DETECTION OF SOME VIRULENCE GENES OF AVIAN PATHOGENIC *E. Coli* BY POLYMERASE CHAIN REACTION.

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ABSTRACT

The present study was conducted on 44 commercial broiler chicken farms (20-30 days old) located in four different centers at Kaliobia Governorate (Benha, Kafer-Shoker, Toukh and Shebin El-kanater), 11 from each were inspected for E. coli infection. Samples were taken from diseased and freshly dead chickens (liver, heart blood, lung, intestine, kidney and spleen from each chicken) for bacteriological examination. The results revealed that 502 out of 1320 samples (38%) were positive for *E. coli* isolation, where 124 isolates (24.7%) from 408 samples of 68 diseased chickens and 378 isolates (75.7%) from 912 samples of 152 freshly dead chickens. Three seogroups of *E. coli* were obtained by serological identification (055, 0125, and recently 0146), represented as 6 strains were serotyped 0146 (40%), 3 stains 0125 (20%), 2 strains 055 (13.3%) and 4 isolates were untyped by the available antisera. Moreover, the serogroups 055 and 0146 were positive for K99 while 0125 was negative. Gentamycin, Cefotaxime, Ampicillin / Clavulinic acid and Enrofloxacin were the most proper antibiotics with the highest in vitro efficiency against isolated *E.coli.* Finally, Multiplex PCR showed that eaeA, ompA, kpsMTII, tsh, iutA and iss virulence genes were detected in all serogroups. While papC virulence gene was detected in serotypes 055 only. Moreover, stx2 virulence gene was detected in both serotypes 055 and 0125, meanwhile, it was not amplified in 0146 serotype.

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1. INTRODUCTION

Pathogenic *E. coli* strains have been divided into intestinal pathogenic *E. coli* and extra intestinal pathogenic *E. coli* (ExPEC) depending on the location of the infection. Avian pathogenic *E. coli* (APEC) strains belong to the ExPEC group is a major pathogen responsible for morbidity and mortality in chickens. It induces different syndromes in poultry, including systemic and localized infections, such as respiratory colibacillosis, acute colisepticemia, yolk sac infection, enteritis, arthritis, omphilitis, swollen-head syndrome, coli granuloma, salpingitis and oophritis. The most common form of colibacillosis is characterized by an initial respiratory disease in 3-6 week-old broiler chickens. It is usually followed by a systemic infection with characteristic fibrinous lesions (airsacculitis, perihepatitis, and pericarditis) and fatal septicemia (Ewers et al., 2003; Roy et al., 2006; Sharada et al., 2010). The pathogenicity of *E. coli* is generally enhanced or initiated by predisposing factors, such as mycoplasma infections, viral infections, environmental factors and immune-suppressive diseases (Ewers et al., 2003; Bopp et al., 2005; Gomes et al., 2005). The multiplex PCR technique is capable of identifying the most highly pathogenic *E. coli* isolates in a flock. These isolates can be used as the basis for the production of a powerful vaccine to be used against APEC infections (JanBen et al., 2001). Based on the fact that virulence varies not only among different species but also among strains of the same species.

Thus, numerous studies have been conducted to identify virulence factors of isolated pathogenic *E.coli* strains (Kaipainen et al., 2002; Zaki et al., 2004; Ewers et al., 2007). Avian pathogenic *E.coli* for poultry commonly belong to certain serogroups particularly serogroups O1, O2, O11, O15, O55, O78, O79 and O111 (Gross, 1994; Bopp et al., 2005).The pathogenic and non- pathogenic strains in poultry are differentiated based on the virulence, which has been attributed to various factors including those encoding for adhesions (F1, P, and stg fimbriae, curli, and EA/I), anti-host defense factors (ompA, iss, lipopolysaccharide, and K1), iron acquisition systems (aerobactin, iro proteins, yersiniabactin, and the sit iron acquisition locus), auto transporters (tsh, vat, and aatA), the phosphate transport system, sugar metabolism, the ibeA protein and motility (Dho and Lafont,1984). Detection of pathogenic *E.coli* strains that causes colisepticemia becomes important for effective treatment with antimicrobial therapy and control resulting in reducing both the incidence and mortality which associated with avian colibacillosis (Dho et al., 1990 ; Susantha et al., 2001 ; Jeffrey et al., 2002 ; Georgnaras et al., 2004 ; Zhao et al., 2009; Qabajah et al., 2010). This study was planned for bacteriological characterization of chichen *E.coli* isolates and detection of some virulence genes of the isolated strains by using PCR.

2. 2. MATERIAL AND METHODS

2.1. Samples collection

A total of 44 commercial broiler farms (20-30 days old) were inspected for E.coli infection from four different centers at Kaliobia Governorate (Benha; kafer-Shoker; Toukh and Shebin El- Kanater) . Bacteriological Samples were taken from 68 diseased and 152 freshly dead chicken (liver; heart blood; lung; intestine; kidney and spleen from each chicken) after clinical and postmortem examination. Each examined organ was taken alone in sterile plastic bags, kept in icebox and transferred with minimum delay to the laboratory.

2.2. Bacterteriological examination

The surface of organs was seared by hot spatula, and then a sterilized loopfuls were inoculated onto nutrient broth and incubated aerobically at 37ºC for 12 hours. Loopfuls from incubated nutrient broth were streaked onto MacConkey's agar plates and incubated for 24 hours at 37ºC. Suspected lactose fermented colonies were picked up and streaked on the following media: Eosin methylene blue (EMB) ; Brilliant Green and Xylose Lysine Deoxycholate (XLD) agar plates then incubated for another 24-48 hours at 37ºC. The suspected purified colonies were picked up and kept in Semi-solid agar for morphological and biochemical identification (Konemann et al., 1997; Quinn et al., 2002).

2.3. Serological typing of E.coli

Fifteen isolates that were preliminary identified biochemically as *E. coli*, taken randomly, were subjected to serological identification (Edward et al., 1972) using slide agglutination test.

2.4. Antibiotic susceptibility testing

The isolated *E.coli* strains were subjected to the sensitivity test against different antibiotics, using the disc and agar diffusion method (Konemann et al., 1997).

2.5. Virulence genes of E.coli detection by PCR

Multiplex PCR was applied by using eight sets of primers for detection of eight virulence genes that may play a role in virulence of APEC. These genes were eaeA (intimin or *E.coli* attaching and effacing gene); ompA (outer membrane protein); stx_2 (shiga-$toxin_2$ gene); papC (pyelonephritis associated Pili gene); kpsMTII (capsular lipopolysaccharide gene); tsh (temperature sensitive heamagglutinin gene) iutA (ferric aerobactin outer membrane receptor gene) and iss (increased serum survival gene).

It was applied on isolated *E.coli* Following QIA amp DNA mini kit instructions (Catalogue no.51304); Emerald Amp GT PCR mastermix (Takara) Code No. RR310A kit and agarose gel electrophoreses (Sambrook et al.,1989).

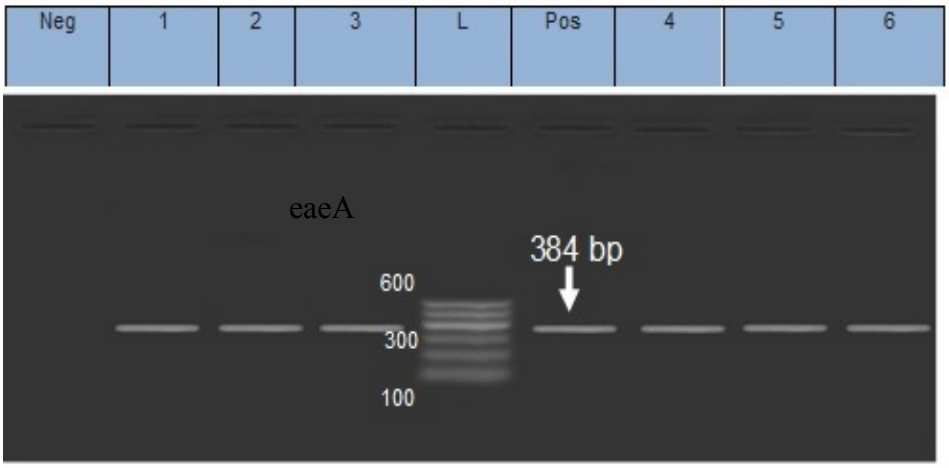
3. RESULTS

The results of *E.coli* isolation (Table 1) showed that 502 out of 1320 samples (38%) were positive for *E.coli* isolation, where 124 isolates (24.7%) were isolated from 408 samples of 68 diseased chicken and 378 isolates (75.7%) from 912 samples of 152 freshly dead chicken represented as 145 (11%); 134 (10.1 %); 115 (8.7%) and 108 (8.2%) from Benha; kaferShoker; Toukh and Shebin El-Kanater respectively. The bacteriological examination of studied organs revealed that *E.coli* were isolated from 157 intestine samples (31.3%); 141 liver samples (28.1%); 81 heart blood samples (16.1%); 48 spleen (9.6%); 40 kidney samples; (8.0%) and 35 lung samples (7.0%) (Table 2). The results of serological identification (Table 3) showed that out of 15 *E.coli* isolates. 11 isolates (73.3%) were positive with polyvalent antisera (2) while the other 4 isolates (26.7%) were negative (untyped). By using monovalent antisera, only 3 serogroups of *E. coli* (O55, O125 and O146) were identified and represented as 6 strains that were serotyped; one as O146 (40%), three as O125 (20%) and two as O55 (13.3%). The four isolates were untyped by the available antisera. The serogroups O55 and O146 were positive for K99 while O125 was negative. The in- vitro sensitivity tests (Table 4) showed the isolated *E.coli* were Highly sensitive for Gentamycin (94.0%), Ampicillin/Clavulinic acid (92.4%) ,Cefotaxime (92.4%) and Enrofloxacin (92.0%) and Moderately sensitive for Ciprofloxacin (80.7%) , Norfloxacin (80.7%) and Doxycycline (79.7%). Meanwhile, they were weakly sensitive for Chloramphenicol (29.9%), Erythromycin (21.9%), Trimethoprim/Sulphamethoxazol (20.7%) and Neomycin (20.3%). Moreover, they were resistant for Pefloxacin (5.4%), Streptomycin (4.4%), Oxytetracycline (3.4%) and Amoxillin (3.2%). Multiplex PCR results (Table 5) recovered that eaeA; ompA; kpsMTII; tsh; iutA and iss virulence genes were detected in all serogroups. While papC virulence gene was detected only in serotype O55. Moreover, stx_2 virulence gene was detected in both serotypes O55 and O125 meanwhile it was not amplified in O146. The eaeA gene was amplified in serogroups O55; O125 and O146 giving a PCR product of 384 bp (photo 1).The ompA gene was amplified in O55, O125 and O146 giving a PCR product of 919 bp (photo 2).The stx_2 gene was amplified in both serotypes O55 and O125 giving a PCR product of 779 bp (photo 3). Meanwhile it was not amplified in O146 serotype under the same condition. The papC gene was amplified in O55 giving a PCR product of 501bp (photo 4). On the other hand both O125 and O146 were negative under the same condition. The kpsMTII gene was amplified in serogroups O55, O125 and O146 giving a PCR product of 280 bp (photo 5).The tsh gene was amplified in serogroups O55, O125 and O146 giving a PCR product of 620 bp (photo 6).The iutA gene was amplified in serogroups O55; O125 and O146 giving a PCR product of 300 bp (photo 7). The iss gene was amplified in serogroups O55, O125 and O146 giving a PCR product of 266 bp (photo 8).

4. DISCUSSION

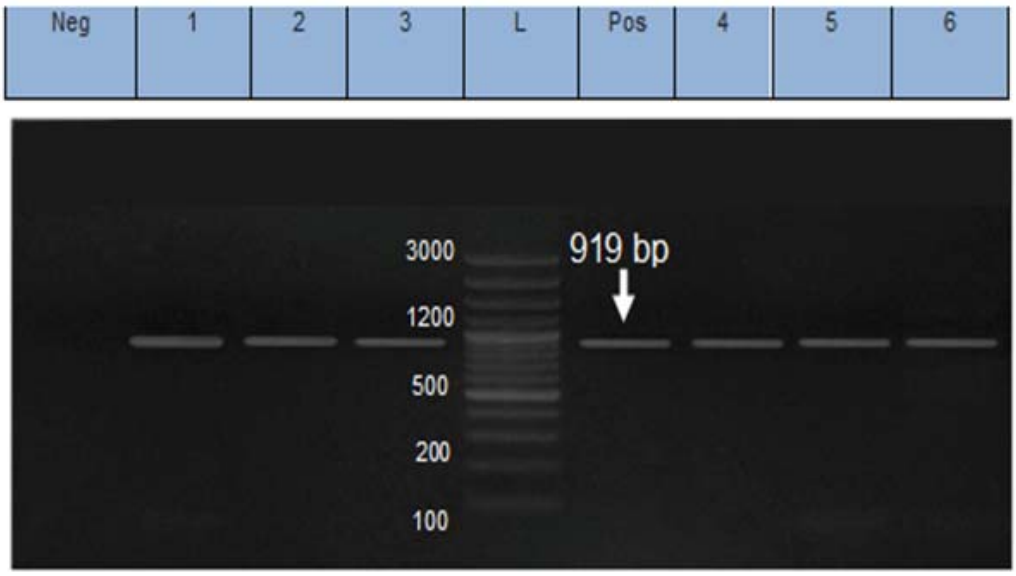
Escherichia. coli infections in birds cause many clinical manifestations which characterized by a respiratory disease that is frequently followed by a generalized infection which ended by death. Avian pathogenic *E. coli* (APEC) strains fall under the category of extra intestinal pathogenic *E.coli*, which are characterized by the possession of virulence factors that

Photo (1): Amplification of eaeA gene of *E. coli* serogroups



Lane L: 100-600bp DNA Ladder. Neg.: Negative control. Pos.: Positive control. Lane 1 & 6:*E.coli* O55 (Positive). Lane 2 & 3: *E.coli* O146 (Positive). Lane 4 & 5: *E.coli* O125 (Positive).

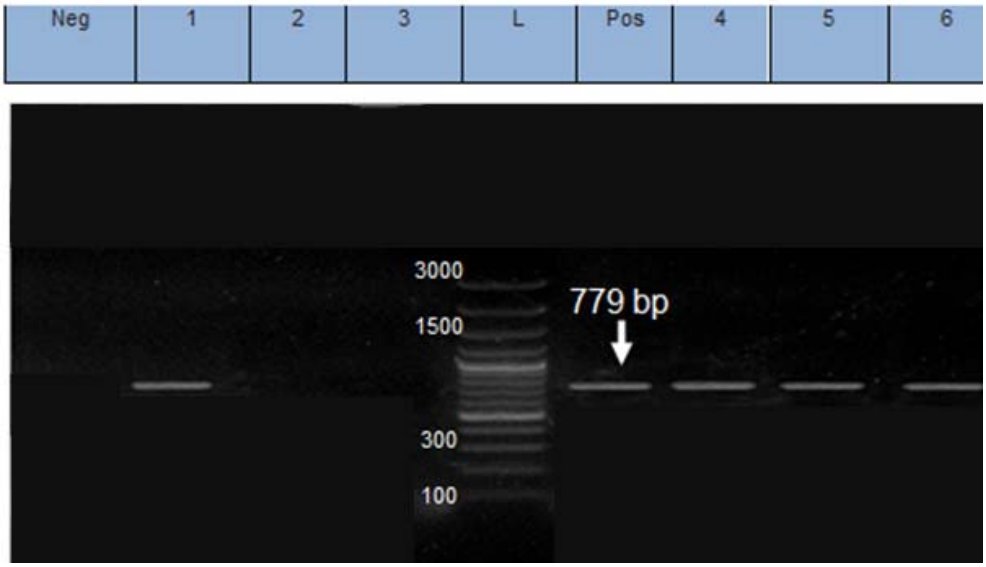
Photo (2): Amplification of ompA gene of *E. coli* serogroups



ompA

Lane L: 100-3000bp DNA Ladder. Neg.: Negative control. Pos.: Positive control. Lane1 & 6: *E.coli* O55 (Positive). Lane 2 & 3: *E.coli* O146 (Positive). Lane 4 & 5: *E.coli* O125 (Positive)
.

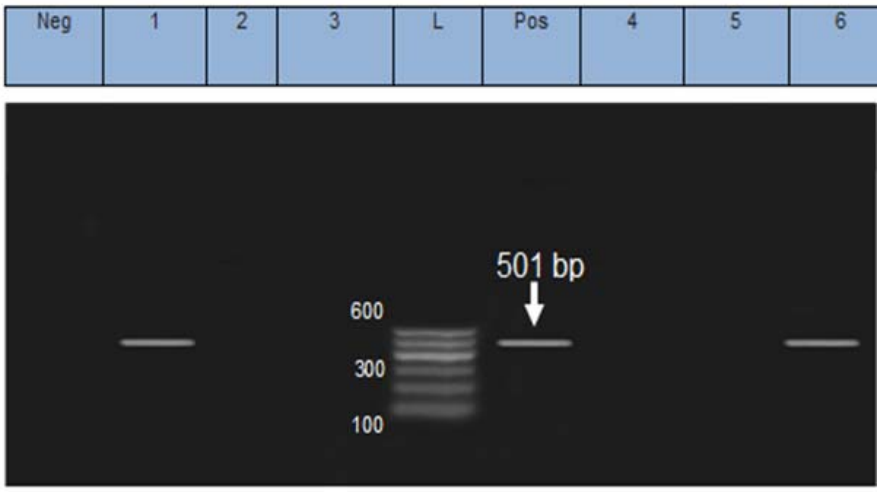
Photo (3): Amplification of stx2 gene of *E. coli* serogroups



stx2

Lane L: 100-3000bp DNA Ladder. Neg.: Negative control. Pos.: Positive control. Lane 1 & 6: *E.coli* O55 (Positive). Lane 2 & 3: *E.coli* O146 (Negative). Lane 4 & 5: *E.coli* O125 (Positive).

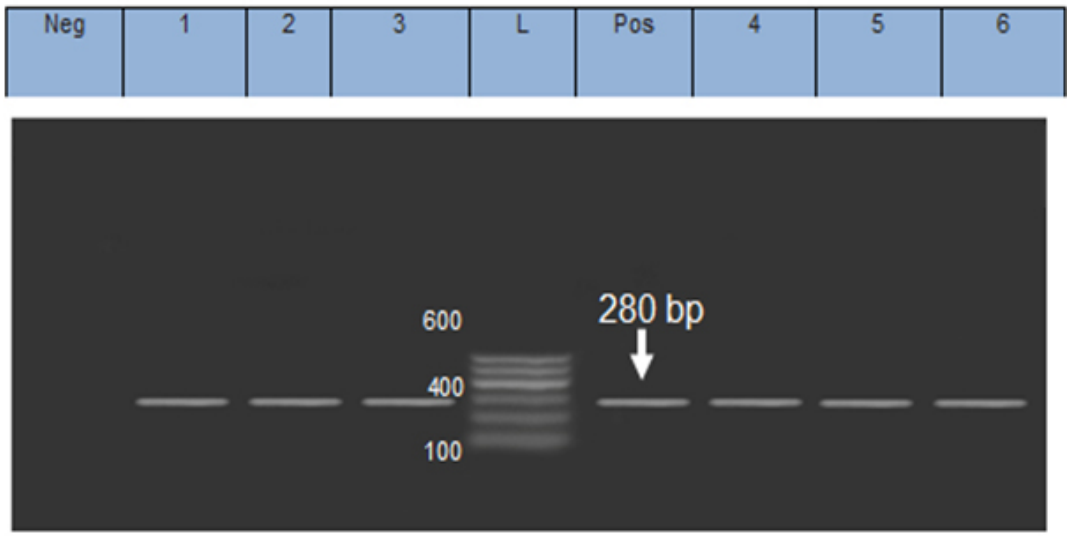
Photo (4): Amplification of papC gene of *E. coli* serogroups



papC

Lane L: 100-600 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control. Lane 1 & 6: *E.coli* O55 (Positive). Lane 2 & 3: *E.coli* O146 (Negative). Lane 4 & 5: *E.coli* O125 (Negative).

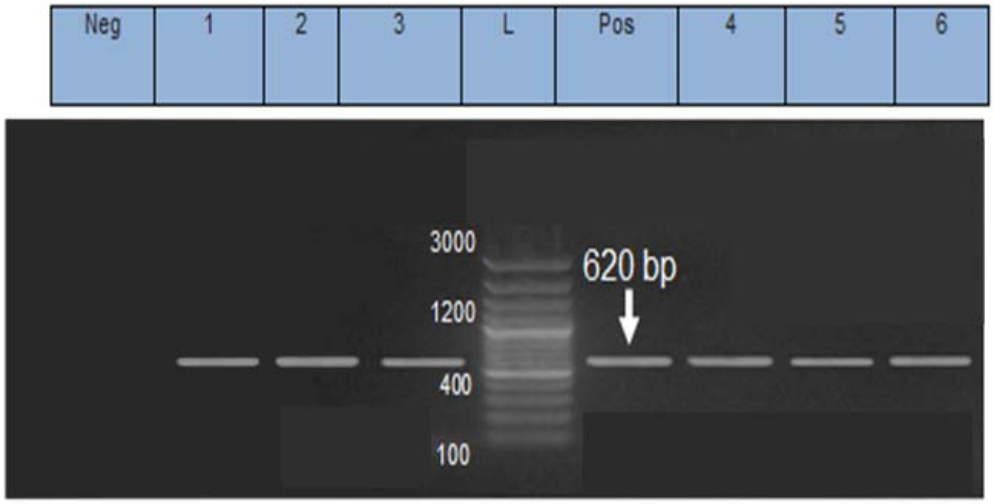
Photo (5): Amplification of kpsMTII gene of *E. coli* serogroups



kpsMTII

Lane L: 100-600 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control. Lane 1 & 6: *E.coli* O55 (Positive). Lane 2 & 3*: E.coli* O146 (Positive). Lane 4 & 5: *E.coli* O125 (Positive).

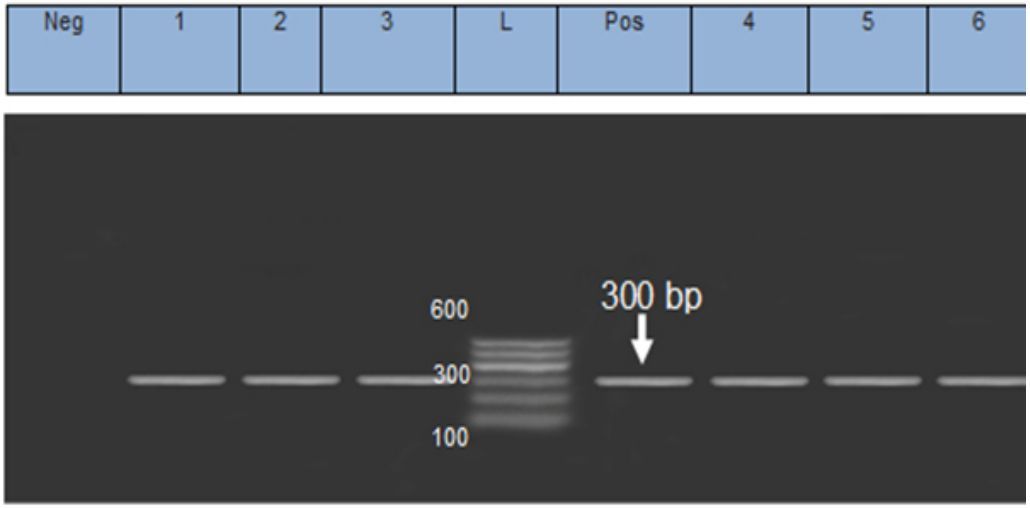
Photo (6): Amplification of tsh gene of *E. coli* serogroups



tsh

Lane L: 100-3000 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control. Lane 1 & 6: *E.coli* O55 (Positive). Lane 2 & 3*: E.coli* O146 (Positive). Lane 4 & 5: *E.coli* O125 (Positive).

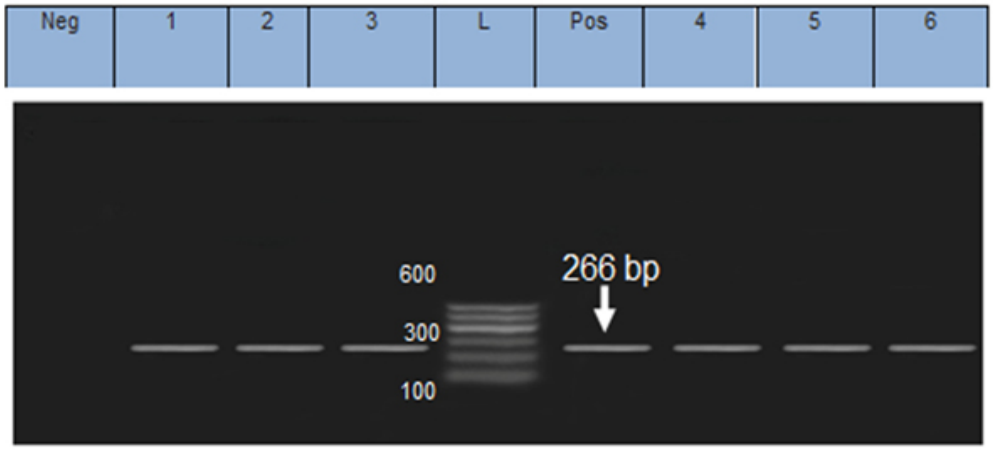
Photo (7): Amplification of iutA gene of *E. coli* serogroups



iutA

Lane L: 100-600 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control. Lane 1 & 6: *E.coli* O55 (Positive). Lane 2 & 3: *E.coli* O146 (Positive). Lane 4 & 5: *E.coli* O125 (Positive).

Photo (8): Amplification of iss gene of *E. coli* serogroups



iss

Lane L: 100-600 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control. Lane 1 & 6: *E.coli* O55 (Positive). Lane 2 & 3: *E.coli* O146 (Positive). Lane 4 & 5: *E.coli* O125 (Positive)

Table (1): Percentages of E. coli isolated from different places in Kaliobia Governorate in comparison with overall total

	Benha			Kafr Shokr			Toukh			Shebin El-kanater			Overall		
	Dis.	*Dead*	*Total*	*Dis.*	*Dead*	*Total*	*Dis.*	*Dead*	*Total*	*Dis.*	*Dead*	*Total*	*Dis.*	*Dead*	*Grand Total*
No. of samples	102	228	330	102	228	330	102	228	330	102	228	330	408	912	1320
+ve samples No.	30	115	145	31	103	134	30	85	115	33	75	108	124	378	502
% [1]	29.4	50.4	43.4	30.4	45.2	40.6	29.4	37.3	34.9	32.4	32.9	32.7	30.4	41.5	38.0*
% [2]	6.0	22.9	28.9	6.2	20.5	26.7	6.0	16.9	22.9	6.6	14.9	21.5	24.7	75.3	100.0
% [3]	2.3	8.7	11.0	2.4	7.8	10.2	2.3	6.4	8.7	2.5	5.7	8.2	9.4	28.6	38.0

[1]Percentage in relation to total number of cases in each raw
[2]Percentage in relation to total number of positive samples (502)
[3]Percentage in relation to total number of collected samples (1320)
*Total percentage of positive samples (502) to total collected samples (1320)
Dis. : diseased

Table (2): *E. coli* isolated from studied chicken's cases in Kaliobia Governorate

Centers		Number of birds	Samples						Total			
			Liver	Heart Blood	Lung	Intestine	Kidney	Spleen				
			NO.	NO.	NO.	NO.	NO.	NO.	Number of samples	Number of Positive samples	Positive percentage of *E. coli* %[1]	%[2]
BENHA:												
Diseased		17	10	4	2	11	1	2	102	30	29.4	6.0
Freshly Dead		38	31	22	8	32	10	12	228	115	50.4	22.9
TOTAL		55	41	26	10	43	11	14	330	145	43.9	28.9
KAFR SHOKER:												
Diseased		17	9	4	3	11	2	2	102	31	30.4	6.2
Freshly Dead		38	27	18	8	30	10	10	228	103	45.2	20.5
TOTAL		55	36	22	11	41	12	12	330	134	40.6	26.7
TOKH:												
Diseased		17	9	5	2	10	2	2	102	30	29.4	6.0
Freshly Dead		38	25	10	5	29	7	9	228	85	37.3	16.9
TOTAL		55	34	15	7	39	9	11	330	115	34.9	22.9
SHEBIN EL-KANATER:												
Diseased		17	10	6	3	11	1	2	102	33	32.4	6.6
Freshly Dead		38	20	12	4	23	7	9	228	75	32.9	14.9
TOTAL		55	30	18	7	34	8	11	330	108	32.7	21.5
OVERALL TOTAL Diseased		68	38	19	10	43	6	8	408	124	30.4	24.7
OVERALL TOTAL Freshly Dead		152	103	62	25	114	34	40	912	378	41.5	75.3
Grand Total	NO.	220	141	81	35	157	40	48	1320	502	38.0*	100.0
	%[3]	---	28.1	16.1	7.0	31.3	8.0	9.6		---		

[1]Percentage in relation to total number of cases in each row

[2] Percentage in relation to tot al number of positive samples (502)

[3]Percentage in relation to total number of positive samples (502).

* Total percentage of positive samples (502) to total collected samples (1320)

Table (3): Serological typing of the isolated *E.coli* strains from different farms

Serial No.	Isolate No.	Polyvalent antisera	Monovalent antisera	K99
1	201	2	O146	+
2	87	2	O125	-
3	6	-	Untyped	Undo*
4	152	2	O146	+
5	75	2	O55	+
6	14	2	O125	-
7	186	-	Untyped	undo
8	169	2	O146	+
9	23	2	O146	+
10	180	-	Untyped	undo
11	153	-	Untyped	undo
12	90	2	O125	-
13	77	2	O55	+
14	205	2	O146	+
15	170	2	O146	+

*Undo means untyped monovalent antisera were not tested for K99

Table (4): In-vitro anti-microbial Sensitivity test for the isolated *E. coli* strains.

Antibiotics	Disputant	highly sensitive	Moderately sensitive	weakly sensitive	Total sensitive	Resistant
		%				
Gentamycin	10 mcg	79.3	14.7	3.8	94.0	2.2
Enrofloxacin	5 mcg	77.6	14.3	4.9	92.0	3.2
Norfloxacin	10mcg	61.8	18.9	13.7	80.7	5.6
Trimethoprim/ Sulphamethoxazol	(1.25/23.75) mcg	7.0	13.7	26.9	20.7	52.4
Doxycycline	30 mcg	60.0	19.7	13.9	79.7	6.4
Ciprofloxacin	5mcg	60.4	20.3	13.3	80.7	6.0
Erythromycin	15 mcg	7.6	14.3	26.1	21.9	52.0
Neomycin	30 mcg	6.6	13.7	26.5	20.3	53.2
Pefloxacin	5 mcg	2.0	3.4	16.9	5.4	77.7
Streptomycin	10 mcg	1.2	3.2	16.7	4.4	78.9
Cefotaxime	30 mcg	78.1	14.3	5.0	92.4	2.6
Ampicillin/ Clavulinic acid	(20/10) Mcg	77.3	15.1	4.2	92.4	3.4
Oxytetracycline	30 mcg	1.0	2.4	6.8	3.4	89.8
Chloramphenicol	30 mcg	7.8	22.1	27.3	29.9	42.8
Amoxicillin	25mcg	0.8	2.4	16.1	3.2	80.7

Percentage in relation to total number of isolated *E. coli* (502)

Table (5): The results of PCR amplifications of different used genes of *E. coli* serogroups

Serial	serotype	Virulence genes							
		iss	iutA	tsh	kpsMTII	papC	stx_2	ompA	eaeA
1 , 6	O55	+	+	+	+	+	+	+	+
*2 , 3	O146	+	+	+	+	-	-	+	+
**4,5	O125	+	+	+	+	-	+	+	+

- eaeA (intimin or E.coli attaching and effacing gene)
- ompA (outer membrane protein)
- stx_2 (shiga-$toxin_2$ gene)
- papC (pyelonephritis associated Pili gene)
- kpsMTII (capsular lipopolysaccharide gene)
- tsh (temperature sensitive heamagglutinin gene)
- iutA (ferric aerobactin outer membrane receptor gene)
- iss(increased serum survival gene)

*Two serotypes from O146 were PCR amplified.
** Two serotypes from O125 were PCR amplified.

enable to live extra intestinal life (Johnson et al., 2006). The results of *E.coli* isolation, (Table 1) revealed that 502 out of 1320 samples were positive for *E.coli* isolation, where 124 strains (24.7%) isolated from 408 samples of 68 diseased chicken and 378 strains (75.7%) from 912 samples of freshly dead chicken. These results came in accordance with (Kilic et al., 2009; Sharada et al.,2010; Shimaa,2013).Our results disagreed with previous reports, as some were higher (Arara et al.,1987; Saitanu,1990) and others were lower (Ghosh,1987; Mashhoor et al.,1987; Javed et al.,1991).The percentage of isolation in different centers (Table 2) may be attributed to the prophylactic and therapeutic use of antibiotics, vaccination for respiratory viruses and immune status of birds or differences in degree of hygiene and overcrowding in the farms. Moreover, higher rates of isolation of *E.coli* from intestine (31.3%), liver (28.1%), heart blood (16.1%), spleen (9. 6%), kidneys (8.0%) and finally lungs (7.0%) (Table 2) indicated the acute nature of the disease (Krishnamohan et al., 1994; Blanco et al., 1996; Sharada et al., 2010) and also indicated the predominant role of *E.coli* in causing enteritis (El-Boraay et al., 2002; Sharada et al., 2010) . Also, higher incidence in freshly dead samples indicating were recorded by (Ghosh, 1987; Krishnamohan et al., 1994; Sharada et al., 2010). Nearly similar results were recorded by (El-Boraay et al., 2002; Saha et al., 2003; Disouky, 2009; Sharada et al., 2010; Al-Ajmi, 2011). Meanwhile, some reported lower incidence of *E.coli* isolation (Aphukan et al., 1990; Abhilasha et al., 2001), others have reported higher incidences (Sepehri and Zadeh, 2006). The serological identification of random 15 *E.coli* isolates (Table 3) clarified that 11 (73.3%) gave positive results with polyvalent antisera (2) while, other 4 (26.7%) were negative (untyped). By using monovalent antisera, only 3 identified serogroups of *E.coli* (O55, O125 and O146) were identified serologically represented

as six strains were serotype O146 (40%); three O125 (20%); two O55 (13.3%) and four isolates were untyped by the available antisera. Similar *E.coli* serotypes had been also previously isolated from cases of chickens in Egypt as previously reported (Abd El-Galil et al.,1983; Ibrahim et al., 1998; Abd El-Haleem, 2000; Abd El-Salam, 2004; Nashwa et al., 2010; Sharada et al., 2010; Ammar et al., 2011; Shimaa, 2013) Concerning to the recently identified serotype O146 in Egypt. Similar results from cases of colibacillosis were recorded (Arara et al., 1987; Shimaa, 2013). Meanwhile, other serogroups were identified in different countries where O1, O2 and O78 are registered worldwide as the most prevalent pathogenic avian *E.coli* serogroups , in addition to other serotypes as O5, O6, O9, O11, O15, O22, O25, O36, O41, O51, O53, O56, O60, O68, O81, O83, O88, O95, O102, O103, O109, O115, O116, O141, O145, O153, and O174 as reported (Gomis et al., 2001; JanBen et al., 2001; Ewers et al., 2004; Monroy et al., 2005; Jeong et al., 2011). These variations reflect that *E.coli* serogroups are country specific and also it may differ within different localities in the same country and this beneficial in bacterines preparation as it must be specific to the predominant serotypes, also it is clear that avian *E.coli* represented by known and few serotypes. Moreover, the serogroups O55 and O146 were positive for K99 (virulence factor) while O125 was negative. These results came in accordance with (Frank et al., 1998). The results of antibiotic sensitivity tests (Table 4) revealed that Gentamycin; Cefotaxime; Ampicillin/ Clavulinic acid and Enrofloxacin were the most proper antibiotics with the highest efficiency against isolated *E.coli* in vitro. These results are in coincidence with (Raji et al., 2007; Smith et al., 2007; Helal, 2012; Wafaa, 2012). It is also very significant to note that almost all the *E.coli* isolates showed weak sensitivity for Chloramphenicol, Erythromycin, Trimethoprim/ Sulphamethoxazol and Neomycin and very high resistance to Pefloxacin, Streptomycin, Oxytetracycline and Amoxicillin. This is of serious concern because these drugs are still considered the most recommended for the treatment of colibacillosis in both animal and human (Raji et al., 2007). Regarding the occurrence of intimin or *E.coli* attaching and effacing gene (eaeA) virulence gene in *E.coli* isolates. The result revealed that it was amplified in all serogroups (O55; O125 and O146) giving a PCR product of 384bp (photo 1).These results came in accordance with those recorded (Frank et al., 1998; Osek, 2003; Ahmed et al., 2007; Fujioka et al., 2009; Hideki et al., 2009; Dutta et al., 2011; Al-Ajmi, 2011). Meanwhile these results disagreed with others who found no eaeA gene detected in all APEC isolates (Olsen and Christensen, 2011; Shimaa, 2013). The results of PCR for amplification of iss gene of *E. coli* serogroups cleared that the iss gene was amplified in O55; O125 and O146 serogroups, giving a PCR product of 266bp (photo 8). Similar reports were recorded (Gomis et al., 2001; Qabajah and Yaqoub, 2010; Ammar et al., 2011; Jeong et al., 2011; Helal, 2012) who stated that Iss gene is the most important and widely distributed virulence marker of APEC. The results of PCR for amplification of stx_2 gene of *E. coli* serogroups (photo 3) revealed that the stx_2 gene was amplified in both serotypes O55 and O125 giving a PCR product of 779bp, meanwhile, it was not amplified in O146 serotype. These results were nearly agreed with those obtained (JanBen et al., 2001; Fujioka et al., 2009; Hideki et al., 2009; Zhao et al., 2009; Dutta et al., 2011; Al-Ajmi, 2011). On the contrary, these results disagreed with (Kobayashi et al., 2005; Shimaa, 2013) who could not detect shiga toxin genes in chicken samples. The results of PCR for amplification of ompA gene of *E. coli*serogroups (photo 2) showed that, the ompA gene was amplified in O55, O125 and O146 giving a PCR product of 919bp. Similar findings were recorded (Johnson et al., 2008; Zhao et al., 2009) who reported

that ompA gene was found in all APEC isolates. The results of PCR for amplification of papC gene of *E. coli* serogroups (photo 4) cleared that the papC gene was amplified in serotype O55 only giving a PCR product of 501bp. Similar results obtained (JanBen et al.,2001; Zhao et al., 2009; Qabajah and Yaqoub, 2010). The results of PCR for amplification of kpsMTII gene of *E. coli* serogroups (photo 5), the kpsMTII gene was amplified in serogroups O55; O125 and O146 giving a PCR product of 280bp . These results go in parallel with (Zhao et al., 2009) who reported that the kpsMTII gene involved in the synthesis of capsules occurred in most strains of APEC. The results of PCR for amplification of tsh gene of *E. coli* serogroups (photo 6) appeared that the tsh gene was amplified in serogroups O55; O125 and O146 giving a PCR product of 620bp. These results go in parallel with those obtained (Gomis et al., 2001; JanBen et al., 2001; Fujioka et al., 2009; Qabajah and Yaquoub, 2010). The results of PCR for amplification of iutA gene of *E. coli* serogroups (photo7) showed that, the iutA gene was amplified in serogroups O55; O125 and O146 giving a PCR product of 300bp. These results go in parallel with those obtained (Johnson et al., 2008; Zhao et al., 2009) who reported that iutA was found in much higher percentage of *E.coli* associated with avian diseases. From results of the present work, it could be concluded that higher percentage of *E. coli* infection was detected in broilers. Multiplex PCR indicated that all serotypes isolated (O55, O125 and recently O146) had the eaeA; ompA; kpsMTII; tsh; iutA and iss virulence genes, meanwhile, papC virulence gene was detected in serotype O55 only and Stx_2 virulence gene was detected in both serotypes O55 and O125. Moreover, the serogroups O55 and O146 were positive for K99 while O125 was negative, and all of these genes play a role in pathogenicity and virulence of APEC. Also, Gentamycin; Cefotaxime; Ampicillin/ Clavulinic acid and Enrofloxacin were the most proper antibiotics with the highest efficiency against the isolated *E.coli* in vitro and can be used for treatment of *E.coli* infections in broiler chicken farms.

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الكشف عن بعض جينات الضراوة في الميكروب القولونى الممرض للدجاج بواسطة تفاعل البلمرة المتسلسل

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الملخص العربي

عدوى الميكروب القولوني من أهم الأمراض البكتيرية التي تؤثر في صناعة الدواجن والتي تسبب خسائر اقتصادية كبيرة ليس فقط نتيجة النفوق العالي للدواجن والفقد في الإنتاج والإعدامات في المجازر ولكنها عامل مساعد للإصابة بكثير من الأمراض الأخرى. وعلى ذلك فإن هذه الدراسة تلقى الضوء على الايشيريشياكولاى المعزولة من بداري التسمين وزراعتها على الأوساط الملائمة وكذلك الخصائص الكيميائية الحيوية والخصائص السيرولوجية وإجراء اختبارات الحساسية مع تحديد أهم الجينات الأكثر ضراوة بين العترات المعزولة. مت هذه الدراسة على 44 مزرعة من مزارع بداري التسمين (من عمر 20–30 يوم) من أربع مراكز مختلفة في محافظة القليوبية و هي بنها و كفر شكر وطوخ و شبين القناطر و تم فحص11 مزرعة من كل مركز لعدوى الايشيرشيا كولاى وقد تم تجميع 408 عينة من 68 دجاجة مريضة وتم عزل 124 عترة بنسبة 24,7% وتم عزل 378 عترة من 912 عينة من 152 دجاجة نافقة حديثا بنسبة 75,5% وقد تم تجميع العينات من الكبد و دم القلب و الرئة و الأمعاء و الكلى و الطحال من كل دجاجة بعد اجراء الفحص الإكلينيكي و الصفة التشريحية .سجلت أعلى معدلات عزل الميكروب القولوني من الاعضاء المختلفة كالآتي: الامعاء بنسبة 31,3% يليها الكبد بنسبة 28,1% و دم القلب بنسبة 16,1%و الطحال بنسبة 9,6% و الكلى بنسبة 8 % و أخيرا الرئة بنسبة 7%. أظهرت نتائج التصنيف السيرولوجى لعدد 15 معزولة (مختارة عشوائياً) من الميكروب القولوني من إجمالي 502معزولة كالآتي: 11 معزولة صنفت سيرولوجيا بنسبة 73,3% بينما 4 معزولات 26,7% لم يتم التعرف عليهم سيرولوجيا. تم التعرف على ثلاث سلالات فقط من المعزولات المصنفة سيرولوجياً و هم O55, O125, O146 حيث مثلت 6 معزولات من سلالة O146 بنسبة 40% و3 معزولات من O125 بنسبة 20% و2 معزولة من O55 بنسبة 13,3%. كما أوضحت النتائج ان السلالتين O55, O146 كانتا ايجابيتين للعامل الجيني k99 بينما أظهرت السلالة O125 سلبيتها للعامل الجينىk99. ومن ثم تم تطبيق تفاعل البلمرة المتسلسل لجينات الضراوة (*eae*A, *omp*A, *kps*MTII, *tsh, iut*A, *iss*) والتي ثبت تواجدها في المعزولات المصنفة آنفاً بينما تواجد جين الضراوة *pap*C في السلالة O55 فقط اما جين الضراوة stx_2 وجد فقط في السلالتين O55 وO125 ولم يوجد في السلالة O146 . اثبتت نتائج اختبارات الحساسية أن الجنتاميسين و السيفوتاكسيم و الأمبسيللين مع حمض الكلافيونيك اسيد و الأنروفلوكساسين كانوا الاكثر تأثيرا على المعزولات معملياً.

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