

## Phenotypic Characterization of *Escherichia coli* Isolated from Broiler Chicken

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### Abstract

*E. coli* is one of the most common isolates in avian diseases, which causes colibacillosis. In this study a total of 114 *E. coli* isolates were recovered from 500 chicken samples including heart, liver and rectal swabs samples with a percentage of 12.8 % from broilers with a history of respiratory manifestations and postmortem lesions (pericarditis, per hepatitis and air sacculitis) in Sharkia province. The recovered *E. coli* isolates were typed serologically into 10 different 'O' groups including O1, O2, O26, O44, O78, O91, O111, O121, O125 and O128. Untypeable isolates were also recovered. The results of congo red test revealed that (40/500) of *E. coli* isolates from rectal swabs were positive. The susceptibility of identified *E. coli* isolates to a panel of seventeen commonly used antimicrobial agents and *E. coli* isolates showed the the highest resistance against lincomycin (100%), tetracycline (96.8%), streptomycin (93.7%), trimethoprim sulfamethoxazole, ampicillin, erythromycin and florfenicol (92.18%), which being the least effective antimicrobial agent against *E. coli*, while the least resistance rate was detected against apramycin (32.18%).

### 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. 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 asystemic infection with characteristic fibrinous lesions (airsacculitis, perihepatitis, and pericarditis) and fatal septicemia *Ewers et al., (2005) and Sharada et al., (2010)*.

*E. coli* is serologically classified according to its antigenic

composition into somatic (O and flagella (H) antigens *Compos et al., (2004)*.

Thus, the current study was undertaken for (i) isolation and identification of *E.coli* from organs and cloacal swabs samples of chicken morphologically, biochemically and serologically (ii) Antibiotics sensitivity testing for *E.coli* isolates .

### **Material & Methods:**

A total of 500 broilers chickens that had died from colibacillosis with typical preceding symptoms like septicemia, respiratory infections. from different sources in Sharkia Governorate, Egypt. during the period of March 2016 to December 2017. The samples were aseptically collected in sterile containers and immediately transported in an ice box to the laboratory for further bacteriological examination according to the technique recommended by (*Quinn et al.,2002*).

The isolates were serologically identified according to *Kok et al. (1996)* by using rapid diagnostic *E. coli* antisera sets (*DENKA SEIKEN Co., Japan*) for diagnosis of the Enteropathogenic types. Various serotypes were tested for pathogenicity based on Congo red dye binding test as described by *Berkhoff and Vinal (1986)*. The susceptibility of identified *E. coli* isolates to a panel of 17 commonly used antimicrobial agents was performed by the standard Kirby-

Bauer disc diffusion method *Bauer (1966)* and the results were interpreted according to the criteria recommended by the *CLSI (2015)*.

### **Results & Discussion:**

a total of 114 *E. coli* isolates with a percentage of 12.8 % were recovered from 500 specimens from each of liver, heart and rectal swabs.

There is a common isolate (n=50) between liver, heart and fecal swabs so the two isolates from liver and heart, liver and rectal swabs, heart and rectal, liver &heart and rectal of the same chicken sample were considered one strain. Therefore, number of *E. coli* strains 64 by percentage 12.8% out of 114 *E. coli* isolates.

a lower rate was recorded by (*Gomis et al., 2001 and Zhao et al., 2001*) who isolated *E. coli* with a prevalence of 34.6% and 38.7 % respectively. while Higher rates also were recorded by *El-Boraay and Abo-Table (2002)* who examined 110 broilers with a history of colibacillosis ., 82 isolates were obtained from livers, lung and intestine of the autopsied birds with a prevalence rate of (74.54%) . in addition, other researchers isolated *E. coli* from chickens with a percentage of 57.1%, 36.2%, 76.5%, 75%, 55 % and 92 % (*Dutta et al., 2011; Hassan 2011; Sharada et al., 2010; Claudie et al., 2009; Salama et al., 2007 and Johnson et al.,2005 respectively*). *Lyhs et al. (2012), Eid and Erfan (2013),*

*Peer et al. (2013) and Hamza et al. (2016)* recovered *E. coli* with a prevalence rate of 94.5%, 80%, 84% and 60% respectively.

As seen in **table (1)**: The bacteriological examination revealed that fresh heart blood samples, 36 *E. coli* isolates with a prevalence rate of 7.2%. Similar results were recorded by *Ahmed et al. (2013)* who obtained 20 *E. coli* isolates out of 50 samples of examined fresh heart blood with an incidence of 40%.

Regarding examined liver samples, 38 *E. coli* isolates with a prevalence rate of 7.6%. Nearly similar results were recorded by *Sharada et al., (2010)* who recovered maximum isolates of *E. coli* from cases of perihepatitis (44.61%).

Isolation percentage from liver and heart is nearly close (38 % and 36 %) because sampling may be during septicemia. Similar observation was recorded by *Antao et al. (2008)* who reported that Colibacillosis is often lethal to poultry, particularly broilers. The causative agent, *E. coli* gains entry into the bloodstream from an infected site, primarily the respiratory tract, via translocation across air capillary walls, causing bacteria spread to various internal organs, resulting in septicemia and death of the birds.

examined rectal swabs samples 200 *E. coli* with a prevalence rate of 40% out of 500 chicken samples

also *El-Jakee et al. (2012)* isolated 12 (12 %) *E. coli* isolates from 100 cloacal swabs of diarrheic chicken. *Dipineto et al. (2006)* detected *E. coli* in 26 (3.6 %) of the 720 cloacal swabs.

As seen in **table (2)**:

The recovered *E. coli* isolates were typed serologically into 10 different 'O' groups including O1, O2, O26, O44, O78, O91, O111, O121, O125 and O128. Untypeable isolates were also recovered. Nearly similar results were obtained by *Ammar et al. (2014)* who recovered 8 different 'O' groups including O26, O44, O55, O78, O111, O114, O125 and O127. One untypeable strain was also recovered.

The most prevalent *E. coli* serotypes were untypeable with 28% of the total isolates, followed by O78 with 21.9 %, O2 with 9.6 %, O91 with 7 %, O125 with 6.1 %, (O1, O44, O128 and O111) which had the same isolation rate with 5.3 % each, O26 with 3.5 % and finally the lowest prevalent serotypes were detected to O121 with 2.6 %. Similar results were recorded by *El-Morsi (1998)* who recovered 5 *E. coli* strains from 25 livers of poultry. The isolated serotypes of *E. coli* from liver samples were 2 untypeable (40%), 2 belonged to O111:K58 (40%) and one was O126:K71 (20%).

High percentage of untypeable isolates in APEC was previously recorded by numerous studies

regardless of their geographic location *Zhao et al., (2005), Ewers et al. (2009), Hussein et al. (2013) and Yousef et al. (2015).*

*Oh et al. (2012)* found that 30 strains (51.7%) were untypeable by O serogrouping because of autoagglutination and an incomplete antisera panel.

Serotyping in this study revealed that the most prevalent serogroups belonged to serogroups O78 which go in parallel with *Dho-Moulin and Fairbrother (1999), La Ragione and Woodwar (2002), Yaguchi et al. (2007) and Ozaki and Murase (2009)*. Much more than half of the isolates could not be assigned to the common serogroups. This supports the suggestion that serotyping is not recommended as a specific diagnostic tool for the identification of avian pathogenic *E. coli* (*Ewers et al., 2005*).

The results of congo red test revealed that (40/500) of *E. coli* isolates from fecal swabs were positive. These results agreed with those obtained by (*Sharda et al., 2010*) who also reported a clear relationship between the expression of congo red and the pathogenicity in avian *E. coli* and stated that it was due to presence of B-D-glucan in bacterial cell wall. (*Yoder, 1989*) has reported that Congo red binding did not correlate well with pathogenicity.

The negativity of all the isolates to hemolysis on 5% sheep blood agar is in accordance with (*Erganis et al., 1989*) who attributed heavy

mortality in chicks due to non-hemolytic strains indicating that avian pathogenic *E. coli* to be independent of hemolytic activity, (*Sharada et al., 1999*) who reported that avian *E. coli* to be pathogenic needed not to be hemolytic and (*Rodriguez et al., 2005*) who reported that none of their isolates from colisepticemic cases was positive for hemolysis on 5% sheep blood agar.

As revealed in **table (3)** antibacterial susceptibility profiles of *E. coli* isolates showed the highest resistance against Lincomycin (100%), tetracycline (96.8%), streptomycin (93.7%), trimethoprim sulfamethoxazole, ampicillin, erythromycin and florfenicol (92.18%), which being the least effective antimicrobial agent against *E. coli*, while the least resistance rate was detected against apramycin (32.18%). Which agreed with *Eid and Erfan (2013)* found that the highest resistance rates were recorded against Doxycyclin (100%), while the resistance against Penicillin, Lincomycin, tetracycline and Oxitetracycline were (96.4% each). The highest sensitivity rates were recorded to Ciprofloxacin (75%) and Gentamycin (50%).

also *Subedi et al. (2018)* reported maximum resistance to ampicillin (98%), followed by co-trimoxazole (90%), and doxycycline (62%). The highest intermediate resistance was shown by colistin (50%) and the highest sensitivity was against

amikacin (84%), followed by nitrofurantoin (55%).

The results of antibiotic susceptibility testing of this study are invariance with some studies and in accordance with others, indicating that antibiotic susceptibility pattern varies with different isolates, time and development of multiple drug resistant *E. coli* (*Eid and Erfan, 2013*).

Failure of antibiotic treatment in controlling some disease cases caused by *E. coli* may be partially explained by the spread of drug resistance. Multiple drug resistance (MDR) is a serious problem and has attained a hazardous level. Practices such as indiscriminate use of antimicrobial drugs, exhaustive use of certain antibiotics as feed additives for growth promotion, their supply without prescription and their use in sub curative doses can constitute the root of their drug

resistance problem (*Salwa et al., 2007*).

In the current study, all tested *E. coli* isolates showed multidrug resistance pattern. The noticed results are partially similar to those obtained in a previous scientific literature, where 24.5% of *E. coli* isolates from different sources were MDR against ten antimicrobial agents (*Kurutepe et al., 2005*). Moreover, MDR *E. coli* isolates were highly detected by several researches in Bangladesh, Canada and Swiss (36.6%, 2.5% and 87.5%, respectively) (*Hassan et al., 2011; Mainali et al., 2013 and Abgottspon et al., 2014*).

The present study showed that there is an emerging drug resistance problem in APEC associated with colibacillosis in Egypt. The observed high level multidrug resistance could hamper the treatment of colibacillosis (*Saidi et al., 2013*).

**Table (1): Isolation rate of *E. coli* from different organs**

Item	Heart Blood	Liver	Fecal swabs
Number of samples	500	500	500
Number of isolated <i>E.coli</i>	36	38	40
Isolation rate	7.2 %	7.6 %	40 %

**Table (2): Percentage of detected serotypes based on total number of *E. coli* isolates**

Types of pathogenic <i>E. coli</i>	Serotypes	Number of isolates	Prevalence of serotypes (%)
	O1 : H7	6	5.3
	O2 : H6	11	9.6
	O44 : H18	6	5.3
	O78	25	21.9
ETEC	O125 : H21	7	6.1
	O128 : H2	6	5.3
EHEC	O91	8	7
	O26 : H11	4	3.5
	O111 : H4	6	5.3
	O121	3	2.6
Untypeable		32	28
Total		114	100

**EPEC** : Enteropathogenic *E. coli*

**ETEC**: Enterotoxigenic *E. coli*

**EHEC**: Enterohemorrhagic *E. coli*

**Table (3): Antimicrobial susceptibility profile of *E. coli* isolates**

Chemotherapeutic Agent	Susceptibility profiles of <i>E. coli</i> isolates					
	Sensitive isolates		Intermediate isolates		Resistant isolates	
	Number	%	Number	%	Number	%
Apramycin	36	56.25	7	10.94	21	32.81
Gentamicin	29	45.31	1	1.56	34	53.12
Norfloxacin	28	43.75	3	4.69	33	51.56
Doxycycline	17	26.56	21	32.81	26	40.62
Neomycin	16	25	19	29.69	29	45.31
Ciprofloxacin	16	25	17	26.56	31	48.43
Spectinomycin	8	12.5	0	0	56	87.5
Florfenicol	5	7.8	0	0	59	92.18
Amoxicillin-clavulanic acid	5	7.8	20	31.25	39	60.93
Trimethoprim\sulphmethozole	5	7.8	0	0	59	92.18
Colistin sulphate	5	7.8	15	23.44	44	68.75
Erthromycin	4	6.25	1	1.56	59	92.18
Cefepime	2	3.125	6	9.38	56	87.5
Streptomycin	2	3.125	2	3.13	60	93.75
Lincomycin	0	0	0	0	64	100
Tetracycline	0	0	2	3.13	62	96.87
Ampicillin	0	0	5	7.81	59	92.18

Percentage is calculated from the total number of samples (64)

## Conclusion

### It can be concluded that:

1- Bacterial examination confirmed cases of colibacillosis from which 114 *E. coli* were isolated.

□□□□- All isolates had characteristic biochemical features of *E. coli*. The fact that the majority of APEC strains in this study were untypeable confirms the need to use other characterization methods to describe the APEC pathotype.

□- The increasing alarm of antibiotic resistance observed in indifferent serogroups of *E. coli* isolates from chickens that cause failure of treatment strategies for human beings.

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## الملخص العربي

### "العوامل الضاره والتصنيف الجيني لميكروب الايشيريشيا كولاى المعزوله من الدواجن"

تعد الإيشيريشيا كولاى احدى الكائنات الدقيقة الطبيعية الموجودة فى أمعاء الدواجن ولكن بعض السلالات تمتلك عوامل الضراوة وتسبب مرض الكولي باسيلوزيس والذي يعد أحد أهم الأمراض التي تصيب الدواجن و يؤدى الي خسائر اقتصادية فادحة في صناعة الدواجن في أنحاء كثيرة من العالم، لذا تهدف هذه الدراسة لتحديد مدى انتشار الإيشيريشيا كولاى و مناقشة توزيع جينات الضراوة عبر الإيشيريشيا كولاى المعوية المنزفة في الدجاج. تم تجميع خمسمائه عينة من الدجاج (الطيور المريضة) والتي تعاني من التهاب غشاء التامور ، التهاب حوائط الكبد ، و التهاب الاكياس الهوائية في محافظة الشرقية . وقد خضعت كل العينات للعزل و التصنيف البيوكيميائى للإيشيريشيا كولاى. وقد تبين بالتحليل البكتيرى تواجد الميكروب القولونى بنسبه ( ١٢,٨ ٪ ) من العينات التي تم جمعها. من بين ٥٠٠ عينه تم فحصها من قلوب دجاج مصاب كانت ٣٦ عينه ايجابية للميكروب القولونى بنسبه عزل ٧,٢% بينما كانت نسبه العزل من الكبد ٧,٦% ومن المسحات ٤٠%. تم اجراء التصنيف السيرولوجى لمعزولات الايشيريشيا كولاى وقد أظهرت نتائج التصنيف السيرولوجى ١٠ عترات مختلفة من الميكروب و كان السائد فيها العترات غير المصنفة بنسبة ٣٢ ٪ من مجموع المعزولات ، تليها O78 بنسبة ٢١,٩ ٪ ، O2 بنسبه ٩,٦ ٪ ، O91 بنسبه ٧ ٪ ، O125 بنسبه ٦,١ ٪ ، ( O1, O111 O128 O44) بنفس النسبه ٥,٣ ٪ ، O26 بنسبه ٣,٥ ٪ ، O121 بنسبه ٢,٦ ٪. تم إجراء اختبار الحساسية لكل العترات وقد وجد أن اكثر المضادات مقاومة هي اللينكوميسين والتتراسيكلين و الاستربتوميسين و تراه مسبرين و الامبيسلين و الايسرروميسين والفلوروفينيكول الاقل مقاومه كانت الابراميسين . اوضحت النتائج ان كل المعزولات كانت مقاومة لكل المضادات الحيويه.