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DIARRHEIC SYNDROME IN BROILER AND SOME WILD BIRDS CAUSED BY *ESCHERICHIA COLI*

GHADA, O. EL-DEMERDASH [1]; FATMA AMER [2] AND HEBA ROSHDY [2]

[1]Animal Health Research Institute, Fayoum Branch, Agriculture Researches Center, Egypt

[2] Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Ministry of Agriculture, P.O. Box 246 – Dokki, 12618 – Giza, Egypt

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ABSTRACT

E. coli is a Gram negative bacterium, although it is normal intestinal inhabitant but some strains due to their virulence genes play a major role in causing diarrhea in birds. In the present study, a total of 150 fecal swabs from (crows, egret wild birds and broiler chicken) collected from Giza, Fayoum city governorates, (50 each). The result showed, that isolation rate of *E.coli* was reported to be isolated in higher incidence in Fayoum governorate from crows, 48%, broiler chicken, 40% and egret wild birds 28% while Giza Governorate in where *E.coli* was isolated from crows, egret wild birds and broiler chicken ,an incidence of 20%, 12% and 20% respectively. The serogroups of *E. coli* strains that obtained by serological identification were from crows (O78, O91, O145, O127, O158, O119, O125 and O55), egret bird (O78, O158, O125, O119, O91 and O44) and chicken broiler (O78, O125 and O158). The results of sensitivity test for some *E. coli isolates* showed that they were highly resistant for to streptomycin (83.4%66.4%and 42.8%) in (crows, egret birds and broiler chicken) respectively. The results of multiplex PCR showed that *phoA, virulence* gene was detected in all *E.coli* serogroups while, *Stx2, gene was* detected in serogroups O78, O91 and O125 in crows only. *(hly, eaeA* and *Stx1*) virulence genes were not detected in all tested *E. coli* sergroups. On the other hand *aadA1* gene was detected by some *E. coli* strains (7from crows and 2 from egret).

Keywords: broilers chicken, *E. coli*, resistant genes, and antimicrobial resistance, Wild birds.

INTRODUCTION

Wild birds is important vectors and reservoirs for fecal pathogens in coastal areas. As vectors of many diseases has taken big interest recently, Also these birds have migratory behavior causes dissemination of multi- resistant (MR) bacteria through colonized or infected with resistant bacteria (Guenther *et al.*, 2011; Oteo 2018; Arnold *et al.*, 2016). From bad habits of human help in attracted wild birds to garbage, manure, untreated sewage so those birds carry many pathogens like *Salmonella* enterica *E.coli, and Campylobacter* spp (Moore *et al.*, 2002; Fogarty *et al.*, 2003; Waldenström *et al.*, 2003) *E.coli* The importance as that it found in food, and environment it also harm animal and human as it is opportunistic bacteria (Benskin *et al.*, 2009; Lisa *et al.*, 2013).

Corresponding author: Heba Roshdy
E-mail address: roshdy2019 @yahoo.com
Present address: Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Ministry of Agriculture, P.O. Box 246 – Dokki, 12618 – Giza, Egypt

The nature of life of crows and egret birds as one of wild birds which living near villages and towns, it disseminate *E.coli* through feases to the environment causes spread of infection to animals, birds, human through biological or mechanical way (Clark, 2003; Hbalck, 2004; Mbanga *et al.,* 2015) especial if its aggregation is found near the domestic rearing which causes many economic losses through dissemination of pathogens and also act as carrier and transporter to infection between animals birds and human (Ishii *et al.,* 2007; Maysa *et al.,* 2013). Also its droppings contain nutrient matters that attracts flies which help in transfere microorganisms (Johnson *et al.,* 2007). Recent studies have Proved that wild bird and rooks shedding bacteria which resistant to antibiotics (Hasan *et al.,* 2015; Jamborova *et al.,* 2017; Keya *et al.,* 2019). The molecular differentiation of different *E. coli* strains could give guidance for epidemiological studies of sources of infection and disease transmission. A random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) is quicker and more effective procedure to differentiate variant isolates of *E. coli.* The distinctive DNA patterns generated by RAPD for each *E. coli* isolate reflects genetic diversity present in a bird species (Gomes *et al.*, 2005). The aim of this present work was to characterize and investigate the Prevalence and characterize the *E. coli* isolates from crows, egret birds and broilers chicken (serologically, biochemically, detection of antimicrobial sensitivity to different antimicrobial agents, and detection of some virulence genes of *E.coli* using PCR technique, and detection of some antibiotic resistance genes in *E. coli* isolates by PCR technique.

MATERIALS AND METHODS

1. Samples Collection and Preparation:

A total of 150 fecal swabs were collected from crows, egret birds and diarrhatic fecal swabs from broiler chicken (50 each) and submitted to the Central Laboratory for Veterinary Quality Control on Poultry Production, Dokki and Fayoum to be checked for the presence of *E. coli* infection. The samples were collected from (Giza, Fayoum) governorates. All samples were collected without any contamination by sterile cotton swabs then inoculated in test tube then rapidly transported in ice box to the laboratory. According to (Middleton *et al.,* 2005).

2. Bacteriological examination:

All samples were examined bacteriologically for the presence of *E.coli.* Isolation and identification of *E.coli* were done according to (Lee *et al.*, 2008). Where, all the collected samples were pre-enriched in buffered peptone water (Oxoid) and incubated at 37˚C for 24 hrs under aerobic conditions. Then a loop ful from each broth culture was inoculated onto blood agar, MacConkeys' agar (Oxiod), XLD agar (Oxiod) and Eosin methylene blue agar plates (Oxiod) and incubated at 37ºC for 24 hours. The growing surface colonies were picked up, surfaced and further biochemically tested for growth on triple sugar iron agar and lysine iron agar, citrate utilization, urease production, and indole fermentation were done.

3. Serotyping of *E. coli* isolates:

E.coli isolates were serotyped by slide agglutination test according to (Lee *et al.*, 2009) using standard *E. coli* antisera (Sifin and Denka Seiken Comp.).

4. Antibiotic sensitivity tests:

The antibiogram of some *E. coli* isolates was done by disc-diffusion method according to (Koneman *et al.,* 1997) against (ten) antimicrobials (Oxoid®), and the zones of inhibition were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI/NCCLS, 2017). The used antibiotics were Amoxicllin+ Clavulanic acid (Am+CL, 20-10µg), Chloramphenicol (C^{30}, 30µg), Ciprofloxacin (CF^{5}, 5µg), Gentamicin (G^{10}, 10µg), Nalidixic acid (NA^{30}, 30µg), Nitrofurantoin (F^{300}, 300 µg), Norfloxacin (NX^{10}, 10 µg), Trimethoprim-sulfamethoxazole (SXT,1.25-23.75µg), Tetracycline (T^{30}, 30µg) and Streptomycin ($S^{10,}$ 10 µg).

5. Detection of virulence and antibiotic resistance genes in some *E. coli* isolates by PCR technique:

5.1. Extraction:
DNA of enriched isolates was extracted using commercially available kit, QIAamp DNA Mini Kit, Catalogue no.51304.

5.2. Amplification
Pho-sxt1-sxt2-hyl-eae genes amplification were amplified according to references mentioned in Table (1).

5.3. Analysis of the PCR Products
The products of PCR were separated by electrophoresis on 1% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the PCR products were loaded in each gel slot. A 100 bp and 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table 1: Oligonucleotide primers sequences used for amplification of DNA for the detection of *E. coli.*

Target gene	Primers sequences	Amplified fragment (bp)	Annealing	References
phoA	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	720	55˚C 45 sec	Hu *et al.* 2011
hly	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	1177	60˚C 50 sec.	Piva *et al*., 2003
eaeA	ATGCTTAGTGCTGGTTTAGG GCCTTCATCATTTCGCTTTC	248	51˚C 30 sec.	Bisi-Johnson *et al*., 2011
Stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614	58˚C 40 sec.	Dipineto *et al*., 2006
Stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779	58˚C 40 sec.	
aadA1	TATCAGAGGTAGTTGGCGTCAT GTTCCATAGCGTTAAGGTTTCATT	484	54˚C 40 sec.	Randall *et al.* 2004

phoA: Alkaline phosphatase**. hly:** alpha-haemolysine. **eaeA:** Attachment and Effacement, **Stx1:** shiga- toxin 1**, Stx2:** shiga-toxin 2. **aadA1: (**aminoglycoside 3"-adenylyltransferase activity antibiotic resistance genes**).**

RESULTS

1. Isolation rate of *E.coli* recovered from crows, egret wild birds and chicken broiler feces in different governorates: The isolation rates of *E.coli* were reported to be higher in Fayoum Governorate in (crows, 48%, broiler chicken, 40% and egret wild birds 28%) than Giza Governorate were *E.coli* was isolated from crows, egret wild birds and broiler chicken is an incidence of 20%, 12% and 20% respectively.

Table 2: Isolation rates of *E.coli* recovered from crows, egret birds and Chicken broiler samples in different governorates.

Locality	Crows feces			Egret feces			Chicken broiler feces			Total		
	NO	+ve	%	NO	+ve	%	NO	+ve	%	NO	+ve	%
Fayoum	25	12	48	25	7	28	25	10	40	75	29	38.6
Giza	25	5	20	25	3	12	25	5	20	75	13	17.3
Total	50	17	34	50	10	20	50	15	30	150	42	28

Percentage according to total number of the examined samples in each governorates.

2. Serotyping Results of *E. coli* isolated from crows feces, egret bird feces and chicken broiler feces:

The most commonly detected *E.coli* serogroups isolated were from crows (O78, O91, O145, O127, O158, O119, O125 and O55), egret bird feces (O78, O158, O125, O119, O91 and O44) and chicken broiler (O78, O125 and O158).

Table 3: The serotypes of *E. coli* isolated from crows feces, egret bird feces and chicken broiler feces:

serotype	crows feces		egret feces		chicken broiler feces	
	No.	%	No.	%	No.	%
O78	2	11.8%	1	10 %	3	20 %
O91	2	11.8%	1	10 %	-	-
O145	2	11.8%	-	-	-	-
O127	1	5.9%	-	-	-	-
O158	1	5.9%	1	10 %	1	6.7 %
O125	2	11.8%	1	10 %	3	20 %
O119	1	5.9%	1	10 %	-	-
O55	1	5.9%	-	-	-	-
O44	-	-	1	10 %	-	-
Total serotyped	12	70.6 %	6	60%	7	46.7 %
Un serotyped	5	29.4 %	4	40 %	8	53.3 %
Total	17	-	10	-	15	-

3. Antimicrobial resistance of *E.coli* isolated from crows feces, egret wild bird feces and chicken broiler feces:

The results resistance of the testing of *E.coli* isolates recovered from crows feces, egret feces and chicken broiler feces**.** Against 10 antimicrobial drugs. It is evident that the highest resistances were recorded against Streptomycin (83.4 %, 66.4%and 42.8% respectively).

Table 4: Interpretation of antibiotic resistance test of some *E.coli* isolates.

antibiotics	Crows feces N= 12			Egret bird feces N=6			Chicken broiler feces N=7		
	R	I	S	R	I	S	R	I	S
Am+CL	1(8.3)*	10(83.4)*	1(8.3)*	1(16.6)*	3(50)*	2(33.4)*	2(28.6)*	4(57.1)*	1(14.3)*
C	5(41.7)*	2(16.6)*	5(41.7)*	2(33.3)*	1(16.6)*	3(50)*	2(28.6)*	3(42.8)*	2(28.6)*
CIP	2(16.6)*	7(58.4)*	3(25)*	2(33.3)*	3(50)*	1(16.6)*	2(28.6)*	3(42.8)*	2(28.6)*
GM	3(25)*	6(50)*	3(25)*	1(16.6)*	4(66.8)*	1(16.6)*	2(28.6)*	2(28.6)*	3(42.8)*
NA	6(50)*	2(16.6)*	4(33.3)*	2(33.3)*	3(50)*	1(16.6)*	1(14.3)*	4(57.1)*	2(28.6)*
F	1(8.3)*	9(75*)	2(16.6)*	1(16.6)*	3(50)*	2(33.4)*	2(28.6)*	3(42.8)*	2(28.6)*
NX	1(8.3)*	4(33.3)*	7(58.4)*	2(33.4)*	1(16.6)*	3(50)*	1(14.3)*	2(28.6)*	4(57.1)*
S	10(83.4)*	2(16.6)*	-	4(66.8)*	1(16.6)*	1(16.6)*	3(42.8)*	2(28.6)*	2(28.6)*
SXT	5(41.7)*	3(25)*	4(33.3)*	1(16.6)*	3(50)*	2(33.4)*	1(14.3)*	4(57.1)*	2(28.6)*
T	6(50)*	2(16.6)*	4(33.3)*	3(50)*	2(33.4)*	1(16.6)*	1(14.3)*	4(57.1)*	2(28.6)*

Am+CL= Amoxicillin + Clavulinic acid, **C**= Chloramphenicol, **CIP**= Ciprofloxacin, **GM**= Gentamicin, **NA**= Nalidixic acid, **F**= Nitrofurantoin, **NX**= Norfloxacin, **S**= Streptomycin, **SXT**= Trimethoprim-sulfamethoxazole, **T**= Tetracycline. *(calculated according to the No. of tested *E.coli* isolates). **R**= Resistance, **I**= Intermittent, **S**= sensitivity.

4. Detection of virulence genes and antibiotic resistance genes of some *E.coli* isolated from crows feces, egret wild bird feces and chicken broiler feces by PCR:

To determine the virulence and antibiotic resistance profile of some isolated *E.coli* on a molecular aspects, PCR was performed for related genes, (*phoA*, *Stx2) virulence* genes were detected in tested samples and not detected (*hly*- *eaeA* and *sxt1*) virulence genes. On the other hand *aadA1* antibiotic resistance genes was harbored by some *E.coli* strains in table (5-6) and photo (1), (2), (3), (4) & (5).

Table 5: Result of virulence and antibiotic resistance genes of some *E.coli* isolates.

strain	serotypes	Source	Virulance genes	Antibiotic Resistance genes
1	O55	crows	*phoA*	*aadA1*
2	O119	crows	*phoA*	*aadA1*
3	O127	crows	*phoA*	-
4	O158	crows	*phoA*	*aadA1*
5	O78	crows	*phoA, , Stx2*	*aadA1*
6	O78	crows	*phoA, , Stx2*	*aadA1*
7	O91	crows	*phoA, , Stx2*	-
8	O91	crows	*phoA*	-
9	O145	crows	*phoA*	-
10	O145	crows	*phoA*	*aadA1*
11	O125	crows	*phoA*	*aadA1*
12	O125	crows	*phoA, , Stx2*	-
1	O158	chicken broiler	*phoA*	-
2	O125	chicken broiler	*phoA*	-
3	O125	chicken broiler	*phoA*	-
4	O125	chicken broiler	*phoA*	-
5	O78	chicken broiler	-	-
6	O78	chicken broiler	*phoA*	-
7	O78	chicken broiler	*phoA*	-
1	O119	egret bird	*phoA*	-
2	O78	egret bird	*phoA*	*aadA1*
3	O125	egret bird	*phoA*	-
4	O158	egret bird	*phoA*	*aadA1*
5	O44	egret bird	-	-
6	O91	egret bird	-	-

Table 6: Incidences of virulence and antibiotic resistance genes of *E.coli* isolated from wild birds and broiler chicken by PCR.

Examined genes	Source of examined isolate		
	crows	**egret bird**	**broiler chicken**
phoA	12\12 (100%)	4\6 (66.6%)	6\7 (85.7)
Sxt1	0\12 (0%)	0\6 (0%)	0\7 (0%)
Sxt2	4\12 (33.3%)	0\6 (0%)	0\7 (0%)
hly	0\12 (0%)	0\6 (0%)	0\7 (0%)
eaeA	0\12 (0%)	0\6 (0%)	0\7 (0%)
aadA1	7\12 (58.3%)	2\6 (33.3%)	0\7 (0%)

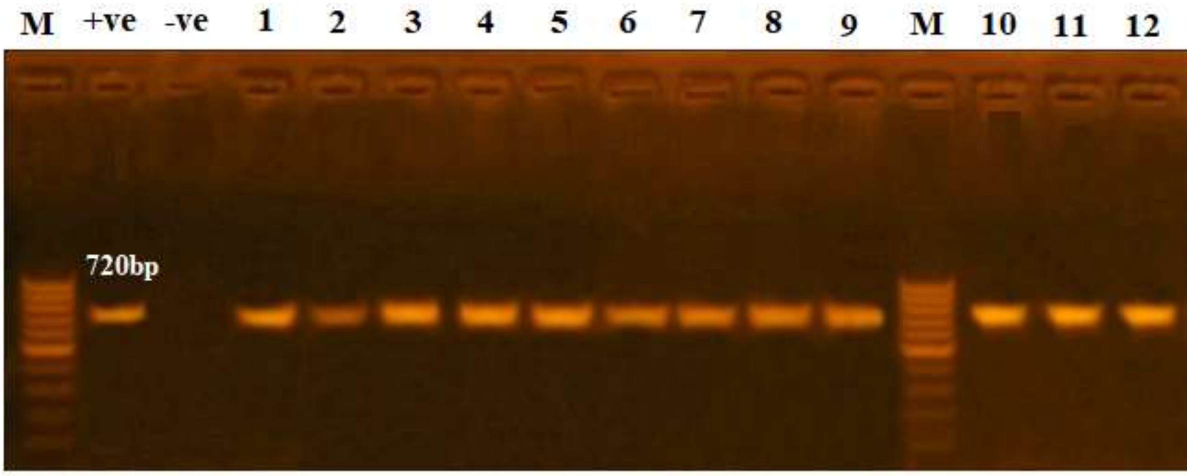

Photo (1): Agarose gel electrophoresis of PCR products after amplification of **(PhoA) gene** at (**720**) bp amplified product. All tested isolated from Crows are positive (1-12).

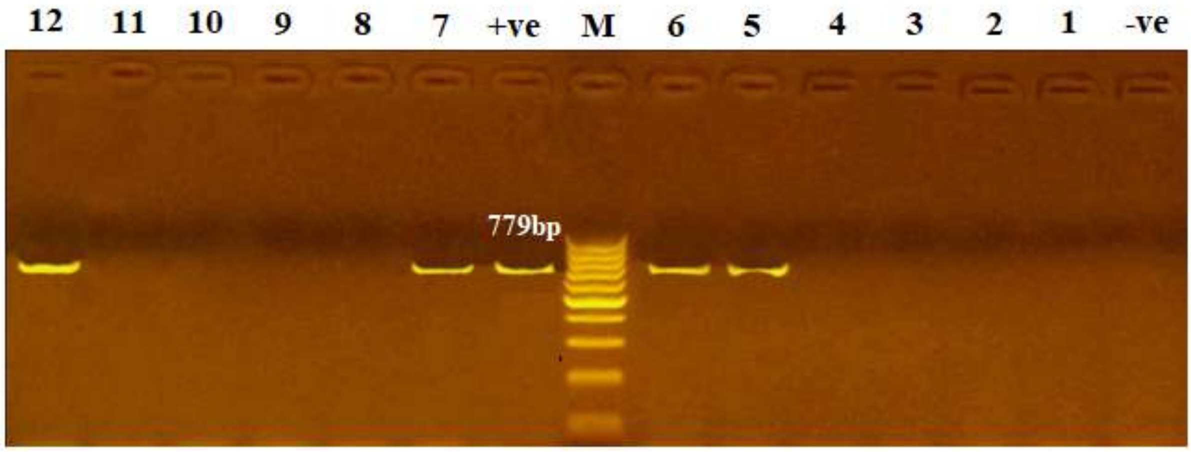

Photo (2): Agarose gel electrophoresis of PCR products after amplification of **(sxt2) gene** at (**779**) bp amplified product. Tested isolated from Crows are positive: O78 (2) -O91-O125.

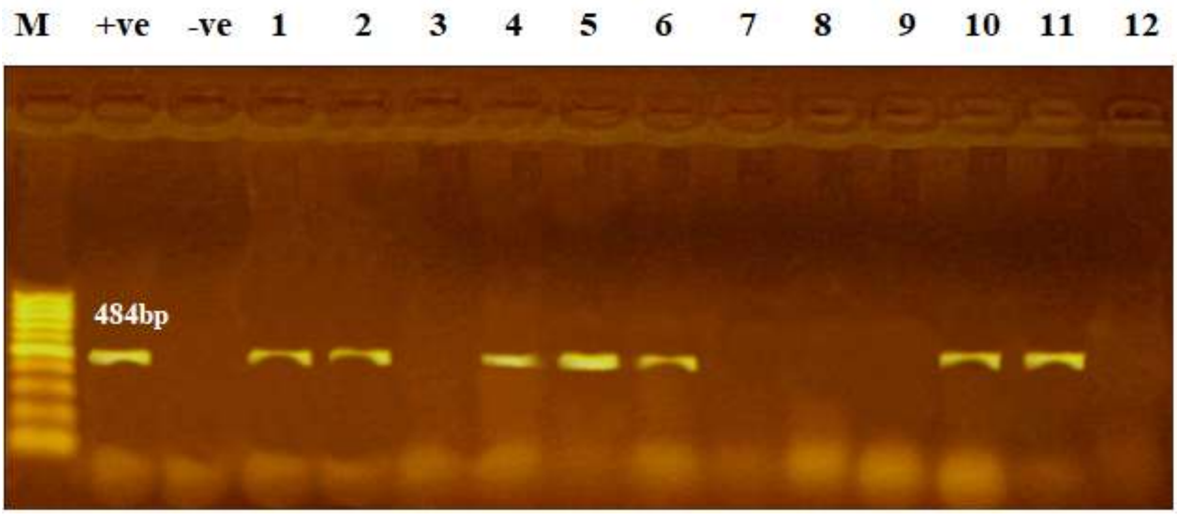

Photo (3): Agarose gel electrophoresis of PCR products after amplification of **(*aadA1*) gene** at (**484**) bp amplified product 7 out of 12. *E.coli* Crows isolates are positive: O55-O119-O158-O78, (2)-O145-O125.

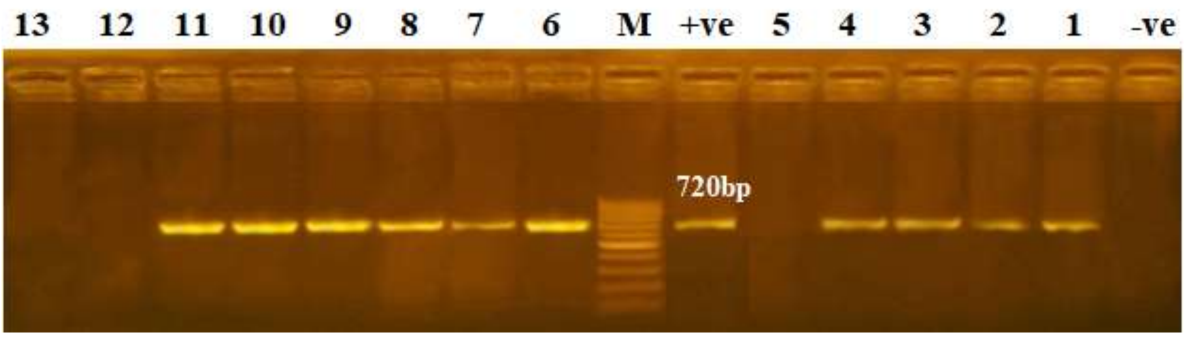

Photo (4): Agarose gel electrophoresis of PCR products after amplification of **(PhoA) gene** at (**720**) bp amplified product. All tested isolated from chicken broiler feces are positive (1-7). {O158- O125 (3) – O78 (2)} expect (5) {O78} negative and four *E.coli* egret wild bird feces tested isolates are positive (8-11) {O119-O78- O125-O158}.while (12-13) negative, {O44-O91}.

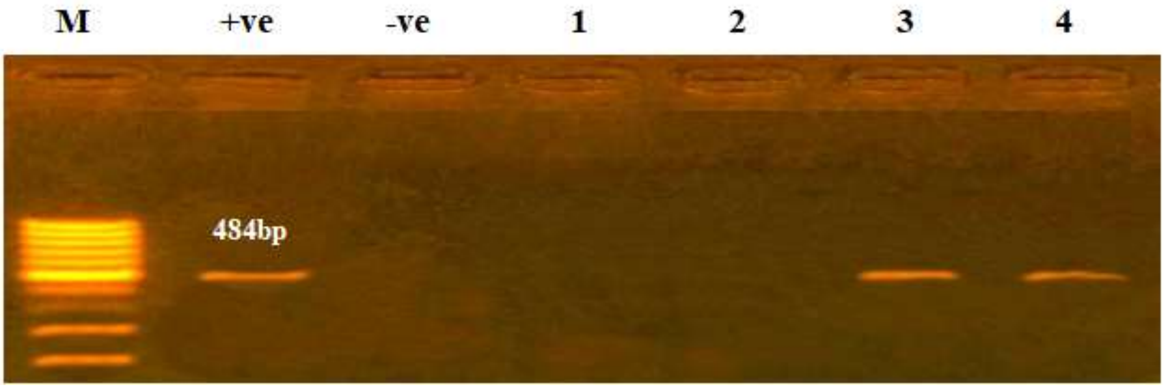

Photo (5): Agarose gel electrophoresis of PCR products after amplification of (***aadA1***) **gene** at (**484**) bp amplified product. Two *E.coli* isolates from egret wild bird feces (O78-O158) are positive.

DISCUSSION

Wild birds act as vectors of many diseases not affect only birds, animals but also human (Gharieb *et al.*, 2013; Badouei *et al.*, 2016). The nature of birds, their ability to cover vast distances within a relatively short period of time, their residence near livestock areas, farms, waste disposal sites, and human habitats made them important vectors of some zoonosis (Gioia *et al.*, 2016 and Oravcova, 2016). The data can be used to monitor trends in the occurrence of pathogenic strains, because multiple serogroups are associated with disease, especially O1, O2 and O78 among many others (Dziva and Stevens, 2008).

In Tables (2), the isolation rate of *E.coli* was reported to be highly in (crows, Egret wild birds and broiler chicken). Where percentage of the isolation rate was 48%, 28%, 40%, 20% and 12%. Respectively. The variation in *E. coli* prevalence rates may be attributed to the species of wild bird examined, localities and bird feeding habits. This result was agree with that described by (Magda *et al.*, 2013 and Aruji *et al.*, 2004) who isolated it from wild birds percentage of 21.6% and 14.52% respectively. Concerning examination of broilers, were positive for *E. coli*. Nearly similar findings were reported by (Ahmed, 2011 and Mona *et al.*, 2013).

It was observed that several serotypes were recovered from crows (O78, O91, O145 and O125), egret bird feces (O78, O158, O125, O119, O91 and O44) and chicken broiler (O78, O125 and O158). Table (3), These results agreed with (Lin *et al.,* 2011 and Hanaa *et al.,* 2017, El-Sheshtawy *et al.,* 2005 and Maysa *et al.,* 2013) who isolated *E. coli* from wild birds in Egypt, nearly the same serotypes with a predominance of O78 have been identified (Reda, 2013).

Table (4), illustrated results of resistance of some testing *E.coli* isolates recovered from crows feces, egret wild birds feces and chicken broiler feces, against 10 antimicrobial drugs. It is evident that the highest resistance was recorded against Streptomycin (83.4 %, 66.4%and 42.8%). These results nearly similar to (Maciel *et al.,* 2017) who Showed that *E.coli* isolates resistant to streptomycin, doxycycline, and Chloramphenicol. The resistance of microorganisms to antibiotics due to inactivation, drug modification, alteration in metabolic pathway, alteration of target site development of new genes and reduced drug accumulation (Blair *et al.*, 2014 and Pruden *et al.*, 2013).

Studies have reported that the environment imposes its own selection on the population of *E.coli* following fecal deposition from its primary habitat within the intestine of animals (Jang *et al.*, 2017).

Table (5-6) illustrated the results of (*PhoA*) gene. Encodes for a hydrolase enzyme which is responsible for removing phosphate groups from molecule. Alkaline phosphatase (*phoA*) gene has been used in PCRs for common *E. coli* strains detection, demonstrating high specificity (hu *et al.*, 2011; Ke Xin *et al.*, 2009).

In the present study, *E. coli* virulence genes, (stx2) known to be associated with human disease was detected in bird fecal samples. (stx2) was detected more frequently, while none of the isolates from these birds were found to be positive for (*stx*1).which nearly agreed with (Kobayashi *et al.,* 2009, Persad, *et al.*, 2014, Sanches *et al.,* 2017 and Ahmed *et al .,* 2018).

In contrast, other researchers could not detect (*stx1* or *stx2*) in wild birds. Considering that stx-2 toxin is more toxic than stx-1 and is often associated with wild birds and chicken Similar results were obtained by (Sanches *et al.,* 2017). Found no stx1 samples among fecal samples from gulls, pigeons, and chickens that were, obtained by (Koochakzadeh *et al.,* 2015 and Ahmed, 2011). Other researchers could not detect (eaeA and hly) gene in wild birds similar results were obtained by (Mona *et al.,* 2013 and Indranil, *et al.,* 2004). Meanwhile these results disagreed with others who found no eaeA and hly gene detected in broiler chickens (Shimaa, 2013).

Antimicrobial resistance has been known as an emerging worldwide problem in both human and veterinary medicine, and antimicrobial use is considered the most important factor for the emergence, selection, and distribution of antimicrobial-resistant bacteria (Mohammed *et al.,* 2014). the current study, we also screened the isolates for the presence of selected antimicrobial resistance genes, including those for streptomycin (*aadA1*), The prevalence of these genes was generally higher in the present study than in previous studies (Dehkordi *et al.,*2014 ., Marcelino *et al.,* 2019 and Kar *et al.,* 2020). And these results differ from (Momtaz *et al.,* 2012).

CONCLUSION

These findings show that wild birds, may constitute an environmental carrier of these pathogens representing a source of infection for other birds, livestock, and humans. Wild birds may spread pathogens over a wide range, thus enhancing their carrier role. Further investigations should continue to characterize the antibiotic resistance genes and the epidemiology link between poultry and human. Biosecurity on the poultry farms should be the first line of defense against infectious diseases.

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متلازمة الإسهال في دجاج التسمين وبعض الطيور البرية التي يسببها الميكروب القولوني

غادة عمر الدمرداش ، فاطمة عامر ، هبة رشدي

E-mail: roshdy2019 @yahoo.com Assiut University web-site: www.aun.edu.eg

الميكروب القولوني هو بكتيريا سالبة الجرام ، وهي تعيش في الأمعاء بشكل طبيعي ، إلا أن بعض السلالات بسبب جينات الضرواة تلعب دورًا رئيسيًا في التسبب في مرض للطيور.

في هذه الدراسة تم جمع ١٥٠ عينة براز وتم فحص العينات بكترلوجيا للميكروب القولوني من (الغربان ، والبلشون البري ، والدجاج التسمين) من (٥٠ عينة لكل نوع من الطيور) .تم وفحص جميع العينات الجرثومية

واظهرت النتائج الميكروب القولوني وكانت تسجيل نسبة عزل بكتيريا الميكروب القولوني مرتفعة في محافظة الفيوم في (الغربان ، ٤٨٪ ، الدجاج التسمين ، ٤٠٪ والبلشون البري ٢٨٪) مقارنة بمحافظة الجيزة حيث تم عزل الميكروب القولوني من (الغربان ، والبلشون والطيور البرية. والدجاج التسمين) بنسبة ٢٠٪ و ٢٥٪ و ٢٨٪ على التوالي.

واظهرت المجموعات المصلية الميكروب القولوني التي تم الحصول عليها عن طريق التعرف المصلي من الغربان (O78 ، O91، O145، O127، O158، O119، O125، O55) ، براز طيور البلشون (O78، O158، O125، O119، O91، O44) و دجاج التسمين (O78، O125، O158). أظهرت نتائج اختبار الحساسية للمضادات الحيوية لبكتريا الميكروب القولوني المعزولة مقاومة عالية للستربتومايسين (٨٣,٤٪ و ٦٦,٤٪ و ٤٢,٨٪) في كل (الغربان والبلشون و دجاج التسمين) على التوالي. أظهرت نتائج تفاعل البوليميراز المتسلسل المتعدد أن جينات phoA والضرواة تم اكتشافها في جميع المجموعات المصلية لبكتريا الميكروب القولوني التي عزلت الجين Stx2 المكتشف في (O78 و O91 و O125) في الغربان فقط. ولم يتم الكشف عن جينات الضرواة (hly و eaeA و Stx1) في جميع مجموعات الميكروب القولوني المصلية. من ناحية أخرى تم الكشف عن جينات aadA1 بواسطة بعض سلالات الميكروب القولوني (7من الغربان و ٢ من البلشون الأبيض).