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Isolation and characterization of avian pathogenic *Escherichia coli* from broiler chickens in some Governorates of Egypt

***Amer, M.M.*[1]*; Bastamy, M.A.* [1]*; Ibrahim, H. M.* [2] *and Salim, Mervit M.* [3]

[1]Department poultry and Rabbit diseases, Fac. Vet. Med., Cairo Univ., Giza 12211, Egypt.
[2]Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.
[3]Master degree student.

Abstract

A total of 80 samples (liver, heart, lung and gall bladder) were collected from diseased and freshly dead broiler chickens from different poultry farms reared in (Zagazig) Sharkia, Ismailaia, Sinai, (Dahshor) Giza and Kaluobaia Governorates for bacteriological examination for *E-coli* infection. The results revealed that 49 out of 80 samples (61%) were positive for *E-coli* isolation. The result of in vitro Pathogenicity testing by Congo red (CR) binding assay indicates that 14 isolates (28.6 %) were CR positive. The most predominant serotypes were O125,O114 and O44 as each represent (2) isolates out of (14) followed by O78, O86, O158, O127, O91, O25, O119 and untypable as each represent (1) isolate out of (14) isolates. Now in this study we are straining and updating available circulating strains of avian pathogenic *Escherichia coli* (APEC) micro-organisms to control colibacillosis in broiler chickens either by treatment or vaccination.

Key Words: Pathogenic -*E-coli* – Broiler – Isolation – Congo red assay.
corresponding author: **Amer, M.M.** **Email:** profdramer@yahoo.com

Introduction

Escherichia Coli strains designated as avian pathogenic *E-Coli* (APEC) are responsible for avian Colibacillosis, acute and largely systemic disease that promotes significant economic losses in poultry industry worldwide because of mortality increase, medication costs, and condemnation of carcass. APEC is a subgroup of extra-intestinal pathogenic *E-Coli* pathotype, which includes uropathogenic *E-Coli*, neonatal meningitis *E-Coli* and septicemic *E-Coli* (**Kobayashi *et al.*, 2011**). Several reports are available on the involvement of serotypes of *E-coli* in poultry diseases **(Roy *et al.*, 2004)** and so on. The pathogenic and non-pathogenic strains in poultry are differentiated based on the virulence which has been attributed to various factors like fimbriae, production of colicin, motility and embryo lethality. Hence, detection of these strains become important for effective treatment and control **(Susantha *et al.*, 2001).** In view of the significance of *E. coli* infection in poultry.

This study has been undertaken to isolate and characterize *E-coli* form different pathological conditions and to determine the prevalent serotypes and there vitro pathogenicity.

Material and Methods

2.1. Sample collection & Transportation:

A total of 80 samples (liver, heart, lung and gall bladder) were collected from diseased freshly dead broilers chickens from 80 different poultry farms located in Sharkia, Ismailaia, Sinai, Giza and Kalubaia Governorates in separate zipper lock bag, kept in ice box and immediately transported to the laboratory. Dead chickens showed lesions of Colibacillosis or chronic respiratory disease (CRD). The available flocks history with their relation to the previous work researches are shown in table (3).

2.2. Bacterteriological examination:

A loopfuls from each were organs inoculated onto nutrient broth and incubated aerobically at 37ºC for 12 hours. Loopfuls from incubated nutrient broth were streaked onto Eosin methylene blue (EMB) agar plates and incubated for 24 hours at 37ºC. Suspected colony was picked up and streaked on the MacConkey's agar plates then incubated for another 24-48 hours at 37ºC. The suspected lactose fermented colonies were picked up and kept in Semi-solid agar for morphological and biochemical identification **(Konemann *et al.*, 1992; Quinn *et al.*, 2002).**

2.3. In Vitro Pathogenicity Testing:

Various serotypes were tested for pathogenicity based on Congo red dye binding test as per the technique of **Berkhoff and Vinal (1986)**. Each isolate was cultured on a separate plate of Trypticase soy agar supplemented with 0.003% Congo red dye (Sigma) and 0.15% bile salts. Appearance of deep brick red colonies after incubated at 37°C for 24 hrs was recorded as positive.

2.4. Serological typing of *E-coli:*

The obtained 14 biochemically and Congo red positive *E-coli* isolates were subjected to serological identification **(Edward *et al.*, 1972)** using slide agglutination test.

Results

On Eosin Methylene blue (EMB) agar showed distinctive green metallic sheen with a black center colonies, medium sized rounded pink colonies on MacConkey's agar media while showed small sized dark brick red colonies on Congo red dye media as a result of in vitro pathogenicity test. *E-coli* isolates were Gram negative, motile, non-sporulated, medium sized bacilli arranged single, pairs and in groups when stained with Gram's stain. The suspected *E-coli* isolates were confirmed by biochemical tests and showed negative Urease test, negative Citrat utilization test, positive Indole test, (A/A with H_2S gas production and A/A without H_2S gas production) Triple sugar(TSI), (+ve) Motility on semisolid agar media. There were found inactive biotypes that were an aerogenic.

It was found that 2 serotype was isolated from the same bird; serotype (O114) and serotype (O158); suspected colibacillosis diseased freshly dead broiler chicken (samples no. 5 and 6). The most predominant serotypes were O125, O114 and O44 as each represent two isolates out of fourteen followed by O78, O86, O158, O127, O91, O25, O119 and untypale as each represent one isolates out of fourteen. There was found that we could isolate serotype (O125) and serotype (O44) from liver of sample no. (8) & heart of sample no. (7) of suspected colibacillosis diseased dead native breed broilers of the same flock. The results of the Congo red (CR) binding assay indicates that 14 isolates (28.6 %) were positive and 35 isolates (71.4%) were negative.

Table (1): Serogrouping of biochemically positive *E-coli* isolated from broilers chickens:

Isolate No.	Somatic Antigen	Capsular Antigen	Serotype
1	O125	K70	O125(K70)
2	O119	K69	O119(K69)
3	O78	K80	O78(K80)
4	O86	K-	O86(K-)
5	O114	K90	O114(K90)
6	O158	K-	O158(K-)
7	O125	K70	O125(K70)
8	O44	K74	O44(K74)
9	O127	K63	O127(K63)
10	O114	K90	O114(K90)
11	O91	K-	O91(K-)
12	O44	K74	O44(K74)
13	O25	K11	O25(K11)
14	Untypale		

No. tissues samples	No. of +ve Congo red	O 25	O 114	O 44	O 78	O 86	O 127	O 158	O 119	O 91	O 25	NT	%
Liver (23)	9	2	2	1	-	1	1	-	1	-	1	-	39%
Heart (20)	3	-	-	1	-	-	-	-	-	1	-	1	15%
Lung (3)	1	-	-	-	1	-	-	-	-	-	-	-	33.3%
Gall bladder (3)	1	-	-	-	-	-	-	1	-	-	-	-	33.3%
Total (49)	Total (14)	2	2	2	1	1	1	1	1	1	1	1	28.6%

Table (2): Distribution of Congo red positive *E-coli* Serotypes isolates in relation to tissues of broiler chickens

Table (3): The relationship between the isolated serotypes from field case and the previous work researches :

Isolate no.	O type & organ	Breed	Age / history	PM lesions	Previous Work Researches
1	O125 liver	White broilers	32days 8,000, 60 dead daily	perihepatitis, pericarditisa and airsaculitis.	Andrawis(1980), Zahdeh(1982), Sahar Zou El-Fakar (1994).
2	O119 liver	White broilers	35days, 5.000	CCRD	Awaad(1972),Bozorgmerhri and Gilani(1980), Zahdeh(1982), Farid et al(1983),El-Sayed(1987), Aly(1989), Khalid(1990)
3	O78 lung	Sasso	45 days, 8.000, 15 dead daily	perihepatitis, pericarditis, airsaculitis and pneumonia.	Abd El Nasser(1976), Bassiouni et al (1979), Hassanain (1983), Karmy et al (1987), Singab(1989), Mukherjee et al., (1997), Blanco et al(1998), Gomis et al(2001), Salama et al (2007), Xlang et al (2012)
4	O86 liver	Sasso	35 days, 15 dead daily	perihepatitis	Awaad(1972), Azzam(1983), Hassanain (1983), Torky et al (1995)
5	O114 liver	Sasso	56days, 15 dead daily	CRD	Awaad (1972), Farid et al(1983), Hassanain (1983), Sahar Zou El- Fakar (1994), Nagwa (1995), Torky et al(1995), Ghosh et al., (2002), Xiang et al.,(2012).
6	O158 gall bladder	Sasso	56days, 15dead daily	CRD	Mukherjee et al.,(1997), Shahin et al., (2011), Jana et al.,(2013)
7	O125 liver	native bread	42days, 10.000, 25 dead daily	CCRD	Andrawis (1980), Zahdeh (1982), Sahar Zou El- Fakar (1994).
	O44 heart	native bread	42days, 10.000, 25 dead daily	CCRD	Nagwa (1995), Shahin et al. (2011)
9	O127 liver	White broiler	32days, 5.000,12 dead daily		Awaad (1972)), Zahdeh (1982), Farid et al (1983), Sahar Zou El- Fakar (1994), Nagwa (1995), Ibrahim(1997) Taha et al.,(2002), Chen et al., (2012).
10	O114 liver	Layers	80 days, 9.000, 10 daily dead	prolapsed oviduct	Awaad (1972), Farid et al(1983), Hassanain (1983), Sahar Zou El- Fakar (1994), Nagwa (1995), Torky et al (1995)
11	O25 liver	White broiler	16 days, 8,000, 8 dead daily	CRD	Sinha et al (1985), Musumeci et al(2012), Jana et al.,(2013).
12	O91 heart	White broiler	22 days, 5,000, 10 dead daily	perihep. and mild CRD)	Dutta et al (2011), Xiang et al.,(2012).
13	O44 liver	White broilers	25 days, 6.000, 30 dead daily		Nagwab(1995), Shahin et al.(2011)
14	Untypable heart	Sasso	56 days, 15 dead daily	CRD	Taha et al.,(2002), Shahin et al.(2011)

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 end 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 enable to live extra intestinal life **(Johnson *et al.*, 2006).**

The serotypes isolated in this study were in accordance with **Ibrahim *et al.* (1998)** and **Singh and Gupta (1996).** The very low per cent Serotypes O2 and O78 may probably be due to variation in serotypes over a period of time in a particular area **(Belitski and Panika, 1969).** The correlation between the isolates and the disease condition could not be established **(Mukherjee and Mishra, 1995).**

The results of *in vitro* pathogencity testing were in agreement with **Berkhoff and Vinal (1986),** who also reported a strong correlation between expression of CR phenotype and virulence in avian *E-coli* and suggested that it was associated with the presence of p-D-glucan in bacterial cell wall. Previous studies also indicated that isolates of virulent avian *E-coli* can be identified by their ability to bind Congo red **(Singh and Gupta, 1996).** The characteristic of CR binding constitutes a moderately stable, reproducible and easily distinguishable phenotypic marker. Nevertheless, **Yoder (1989)** has reported that Congo red binding did not correlate well with pathogenicity.

In conclusion, the present study clearly demonstrates that *E-coli* is one of the major pathogen responsible for various types of disease conditions in poultry leading to

economic losses to poultry industry. Almost serotypes of *E-coli* isolated have been found to be pathogenic, but no particular serotype could be attributed to a particular disease condition or a particular age group.

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عزل وتوصيف الميكروب القولونى الممرض للدجاج من دجاج التسمين في بعض محافظات مصر

محمد محروس عامر [1] ، مصطفى احمد بسطامى[1] ، حازم محمد ابراهيم [2] ، ميرفت محمد سالم [3]

الملخص

فى هذه الدراسه تم جمع عدد 80 عينة (الكبد والقلب والرئة والمرارة) من دجاج التسمين المريضة والنافقة حديثا من مزارع الدواجن مختلفة تربى في الشرقية و الاسماعلية و سيناء و الجيزة و القليوبية و ذلك لفحصها بكتريولوجيا لعدوى الميكروب القولونى. و أظهرت النتائج عزل 49 معزولة من 80 عينة بنسبة 61%. تم إجراء اختبار الضراوة للمعزولات باستخدام اختبار الكونغو ريد و وجد 14 معزولة ايجابية بنسبة 28.6%. أظهرت نتائج التصنيف المصلى لعدد 14 معزولة من الميكروب القولونى الأتى: تم التعرف على سلالات O44 وO114,O125 حيث مثلت كل سلالة بمعزولتين من 14 معزولة. تم التعرف أيضاً على سلالاتO119, O25, O91, O127, O158, O86, O78 حيث مثلت كل سلالة بمعزولة واحدة من 14 معزولة. بينما معزولة واحدة فقط لم يتم التعرف عليها مصلياً.