaxime might be attributed to the intensive bird farming as well as short life span of broilers where antibiotics used as growth promotor and in sub-therapeutic doses which facilitate the development of resistance to β-lactam group of antibiotics which is known as Extended spectrum β-lactamase phenotype (ESBL) [46, 51, 52].

Lincomycin resistance was reported with a percentage of 100% which agreed with results obtained by Eid and Erfan, [8] who recorded 96.4% lincomycin resistance.

Similarly ciprofloxacin showed high resistance rate 90.9% which in agreement with Tong *et al*. [49]; Awad *et al.* [11] and Majhi *et al.* [50] who recorded ciprofloxacin resistance (81%, 41.4% and 60%) respectively. Other researchers [8, 41] reported lower resistance 25% and 26% respectively.

Gentamycin revealed low resistance rate (36.4%). Lower gentamicin resistance rate (10%) [11, 50] were reported and other studies revealed no resistance [50]. On the other hand, Mohamed *et al*. [48] showed complete resistance to gentamicin (100%). Doxycycline in our results showed lower resistance rate (32.7%). Previous incidence of doxycycline resistance was reported by Ammar *et al.* [33] who detected 51.02 % resistance in broilers in Sharkia province. On the other hand, Eid and Erfan, [8] recorded 100% resistance.

The miss use of antimicrobial at sub therapeutic doses or unneeded doses contribute to emergence of multidrug resistance (MDR) [51]. A high incidence of MDR was detected in this study; all isolates were at least resistant to five anti-microbial which agreed with Zhao *et al*. [53]. Xia *et al*. [54] reported that over 58% of *E. coli* isolates showed resistance to four or more antimicrobial agents. The growing incidence of MDR is of public health importance due to the danger of entering the human food chain [55].

ESBL producing organisms are becoming a major threat for poultry and patients in the hospital and community [56, 57].This attributed to the ability of these bacteria to hydrolyze third-generation cephalosporins that are commonly used to treat serious infections as well as the potential transfer of these resistance genes to human through food chain, direct contact, or the environment [58, 59]. In the present study, ESBL antibiotic resistance genes (*bla*CTX-M, *bla*TEM and *bla*OXA) were selected based on their resistance against cefotaxime, amoxicillin-clavulanic acid and oxacillin respectively. Widespread β-lactam antibiotic prescription in the field of veterinary medicine is one of the factors causing the presence of ESBL antibiotic-resistant bacteria to reach epidemic proportions in recent years [60, 61].

Kim *et al.* [62] suggested *bla*TEM gene to be the most common β-lactamase responsible for ampicillin resistance. The rate of detection of *bla*TEM among the tested isolates was 87.3% which is in agreement with Awad *et al*. [11] who recorded *bla*TEM gene in 87.9% of the tested APEC isolates. The prevalence of

*blaC*TX-M gene was 85.5%. Lower detection rate (8.7, 24, 57.9 %) were recorded by Ahmed *et al*., [47]; Yaqoob *et al.*, [16]; Tong *et al*. [49] respectively.

The *bla*OXA is responsible for oxacillin resistance. The prevalence of *bla*OXA was 5.5% which is nearly similar to that recorded by Yaqoob *et al.* [16] (3%). In another investigation, only one strain harbored a *bla*OXA gene (1.96%) [63].

Rapid identification of APEC by PCR allows rapid and reliable results through the detection of some important virulence genes [8]. The pathogenicity of APEC strain is determined by presence of at least five virulence genes [64]. Among 55 tested isolates, *iucD* gene was the most prevalent virulence gene (96.4%). It represents outer membrane protein aerobactin receptor and is important for *E. coli* to grow in iron free media enabling multiplication and invasion [65]. The aerobactin system enables microorganisms to capture and transport iron [66, 67]. The higher prevalence of *iucD* gene was in accordance with that recorded by Subedi *et al*., [68] who detected *iucD* gene presence in 97.8% of tested strains that indicates the importance of *iucD* gene for pathogenicity. Our finding is much higher than recorded in Sri Lanka (55%) [69].

The type 1 fimbrial adhesin gene, *fimH*, contributes to adhesion and protection from host heterophils [70]. In this study, *FimH* prevalence was 92.7%. Other studies revealed 98.1% in 524 APEC isolates [29]. Other research revealed low prevalence of *fimH* (33.3%) [71].

The ColV operon consists of genes for ColV synthesis (cvaC) and ColV immunity (cvi) and two genes for ColV export (cvaA and cvaB) [72]. In the present study the prevalence of *cvaC* was 9% which is much lower than that recorded by Rodriguez-Siek *et al.* [73]; McPeake *et al*. [74]; and Abd El Tawab *et al*., [7] who recorded *cvaC* percentage 66.8% ,99.1% and 60% respectively. Our findings are similar to results recorded by Kwon *et al*. [75] and Arabi *et al*. [76] who detected *cvaC* percentage 16% and 14.2% respectively. Other investigations found variable prevalence rates of *cvaC* (28.1%) [9].

Increased serum survival (*iss*) gene is known to be associated with serum resistance [69]. It was identified in pColV-I-K94 plasmid, it is related to cytotoxic complex inhibition [77]. In this study, the prevalence of *iss* gene was 76.4% which comes in agreement with previous investigations [49, 75]. Our findings are similar to results recorded by McPeake *et al*. [74]; Rocha *et al*. [78] and Mohamed *et al.* [48] who detected *iss* prevalence 72.8%, 73.8%, 72.2% respectively. Higher prevalence of *iss* was reported by Hussein *et al*. [9] who recorded 89.5 % among APEC isolates. The *ompT* gene encodes the episomal outer membrane protease that cleaves colicins [79]. The prevalence rate of *ompT* gene in this study was 58.2%. Higher rate was recorded by Hussein *et al*. [9] and De Carli *et al.* [64] who detected *ompT* in 94.7 and 100% of the tested strains respectively.

The *tsh* gene is responsible for hemagglutination activity of chicken erythrocytes [80]. In this study the prevalence of *tsh* gene was *(*45.5%). These findings are nearly similar to those recorded by Zhao *et al*. [81]; Rocha *et al*. [78]; Dissanayake *et al.* [69] who reported *tsh* with a percentage of 46.3, 55.7 and 45% respectively. Higher records were reported in Iran (96.4%) [76].

Conclusion

The association between virulence and multidrug resistance genes among *E. coli* isolates was concluded, that hindered the control strategy. Therefore, recent alternative strategies were necessary to minimize the antibiotic use and reduce the load of virulent *E. coli* strains.

Conflict of interest

The authors declared that they have no conflict of interest.

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الملخص العربى

الخصائص الجزيئية لسلالات الميكروب القولونى التي تسبب اعراض تنفسية في بدارى التسمين في مصر

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مرض العصيات القولونية هو مرض معقد يسبب خسائر اقتصادية شديدة وهو من التحديات التى تواجه الأطباء البيطريين والمنتجين. لذلك كان هدف هذه الدراسة تحديد خصائص الايشيريشيا كولاى شديدة الضراوة المسببة للمشاكل التنفسية فى الدجاج. فى عدد ٣٠ قطيع بدارى تسمين عمر ١٧-٣٥ يوم اثناء جائحة عام ٢٠١٣-٢٠١٦ فى محافظات الشرقية والإسماعيلية والدقهلية وسيناء اظهرو اعراض تنفسيه و اسهالات. و باجراء الصفة التشريحية ظهرت مطابقة لحالة عدوى الميكروب القولونى الدموى والتى تشمل التهاب فى الاكياس الهوائية وتكون غشاء فيبريني وغشاء فيبرينى على القلب والكبد. كانت نسبة عزل الميكروب القولونى الممرض ١٠٠ % من القطعان المختبره كما عانت ١٠ قطعان من العدوي المختلطه. فى هذه الدراسة تم تجميع ٢٨٤عينة تشمل الاكياس الهوائية و القلب و الرئه و الكبد وقد كانت اعلى نسبة عزل للميكروب القولونى من الأكياس الهوائية بنسبة (٧٦.١%), يليها الرئه (٧٣.٢ %), القلب (٦٧.٦ %) ثم الكبد بنسبة (٥٤.٩ %). تم عمل التصنيف المصلى وقد أظهر ان النوع السائد فيها O78 وO2 حيث كانت نسبة كل منهما ١٥% لكل واحد. تم اجراء اختبار حساسية للمضادات الحيوية وقد وجد أن اكثر المضادات الحيوية مقاومة هى الاسبيراميسين والاوكساسيلين والأموكسيسيللين والليكومايسين بنسبة (١٠٠%) بينما الاقل مقاومة هو الدوكسي سيكلين (٣٢.٧%). تم اجراء اختبار تفاعل انزيم البلمرة المتسلسل لعدد ٥٥ معزولة ايكولاى مقاومة للمضادات الحيوية لتحديد نسبة تواجد ثلاثة جينات مقاومة للمضادات الحيوية وهم (blaTEM, blaCTX-M, blaOXA) بمعدل (٥.٥% و٨٥.٥% و٨٧.٣% على الترتيب). وتحديد نسبة تواجد ستة جينات ضراوة باستخدام multiplex PCR (iucD, Fim H, iss, ompT, tsh, cvaC) وقد كانت نسبتهم (9.1% و٤٥.٥ % و ٥٨% و٧٦ % و ٩٢.٧% و٩٦.٤%) على الترتيب. ويتضح من النتائج السابقة الارتباط بين جينات الضراوة والجينات المقاومة للمضادات الحيوية والتى بدورها تعرقل استراتيجيات التحكم فى عدوى الايشريشيا كولاى وعليه من الضرورى البحث عن استراتيجيات بديلة لتقليل عدوى الايشريشيا كولاى الضارية والمقاومة للمضادات الحيوية.