Genotypic identification of *E.coli* strains isolated from broilers.

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Abstract

In this study bacteriological examination of 1200 samples were collected from 200 birds (60 recently dead, 80 diseased and 60 apparent healthy broilers). The results revealed that *E. coli* isolates were recovered from 842 samples with overall prevalence 70.16. Incidence of positive *E.coli* from fresh heart blood samples was 75%, liver 83%, kidney 64%, spleen 57%, small intestine74.5% and from bone marrow 67.5 % . Serological identification of randomly selected *E. coli* (20) isolates clarified that, one *E. coli* isolate was serotype O_{111}, one was O44, one was O55, two were serotyped O142, two were serotyped O128, two were serotyped O158, three were serotyped O157, four were serotyped O29 and four were serotyped O115. PCR assay was carried out on six serovars (O_{142}, O_{29}, O_{115}, O_{158}, O_{128} and O_{157}) to detect the presence of *phoA, iss* and *iutA* gene. All serovars have the three genes except (O_{29}) does not possess *iss* gene.

Introduction

E. coli is a Gram-negative, non-spore-forming rod, which belongs to the family *Enterobacteriaceae*. The cell wall of Gram-negative bacteria typically consists of three layers, the cytoplasmic membrane and the outer membrane, separated by a peptidoglycan layer. The outer cell membrane contains phospholipids, membrane proteins and lipopolysaccharide (LPS). LPS comprises lipid-A, lipopolysaccharide core and repeated polysaccharide units called O-antigens. Lipid-A is the lipophilic inner part of LPS. The toxic effects of LPS known as endotoxin. Here, the terms LPS and endotoxin are used synonymously ***(Hogan and Smith, 2003).***

Colibacillosis refers to any localized or systemic infection caused entirely or partly by *E. coli* including colisepticaemia, coligranuloma, chronic respiratory disease (CRD), peritonitis, swollen-head syndrome, Artheritis, synovitis, panophthalmitis, perihepatitis and pericarditis ***(Gross, 1991).***

Many species of *E. coli are* intestinal pathogens or commensals in the intestine of man and animals, birds, few are saprophytes in soil and water. some species are also transmitted between man and animals ***(WHO, 2000).***

Escherichiacoli is a commensal bacterium of chickens' intestine. However, some strains, extra-intestinal avian pathogenic *E. coli* (APEC), are able to trigger invasive infections outside the intestine, namely colibacillosis ***(Barnes and Gross, 1997; Zhao et al, 2005).*** In avian strains of *E. coli* as with other bacterial pathogens, virulence is multifactorial and is associated with adherence factors (F1 and P-pili, and curli), the aerobactin iron-sequestering system, serum resistance, capsule production, and temperature sensitive haemagglutination (*tsh*) ***(Dho-Moulin et al, 1999).***

The virulence associated genes such as *fimC, astA, papC, tsh, fyuA, irp2, iucD, iss, hlyE, eaeA, vat, colV* and *stx2F* play important roles individually or in combination in adhesion, ferric transport system, hemolyzation and toxin production of avian pathogenic *E.coli* ***(Yaguchi et al, 2007)***

Multiplex PCR was used to identify traits that predict avian pathogenic *Escherichia coli* (APEC) virulence**.** five genes carried by plasmids were identified as being the most significantly associated with highly pathogenic APEC strains: *iutA, hlyF, iss, iroN,* and *ompT* ***(Johnson et al, 2008)***

This study was planned to identify biochemically and serologically to determine O- antigen in the prevalent *E. coli* in broilers farms in Dakahlia Governorate, Egypt. Also, for detection of virulence genes of *E. coli* using Polymerase Chain Reaction.

Material and methods

Samples collection according to *Waltman et al (1998).*

The samples were collected from 200 broiler chickens (60 recently dead, 80 diseased and 60 apparently healthy chicken). And these samples include liver, spleen , kidney, fresh heart blood, bone marrow and intestine. All samples were put in sterile plastic bags in ice box and transported directly to Mansoura laboratory (Animal Health Research Institute).

Isolation of the *E. coli* isolates.

The tested sample was initially inoculated into a non-inhibitory liquid medium to favour the repair and growth of stressed *E. coli*. The internal organs included liver, spleen , kidney, fresh heart blood, bone marrow and intestine were collected and pre-enriched in buffered peptone water as a 1:10 dilution and incubated at $37^{o}C\pm 1^{o}C$ for 18 h.

Pre-enrichment culture was streaked onto non selective and selective agars for the isolation of *E. coli* 10 µl loop-full from the inoculated and incubated pre enrichment culture were streaked on the surface of Nutrient agar, MacConkey agar, Xylose Lysine Deoxycholate agar (XLD agar) and Eosin methylene blue agar (EMB) and incubated at 37.0 ± 1^{o} C for 24 h.

Identification of *E. coli* isolates:

Microscopic examination

Suspected purified colonies were prepared, fixed and stained with Gram's according to ***Murray et al (2003).*** Then examined microscopically.

Biochemical Identification according to *Murray et al (2003):*

Purified isolates were examined by different biochemical reactions either by oxidase, urea hydrolysis, H_2S production on TSI, lysine decarboxylation, indole, methyl red test, Voges-Proskauer, citrate utilization, motility test and Analytical profile index 20 E (API 20 E)

Serological identification:

The preliminarily identified isolates biochemically as *E. coli* were subjected to serological identification according to ***Koneman et al (1999)*** for determination of (O) antigen using slide agglutination test.

Antibacterial sensitivity testing:

The disk diffusion technique was applied according to ***Cruickshank et al (1975).*** Results were recorded and compared with the standard levels to know whether *E. coli* isolates were sensitive, intermediate, or resistant. The interpretation of inhibition zones of test culture was according to ***NCCLS (2002)***

Detection of virulence genes in *E. coli* isolates using PCR:

1. Extraction of DNA according to **QIAamp DNA mini kit instructions**
2. Preparation of PCR Master Mix according to Emerald Amp GT PCR mastermix (Takara).
3. Cycling conditions of the primers during cPCR.
4. DNA Molecular weight marker.

Agarose gel electrophoreses ***(Sambrook et al, 1989).***

Table (1): *Cycling conditions of the different primers during cPCR.*

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
phoA	94˚C 5 min.	94˚C 45 sec.	58˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
iss	94˚C 5 min.	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	35	72˚C 10 min.
iutA	94˚C 5 min.	94˚C 30 sec.	63˚C 30 sec.	72˚C 30 sec.	35	72˚C 10 min.

RESULTS

The results illustrated in Table (2) demonstrated the Incidence of *E. coli* in examined broilers.

Results of positive cases showed high incidence of suspected *E. coli* isolates from dead chickens 92% (56 / 60), followed by diseased chickens 85% (86 / 80) and from apparently healthy chickens 70% (42 / 60) as shown in Table (2). The recovery rate of *E. coli* from

internal organs is clarified in Table (3).

As shown in Table (3), the highest incidence of *E. coli* was recovered from liver 83 % (166 / 200), followed by fresh heart blood 75 % (150 / 200), small intestine 74.5 % (149 / 200), bone marrow 67.5 % (135 / 200), kidney 64 % (128 / 200) and the lowest incidence was recovered from spleen 57 % (114/ 200).

The morphological characters of *E. coli* clarified that *E. coli* isolates were G-ve rods appeared as pink colonies when cultured on MacConkey media, yellow on XLD and green metallic colonies on EMB medium.

Biochemically, all *E. coli* suspected isolates were lactose fermenting colonies and positive indole, methyl red, and Catalase. Meanwhile all isolates were negative oxidase, urea hydrolysis, citrate utilization, Voges-Proskauer and not produced H_2S.

Results of serotyping of 20 *E.coli* isolates as shown in Table (4); revealed the high incidence of serotypes O_{29} and O_{115} 4 serotypes (20%), followed by O_{157} 3 serotypes (15%), then O_{142}, O_{128} and O_{158} 2 serotypes (10%) and 1 serotype from O_{111}, O_{44} and O_{55}.

Results of antibiotic sensitivity:

From Table (5); the most encountered antimicrobial agents were Ampicillin, Oxytetracycline, Doxycycline, Neomycin and Gentamycin in percentages 65, 55, 55, 55 and 50 % respectively while the lowest resistance percentages were to Erythromycin and Ciprofloxacin in percentages 25 and 20 % respectively.

Result of detection of *phoA* , *iss* and *iutA* genes of isolated *E. coli* by using multiplex PCR were presented in Table (6) and photo (1, 2 and 3).

From Table (6), it was clear that the six tested *E.coli* servoars contain the 3 virulence genes (*phoA* , *iss* and *iutA*) except **O_{29}** which not had *iss* gene.

Photo (1 ,2 and 3): Agarose gel electrophoresis of phoA, iss and iutA genes from randomly selected *E. coli* (6 isolates) L, 100 bp lambda marker; and lane 2 represented the negative amplification of iss gene from *E. coli* isolates recovered from different sources. Positive amplification were presented as follow lane 1 phoA gene (720bp), iss gene (266bp) and iutA gene (300bp) isolated from Liver; lane 2 phoA gene and iutA gene isolated from Liver; lane 3 phoA gene, iss gene and iutA gene isolated from Bone marrow; lane 4 phoA gene, iss gene and iutA gene isolated from Liver; lane 5 phoA gene, iss gene and iutA gene isolated from Liver; lane 6 phoA gene, iss gene and iutA gene isolated from Kidney.

Table (2) *Incidence of E. coli in examined birds:*

case	No. of birds	Positive cases	
		No.	%
Apparently healthy chickens	60	42	70%
Diseased chickens	80	68	85%
Freshly dead chickens	60	56	92%
Total	200	166	83%

Table (3) *Recovery rate of E. coli isolates from examined organs of broilers.*

Case / Organs	Apparently healthy chickens (n = 60)	Diseased chickens (n = 80)	Dead chickens (n = 60)	Total (n = 200)
Fresh heart blood	35	62	53	150 (75%)
Liver	42	68	56	166 (83%)
Kidney	25	57	46	128 (64%)
Small intestine	33	63	53	149(74.5%)
Spleen	17	48	49	114 (57%)
Bone marrow	25	60	50	135(67.5%)

Table (4): *Incidence and frequency distribution of E. coli serovars isolated from examined chickens.*

E. coli isolates	No. of serotyped isolates	*E. coli* serovars	No.	%
166	20	O29	4	20%
		O158	2	10%
		O128	2	10%
		O142	2	10%
		O115	4	20%
		O157	3	15%
		O55	1	5%
		O44	1	5%
		O_{111}	1	5%

Table (5): *Antibiotic susceptibility of 20 E. coli serovars recovered from chickens for 10 Antimicrobial agents.*

Antimicrobial agent	Conc. µg	Symbol	Susceptible		Intermediate		Resistant	
			NO.	%	NO.	%	NO.	%
Ampicillin	10	AMP	2	10	5	25	13	65
Gentamycin	10	CN	5	25	5	25	10	50
Neomycin	30	N	2	10	7	35	11	55
Doxycycline	30	DO	8	40	1	5	11	55
Oxytetracycline	30	OT	7	35	2	10	11	55
Erythromycin	15	E	6	30	9	45	5	25
Colistin	10	CT	10	50	4	20	6	30
Streptomycin	10	S	7	35	4	20	9	45
Ciprofloxacin	5	CIP	3	15	13	65	4	20
Florfenicol	30	Ffc	11	55	3	15	6	30

Table (6): *Detection of virulence genes in recovery 6 E. coli isolates by using multiplex polymerase chain reaction (mPCR):-*

No.	serotype	*phoA*	*iss*	*iutA*
1	O_{142}	+	+	+
2	O_{29}	+	-	+
3	O_{115}	+	+	+
4	O_{158}	+	+	+
5	O_{128}	+	+	+
6	O_{157}	+	+	+

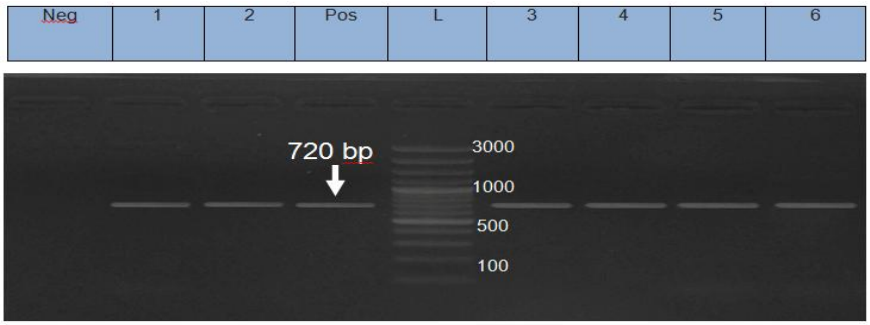

photo (1) Detection of *phoA* gene of *E. coli* at 720bp. lane 1 *phoA* gene (720bp) isolated from Liver; lane 2 isolated from Liver; lane 3 isolated from Bone marrow; lane 4 isolated from Liver; lane 5 isolated from Liver; lane 6 isolated from Kidney.

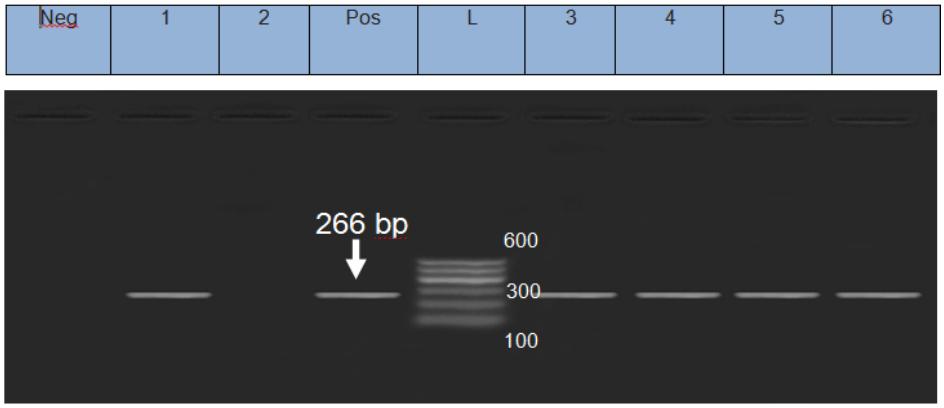

photo (2) Detection of *iss* gene of *E.* coli at 266bp. lane 1 *iss* gene (266bp) isolated from Liver; lane 2 was negative; lane 3 isolated from Bone marrow; lane 4 isolated from Liver; lane 5 isolated from Liver; lane 6 isolated from Kidney.

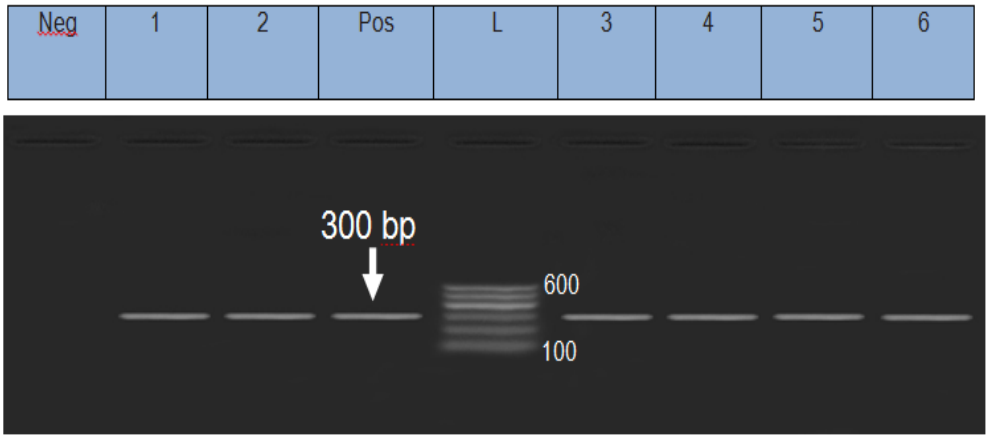

photo (3) Detection of *iutA* gene of *E. coli* at 300bp. lane 1 *iutA* gene (300bp) isolated from Liver; lane 2 isolated from Liver; lane 3 isolated from Bone marrow; lane 4 isolated from Liver; lane 5 isolated from Liver; lane 6 isolated from Kidney.

DISCUSSION

E. coli is considered as a member of the normal microflora of the poultry intestine, but certain strains such as those designated as avian pathogenic *E. coli* (APEC); spread into various internal organs and cause colibacillosis characterized by systematic fatal disease. ***(Someya et al, 2007)***

Bacteriological study was conducted using randomly organ samples from recently dead, disease and healthy broilers including liver, fresh heart blood, kidney, spleen, small intestine and bone marrow from ten

broiler farms located in Dakahlia governorate.

In general, investigation of 1200 organ samples collected from recently dead, disease and healthy broilers revealed that *E. coli* isolates was recovered from 842 samples with overall prevalence (70.16%). Nearly similar results were recorded by ***Abd El Latif (2004)*** who isolated *E.coli* at a percentage of (78.7%), ***Abd El-Salam (2004)*** who isolated 110 *E.coli* from broiler chickens at a percentage of (61%), ***Sharada et al (2009)*** who recovered *E. coli* from 65 (76.47%) samples out of the total 85 samples and ***Nashwa et al (2010)*** who isolated *E.coli* at a percentage of (75%)**.** Meanwhile ***Rahman et al (2004)*** who isolated *E.coli* at a percentage of (40.82%)***, Akond et al (2009)*** who isolated *E.coli* at a percentage of (58%) and ***Momtaz et al (2012)*** reported a lower prevalence for *E. coli* in a percentage ranged from (15.8 – 58%).while ***Abd El Aziz et al (2007)*** reported a higher prevalence for *E. coli* in a percentage 90%. Concerning fresh heart blood samples, 150 out of 200 samples of examined fresh heart blood were *E. coli* positive with an incidence of (75%). Nearly similar results were recorded by ***Saha et al (2003)*** who isolated 96 *E.coli* from of 165 samples (85%). Meanwhile ***Abhilasha and Gupta (2001)*** reported a lower prevalence for *E. coli* in a percentage ranged from (9.5 – 40.5%). Concerning liver samples, 166 out of 200 samples of examined liver were E. coli positive with an incidence of (83%). While lower prevalence was detected by ***Abhilasha and Gupta (2001)*** who isolated *E.coli* from the liver at a percentage of (13.97%)**,** and ***Saha et al (2003)*** who isolated *E.coli* from the liver at a percentage of (54.28%). Also, 128 out of 200 examined Kidney samples were *E. coli* positive with an incidence of 64%. However**,** ***Sepehri and Zadeh (2006)*** recorded higher occurrence of *E. coli* from tested poultry kidney samples (96%). Concerning small intestine samples, 149 out of 200 samples of examined small intestine were *E. coli* positive with an incidence of (74.5%). Nearly similar results were recorded by ***Saha et al (2003)*** who isolated *E.coli* from the small intestine at a percentage of (81.81%); Meanwhile ***Alireza et al (2007)*** reported a lower prevalence for *E. coli* in a percentage 37.5%. Moreover, 114 out of 200 samples of examined spleen were *E. coli* positive with an incidence of (57%). Nearly similar results were recorded by ***Yun-Shifeng et al (1997)*** who recorded the percentage of the isolated *E. coli* was 48.44%.while ***Saha et al (2003)*** reported a lower percentage 39.13%

Finally, bone marrow samples, 135 out of 200 samples of examined bone marrow were *E. coli* positive with an incidence of (67.5%). **Sepehri *and Zadeh (2006)*** recorded higher occurrence of *E. coli* from tested poultry bone marrow samples (96%), while (48.44%) lower

prevalence was reported by ***Yun-Shifeng et al (1997).***

This study revealed that the *E. coli* isolates were isolated from 842 (70.16%) out of 1200 broiler samples originated from different sources including; Fresh heart blood 150 out of 200(75%), Liver 166 out of 200 (83%), Kidney 128 out of 200(64%), Small intestine 149 out of 200 (74.5%), Spleen114 out of 200 (57%) and bone marrow 135 out of 200 (67.5%).

From the above mentioned results, it is obvious that *E .coli* isolates were recovered from poultry farms with higher prevalence from liver samples, followed by Fresh heart blood, Small intestine, Kidney, bone marrow and the lowest prevalence were from spleen. Furthermore, we can conclude that *E. coli* isolates were isolated from different organs at a percentage varied from (57%) to (83%). while the results (39.13%) to (81.81%) recorded by ***Saha et al (2003)***

Typing of isolated bacteria, including *E. coli* could be achieved by Phenotypic and/or genotypic protocols. The phenotypic characteristic method used for identification of *E. coli* includes the morphological and biochemical tests. Most of these techniques are not sufficiently sensitive to distinguish between different strains and they are affected by physiological factors. ***(Fantasia et al, 1990).*** Therefore, serological protocol was established to differentiate *E. coli* isolates. Regarding the morphological characters used for identification of *E. coli*, depend on that *E. coli* isolates are Gram-negative rods appeared as pink colonies when cultured on MacConkey media, green metallic colonies on EMB medium**.** Nearly similar results were noted by ***McClure (2000) and Hogan and Smith (2003).***

On the other aspect, results of biochemical tests by using traditional methods revealed that 90% of suspected isolates were biochemical identical to typical *E. coli* features and by using the API20E system for Identification of suspected isolated *E. coli* strains revealed that 100% of suspected isolates were biochemical identical to typical *E. coli* features. These results are similar to those recorded by ***(Timothy et al, 1985)***, who used the API 20E system for identification of isolated G-ve bacteria and observed that the API20E system identified about 98,9% of the isolated strains.

In the current study, 20 *E.coli* isolates recovered from broilers were serologically examined with available agglutinating sera according to ***Koneman et al (1999).*** The results clarified that , 1 *E. coli* isolate was serotype $\mathbf{O_{111}}$, 1 was **O44**, 1 was **O55**, 2 were serotyped **O142,** 2 were serotyped **O128,** 2 were serotyped **O158,** 3 were serotyped **O157**, 4 were serotyped **O29** and 4 were serotyped **O115**.

These finding were similar to the results that was recorded from the

chickens as the following; ***Taha et el (2002)*** and ***Ammar et al (2011)*** isolated O_{157} and O_{55}, ***Sharaf (2000)*** isolated O_{115} and O_{29}, ***Nashwa et al (2010)*** isolated O_{142}, ***Fatma et al (2008)*** isolated O_{157} and O_{111}, ***Perez-Guzzi et al (2000)*** isolated O_{44}, ***Kumar et al (1996)*** isolated O_{128} and ***Ibrahim et al (1997)*** isolated O_{55},O_{111} and O_{158} .

From the mentioned data, it was clear that the most prevalent *E. coli* serotype isolates recovered from examined broiler chickens samples were **O115** and **O29;** followed by **O157**; then **O158, O128** and **O142**; and finally the lowest prevalent serotype were **O55**, **O44** and O_{111}. These results go in hand with those reported by **Kumar *et al.*, (2003)** who recorded **O115** is one of the most predominant serogroups from many serotypes recovered from chickens (O_{20}, O_{54}, O_{61}, O_{73}, O_{78}, O_{88}, O_{89}, O_{111}, O_{115}, O_{119}, O_{132} and O_{153}).

Antibiotic resistance is increasing among many bacterial species and is rapidly becoming a major world health problem ***Glynn et al (1998) and Roberts (1998)***. Antimicrobials are valuable tools to treat clinical disease and to maintain healthy and productive animals; however the treatment of whole herds and flocks with antimicrobials for disease prevention and growth promotion has become a controversial practice ***Witte (1998); Van Den Bogaard and Stobherinsh (1999).***

Antimicrobial therapy is one of the primary control measures for reducing morbidity and mortality due to APEC associated avian coiibacillosis ***(Dho-Moulin and Fairbrother, 1999; White et al, 2000; Giraud et al, 2001 and Altekruse et al, 2002)***. Results of antibiotic sensitivity of serotyped *E. coli* recovered from broiler chickens showed that the majority of *E. coli* isolates possess resistance to ampicillin (65%). followed by Oxytetracycline, Neomycin and Doxycycline (55%). These results are nearly similar with ***Saha et al (2003)*** and ***El-Sayed and El-Hanafy (2005)*** who reported that the highest resistance was to ampicillin and tetracyclines. These results also were confirmed by ***Zahraei Salehi and Farashi Bonab (2006)*** who proved that the highest rate of resistance was against Oxytetracycline (95%), Doxycycline (88%), Neomycin (81%) and Ampicillin (47%).

The isolates of *E. coli* showed 30% resistence to clostin and florfenicol and 20% to ciprofloxacin nearly similar results was recorded by ***Zahraei Salehi and Farashi Bonab (2006)*** who found that the resistance to clostin and Florfenicol were (6%) and (27%) respectively and was recorded by ***Miles et al (2006)*** who found that the resistance to ciprofloxacin was 11.8%. And ***Bywater et al (2004)*** proved that the resistance was higher in case of old compounds than the newer compounds.

Finally one could conclude that the use of antibiotics is strongly

associated with the prevalence of antimicrobial resistance in *E.coli* isolates in food-producing animals ***(Kang et al, 2005).***

The present study was directed mainly to recognize some virulence genes, such as *(phoA, iss* and *iutA* genes) commonly found in *E. coli* isolated from various broiler farm samples by using one of the recent developments molecular biological techniques (multiplex PCR).Virulence genes of *E. coli* isolates recovered from broiler farms samples are shown in table (5) and photo 1 (A, B and C).

The choice of these genes due to iss and iutA were the most significantly associated with highly pathogenic APEC strains as mentioned by ***Johnson et al (2008)***.while *phoA* gene is a common gene specific to *E.coli*.

Concerning the examination of *E. coli* isolates for the detection of *phoA* gene showed that all isolates, table (5) yielded the expected size of 720 bp PCR amplification products for the *phoA* gene and photo 1 (A). Nearly similar findings were recorded by ***Barry and Patrick (1980)*** who isolated a large number of PhoA gene. PhoA can be used specifically to detect bacterial genes that code for cell envelope proteins that recorded by ***Claude Gutierrez et al (1987)***.

Regarding the occurrence of *iss* gene in *E. coli* isolates. The results revealed that out of 6 *E. coli* isolates recovered from broiler samples, 5 isolates were positive yielded the expected size of 266 bp PCR amplification by percent (83.3%) 5 out of 6 . However, PCR results were negative for *iss* gene in other *E. coli* isolate Table (5) and photo 1 (B). Nearly similar findings were recorded by ***Elaine et al (2003)*** who reported that the iss gene was detected significantly more often amongst colibacillosis isolates. Also, ***Christa et al (2007)*** stated that plasmid-related gene was detected in the majority of avian pathogenic *E.coli* with *iss present* in (74.8 to 86.7%)

Also, ***Catana et al (2008)*** recovered the gene iss which encodes a protein of the external membrane inducing resistance to the complement was present in 53 of the strains tested from 65isolates by percent 81.5%.

Concerning the examination of *E. coli* isolates for the detection of *iutA* gene showed that all isolates, table (5) yielded the expected size of 300 bp PCR amplification products for the *iutA* gene and photo 1 (C). Nearly similar findings were recorded by ***Elaine et al (2003)*** who reported that the *iutA* gene was detected significantly in all colibacillosis isolates.

Finally one could conclude that the detection of *phoA* gene is necessary during detection *E.coli* as *phoA* gene is a common gene specific to *E.coli*.

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التصنيف الجيني لعترات الإكولاى المعزولة من بدارى التسمين

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فى هذه الدراسة تم إجراء فحوصات بكتيرية لـ ١٢٠٠ عينه تم جمعها من ٢٠٠ طائر من بدارى التسمين (سليم ظاهريا - مريض - حديث النفوق) والتى أظهرت عزل الميكروب القولونى الإكولاى من ٨٤٢ عينة بنسبة ٧٠,١٦%. وكانت نسبة العزل من الدم من القلب ٧٥% ، و من الكبد ٨٣ % ، و من الكلية ٦٤ %، ومن الطحال ٥٧ % ، ومن الأمعاء الدقيقة ٧٤,٥ % ، و من نخاع العظام ٦٧,٥ % .و باجراء التصنيف السيرولوجي لعشرين عزلة من عزلات الميكروب القولونى الإكولاى المعزولة من عينات الدواجن أوضحت النتائج أن المعزولات تشمل عترة واحدة من O_{111}، عترة واحدة من O_{44}، عترة واحدة من O_{55}، عترتان من O_{142}، عترتان من O_{128}، عترتان من O_{158}، ثلاثة عترات من O_{157}، أربع عترات من O_{29}، أربع عترات من O_{115}. كما تم اجراء اختبار إنزيم البلمرة المتسلسل التعددي لستة من المعزولات الأتية: (O_{142}, O_{29}, O_{115}, O_{158}, O_{128}, O_{157}) تبين وجود الثلاث جينات (*phoA, iss* and *iutA*) فى جميع العترات باستثناء جين (*iss*) غير موجود فى عترة O_{29}.