
Mansoura Veterinary Medical Journal

ROLE OF DOMESTIC BIRDS IN TRANSMISSION OF ESCHERICHIA COLI AND SALMONELLA SPECIES AS A ZONOTIC PATHOGENS

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ABSTRACT

The role of domestic birds as a zoonotic reservoirs and sources of Escherichia coli (E. coli) and Salmonella species was investigated. For this purpose, a total of 442 samples were collected from 191 poultry (70 chicken, 51 ducks and 70 pigeons) and humans (25 stool specimens and 35 hand swabs). Concerning poultry samples, two samples (one cloacal swab and another feather) were taken from each bird. All samples were subjected for isolation and identification of E. coli and Salmonella spp., the recovered isolates were serologically typed. PCR technique was used for further characterization of some E. coli and Salmonella strains. Occurrence of E. coli isolated from cloacal swabs of birds was 37.4%, while Salmonella spp. was 5.1%. E. coli. Overall percentages of E. coli isolated from feather samples of birds was 37.4%, meantime Salmonella spp. was 4.6%. Regarding the isolated strains from human, E. coli isolated from hand swabs of poultry handlers was 20%, and Salmonella spp. was 2%. While occurrence of E. coli isolated from fecal samples of poultry handlers was 64% and Salmonella spp. was 4%. The typed E. coli serotypes as O91:H21, were characterized strain EHEC (enterohemorrhagic E. coli), O2:H6, O78, O1:H7, O146:H21, O44:H18, O114:H4 and O158 were strain characterized EPEC (enteropathogenic E. coli), O127:H6 were strain characterized ETEC (enterotoxigenic E. coli). It was concluded that domestic poultry in the examined areas considered a significant zoonotic reservoir for E. coli and Salmonella spp. Same serotypes and genotypes of E. coli and Salmonella spp. could be detected in both domestic poultry and humans, suggesting its zoonotic importance and these serotypes are circulated between domestic poultry and humans in the examined areas. The public health importance, healthy education as well as other precautions and preventive measures that recommended to the infection of such zoonotic bacteria in domestic birds and humans were fully discussed.

Keywords: Salmonella spp., E. coli, Serotyping, PCR.

INTRODUCTION

Poultry meat considered the most familiar in the market as it has more features than other meat as easy digestability, inexpensive and have great acceptance among the most of people (Lutful, 2010).

The importance of house breeding to farmers as a source of food in the form of meat

and eggs and a source of employment, moreover source of income to the persons involved in poultry production. Poultry farming linked to rice farming which help controlling water snails and provide a good manure for fertilization of the soil in addition fish farming depends on poultry farming as poultry manure that help growth of phytoplankton which considered a good source for fish feeding (Adziety et al., 2008).

Zoonotic importance of Poultry to humans is dangerous as it transmits viral disease as avian influenza and bacterial disease as *E. coli*, *Salmonella* spp., *Proteus* and *Enterobacter*. *E. coli* and *Salmonella* spp. causing public health hazard worldwide. In United States, 50% of human suffering from diarrhea caused by contaminated food by *E. coli* (Mead et al., 1999). In China 75% of morbidity in humans attributable to contaminated feed by *Salmonella* spp. (Bai et al., 2015). Multiplex PCR is a perfect tool for diagnosis of *Salmonella* spp. and *Escherichia coli* and for determining the virulence genes which has public health significance (Farooq et al., 2009 and Dutta et al., 2011). Multiplex PCR has been stratified to genus *Salmonella* and *E. coli* for detection of its toxins using highly conserved primers to recognize more than one target sequence in a single reaction (Alvares et al., 2004 and Cortez et al., 2006). Information about the potential role of domestic birds in maintaining and disseminating zoonotic agents in Egypt are little. From the zoonotic and economic impact of *E. coli* and *Salmonella* spp., this study was carried out to investigate bacteriologically and molecularly the role of domestic birds as zoonotic reservoir of *E. coli* and *Salmonella* spp. in Dakahlia governorate, Egypt.

MATERIALS AND METHODS

This study was performed to investigate the role of domestic poultry (chickens, ducks and pigeons) as zoonotic reservoir for pathogenic *E. coli* and *Salmonella* spp. by bacteriologically and molecularly approach.

Sampling.

A total of 442 samples were collected from poultry (382) and humans (60) from 37

farmers' houses of different villages, suburban and urban places of Mansoura, Dakahlia Governorate, Egypt.

A. Bird samples:

The samples represented cloacal swabs (191), feather swabs (191) of chickens (70), ducks (51) and pigeons (70).

Cloacal swabs:

Sterile swabs moistened in sterile BPW were inserted into the cloaca of bird and then withdrawn. The swabs were directly immersed into tubes contain BPW under aseptic conditions and transferred to the laboratory (Sadoma, 1997).

B. Human samples:

Human samples were collected from hand swabs (35) and stool specimens (25) of poultry handlers.

Hand swabs:

Sterile swabs moistened in sterile BPW were rolled against the dorsum and palm of the hand. The swabs were directly immersed into tubes contain BPW under aseptic conditions and transferred to the laboratory.

Stool specimens:

Sterile dry swabs were rolled in the stool specimens of human. The swabs were directly immersed into tubes contain BPW under aseptic conditions and transferred to the laboratory.

2. Bacteriological examinations:

A- Isolation of *E. coli*:

Enrichment of the collected samples or swabs in BPW was carried out by incubation at 37°C for 18-24 hours. After enrichment, a loopful from the incubated broth was streaked directly onto EMB (Eosin Methylene Blue) agar and incubated at 37°C for 18-24 hours (*Quinn et al., 1994*). After incubation, the different representative colonies especially metallic shiny colonies from each plate were picked up, purified by streaking onto nutrient agar plates and incubated at 37 °C for 18-24 hours. The purified colonies were streaked onto nutrient agar slants and incubated at 37 °C for 18-24 hours for further identification (*Cruickshank et al., 1975*). Meantime some identified colonies were preserved in glycerol.

B- Isolation of *Salmonella spp.*:

For isolation of *Salmonellae*, the collected swabs in BPW were pre-enriched by incubation at 37°C for 24 hours, after pre-enrichment, 0.1 ml of pre-enriched cultured broth was inoculated into 10 ml RV broth and incubated at 41°C for 24 hours. After enrichment, a loopful from the enriched cultured broth was streaked onto XLD agar and incubated at 37°C for 18-24 hours (*Humphry et al., 1989*). After incubation, (Red colonies with black centers) were picked up and streaked onto nutrient agar slants and incubated at 37°C for 18-24 hours for further identification (*Cruickshank et al., 1975*). Meantime, some identified colonies were preserved in glycerol.

3- Identifications of *E. coli* and *Salmonella spp.*:

The isolated pure colonies from cloacal swabs and man were subjected to microscopical, biochemical and serological identification

Identification morphologically using microscopical examination and motility test according to *MacFaddin (2000)*, while biochemical identification uses Indole test, Methyl Red Test, Voges – Praskauer test, Citrate utilization test, Urease test, Hydrogen sulphide production test, Gelatin hydrolysis test, Oxidation–Fermentation test, Nitrate reduction test, Detection of Ornithine decarboxylase (ODC), Detection of L- lysine decarboxylase (LDC), Detection of Arginine decarboxylase (ADH), Detection of β -galactosidase (ONPG), Fermentation of sugars were identified according to (*Kreig and Holt, 1984*).

4- Serological identification:

A. Serological identification of *E. coli*:

A total of representative 32 *E. coli* strains isolated from chickens (10), pigeons (6), ducks (10) and man (6) were subjected to serological identifications according to *Kok et al. (1996)* by using rapid diagnostic *E. coli* antisera sets (**DENKA SEIKEN Co., Japan**) for diagnosis of the Enteropathogenic types.

B. Serological identification of *Salmonella spp.*:

A total of representative 13 *Salmonella* strains isolated from chickens (4), pigeons (3), ducks (3), and man (3) were subjected to serological identifications according to Kauffman – White scheme (*Kauffman, 1974*) for the determination of Somatic (O) and flagellar (H) antigens using *Salmonella* antiserum (**DENKA SEIKEN Co., Japan**).

5- Molecular identification of the isolated strains by multiplex-PCR:

A total of representative 23 (16 *E. coli* and 7 *Salmonella* spp.) biochemically and serologically identified strains (table 21 and table 22) were selectively subjected for molecular characterization by multiplex PCR.

Regarding, 10 strains of strains were assessed by multiplex PCR for *stx2*, *stx1* and *eaeA* genes.

Concerning 5 strains of *Salmonella* were evaluated by multiplex PCR for *invA*, *hila* and *fimH* genes.

A. DNA Extraction according to (Shah et al., 2009).

Genomic bacterial DNA was extracted from the examined isolates using QIA amp kit according to (Shah et al., 2009).

B. DNA amplification:

B.1. Amplification reaction of *E. coli* isolates (Fagan et al., 1999):

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). PCR assays were carried out using of nucleic acid template prepared by using reference EHEC isolates (approximately 30 ng of DNA) and specific primers table (1).

Table (1): Primer sequences of *E. coli* used for PCR identification system:

Target gene	Oligonucleotide sequence (5' → 3')	Product size (bp)	References
<i>stx1</i> (F)	5' ACACTGGATGATCTCAGTGG '3	614	<i>Dhanashree and Mallya (2008)</i>
<i>Stx1</i> (R)	5' CTGAATCCCCCTCCATTATG '3		
<i>Stx2</i> (F)	5' CCATGACAACGGACAGCAGTT '3	779	
<i>Stx2</i> (R)	5' CCTGTCAACTGAGCAGCACTTTG '3		
<i>eaeA</i> (F)	5' GTGGCGAATACTGGCGAGACT '3	890	<i>Mazaheri et al. (2014)</i>
<i>eaeA</i> (R)	5' CCCATTCTTTTTACCGTCG '3		

The conditions of amplification consisted of an initial 95°C denaturation step for 3 min followed by 35 cycles of 95°C for 20 sec, 58°C for 40 s, and 72°C for 90 sec. The final cycle was followed by 72°C incubation for 5 min.

The reference strains were *E. coli* O157:H7 (positive for *stx1*, *stx2* and *eaeA*) and *E. coli* (a nonpathogenic negative control strain) that does not possess any virulence gene.

B.2. Amplification of virulence genes of *Salmonella* spp. (Singh et al., 2013):

The reaction mixes consisted of 5 µl of the bacterial lysate, 5 µl of 10x assay buffer for Taq polymerase containing 1.5 mM MgCl₂, 2 µl of 10mM dNTP mix 1 µl each of forward and reverse primer (10 pmol) table (2) and 1.25 U of Taq DNA polymerase made up to 50 µl using sterile distilled water. The PCR cycling

protocol was applied as following: An initial denaturation at 94°C for 60 sec, followed by 35 cycles for 60 sec, annealing at 64°C for 30 sec and extension at 72°C for 30 sec, followed by a final extension at 72°C for 7 min. then electrophoresed in 1.5 % agrose gel (Sigma – USA), stained with ethidium bromide and visualized and captured on UV transilluminator.

Table (2) Primer sequences of *Salmonella* spp. used for PCR system:

Target gene	Oligonucleotide sequence (5' → 3')	Product size (bp)	References
<i>invA</i> (F)	5' GTGAAATTATCGCCACGTTTCGGGCA '3	284	<i>Shanmugasamy et al.</i> (2011)
<i>invA</i> (R)	5' TCATCGCACCGTCAAAGGAACC '3		
<i>hilA</i> (F)	5' CTGCCGCAGTGTTAAGGATA '3	497	<i>Guo et al.</i> (2000)
<i>9hilA</i> (R)	5' CTGTCGCCTTAATCGCATGT '3		
<i>fimH</i> (F)	5' GGA TCC ATG AAA ATA TAC TC '3	1008	<i>Menghistu</i> (2010)
<i>fimH</i> (R)	5' AAG CTT TTA ATC ATA ATC GAC TC '3		

Table (3): Occurrence of *E. coli* and *Salmonella* spp. isolated from cloacal swabs of birds.

Source of samples	No of examined	No of positive	%*	<i>E. coli</i>		<i>Salmonella</i>	
				No. of positive	%	No. of positive	%
Chicken	70	29	41.4	25	35.7	4	5.7
Duck	55	17	30.9	14	25.5	3	5.5
Pigeon	70	37	52.9	34	48.6	3	4.3
Total	195	83	42.6	73	37.4	10	5.1

*The percentage was calculated from each total bird samples.

Table (4): Occurrence of *E. coli* and *Salmonella* spp. isolated from feather swabs of bird.

Source of sample	No of examined	No of positive	%*	<i>E. coli</i>		<i>Salmonella</i>	
				No of positive	%	No of positive	%
Chicken	70	33	47.1	29	41.4	4	5.7
Pigeon	70	26	37.1	23	32.9	3	4.3
Duck	55	23	41.8	21	38.2	2	3.6
Total	195	82	42.1	73	37.4	9	4.6

* The percentage was calculated from each total bird samples.

Table (5): Occurrence of enterobacterial strains in hand swabs of 35 poultry handlers.

Isolated organism	Total no. of sample	NO. of positive	%
<i>E. coli</i>	35	7	20
<i>Salmonella</i>	35	1	2

Table (6): Occurrence of Enterobacterial strain in fecal sample of 25 poultry handlers.

Isolated organism	Total no. of sample	No. of positive	%
<i>E. coli</i>	25	16	64%
<i>Salmonella</i>	25	1	4%

There are mixed infections of some examined samples serotypes.

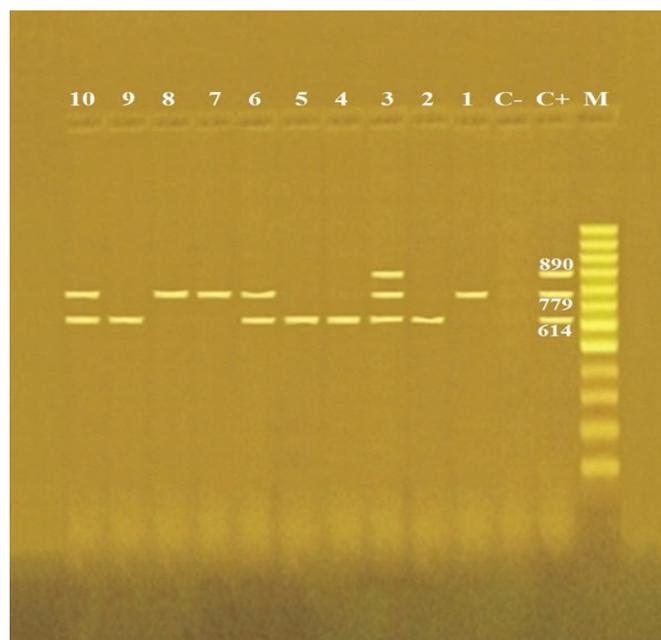
Table (7): Incidence of virulence genes of *E. coli* strains isolated from some representative examined samples isolated from bird, and human.

<i>E. coli</i> strains	<i>stx1</i>	<i>stx2</i>	<i>eaeA</i>
O1 : H7	-	+	-
O2 : H6	+	-	-
O26 : H11	+	+	+
O44 : H18	+	-	-
O78	+	-	-
O91 : H21	+	+	-
O114 : H4	-	+	-
O127 : H6	-	+	-
O146 : H21	+	-	-
O158	+	+	-

Stx1: Shiga- toxin 1 gene

Stx2: Shiga- toxin 2 gene

EaeA: intimin gene



Photograph (1): Agarose gel electrophoresis of multiplex PCR of *stx1*, *stx2* and *eaeA* genes for characterization of *Enteropathogenic E. coli*. *E. coli* showed bands for *stx1* at base pair 614, for *stx2* at base pair 779 and for *eaeA* at 890 bp.

Lane M: 100 bp ladder as molecular size DNA marker.

Lane C+: Positive control *E. coli* for *stx1*, *stx2* and *eaeA* genes at 614, 779 and 890 bp respectively.

Lane C-: Control negative.

Lanes 2 (O2), 4 (O44), 5 (O78) & 9 (O146): Positive *E. coli* strains for *Stx1* gene.

Lanes 1 (O1), 7 (O114) & 8 (O127): Positive *E. coli* strains for *Stx2* gene.

Lanes 6 (O91) & 8 (O158): Positive *E. coli* strains for *stx1* and *Stx2* gene.

Lane 3 (O26): Positive *E. coli* strain for *stx1*, *stx2* and *eaeA* genes.

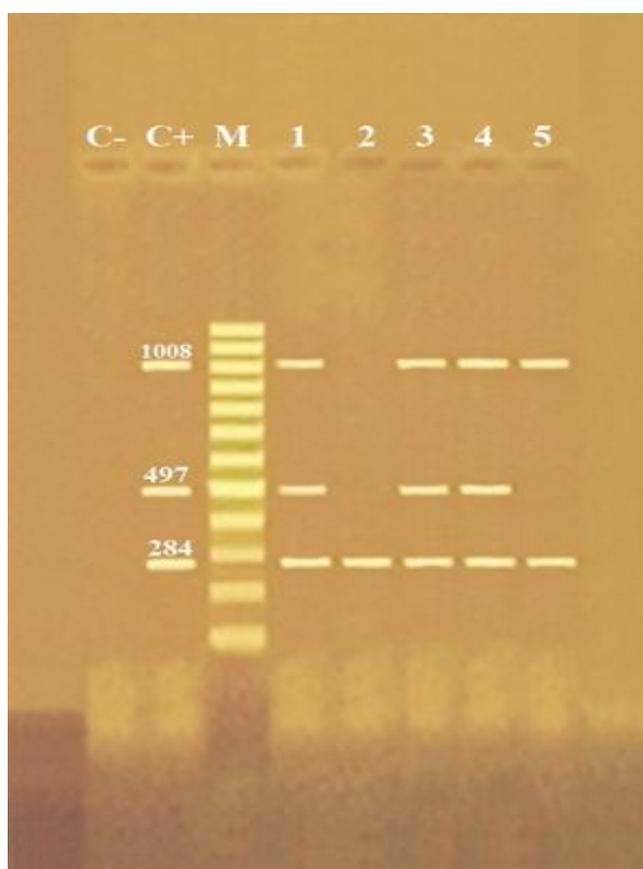
Table (8): Incidence of virulence genes of *Salmonella* strains isolated from some representative examined samples isolated from bird, and humans.

<i>Salmonella</i> strains	<i>invA</i>	<i>hila</i>	<i>fimH</i>
<i>S. Enteritidis</i>	+	+	+
<i>S. Typhimurium</i>	+	+	+
<i>S. Kentucky</i>	+	+	+
<i>S. Inganda</i>	+	-	+
<i>S. Takoradi</i>	+	-	-

invA: invasion A gene
+, presence of gene.

hila: hyper-invasive locus gene
-, absence of gene

fimH: fimbrial gene

**Photograph (2):** Agarose gel electrophoresis of multiplex PCR of *invA*, *hila* and *fimH* virulence genes for characterization of *Salmonella* species. *Salmonella* strains showed bands at 248bp for *invA* gene, 497 bp for *hila* gene, 1008 bp for *fimH* gene.

Lane M: 100 bp ladder as molecular size DNA marker.

Lane C+: Control positive strain for *invA*, *hila* and *fimH* genes.

Lane C-: Control negative.

Lanes 1 (*S. Enteritidis*), 3 (*S. Typhimurium*) & 4 (*S. Kentucky*): Positive strains for *invA*, *hila* and *fimH* genes.

Lane 2 (*S. Inganda*): Positive strain for *invA* and *fimH* genes.

Lane 8 (*S. Takoradi*): Positive strain for *invA* gene.

RESULTS AND DISCUSSION

In Egypt, the rapid growth of the poultry industry which considered to be a source of income to farmers in rural areas has resulted in the production of large quantities of poultry wastes and increasing contacts with birds may lead to spreading of zoonotic pathogens as *E. coli* and *Salmonella* spp. Avian pathogenic *E. coli* (APEC) infections are responsible for large economic perish to the poultry manufacture all over the world and there is increasing hazard of its zoonotic importance (**Ashraf et al., 2013**). Birds and birds products are considered to be the master provenance of non-Typhoidal serotypes of *Salmonella enterica* in the United States (**Braden, 2006**). Among the causative agent of foodborne pathogens, non-typhoidal *Salmonella enterica* is the main cause of morbidity and hospitalizations (**Scallan et al., 2011**)

From the ultimate importance of *E. coli* and *Salmonella* as causative agent of many gastrointestinal disease and illness in humans, this study was undertaken to search the role of domestic birds as zoonotic reservoirs and sources of such enterobacterial agents by microbiological and molecular assessment. One hundred and ninety-one poultry cloacal swabs (70 chickens, 51 ducks and 70 pigeons) were collected. Table (3) clarify the occurrence of *Enterobacterial* strains in poultry cloacal swabs. The overall percentages of the *E. coli* were 37.4 (73 out of 195). It was found that chickens, ducks and pigeons occurrence of *E. coli*, of 35.7%, 25.5% and 48.6%, respectively. These results are nearly similar to the results previously reported by **Taha (2002)**, **Mondal et al. (2008)**, but were not similar to **Hassan and Aml (2014)** and **Amira et al. (2017)** Moreover, lower than the result reported by **Halfaoui et al. (2017)**. Table (3) illustrate that 10 cloacal

samples of poultry out of 195 samples were positive to *Salmonella*. The occurrence of *Salmonella* spp. has percentages of 5.7 for chicken, 5.5 for ducks and 4.3 for pigeons. These results are nearly similar to the results previously reported by other previous researchers (**Mondal et al., 2008** and **Amira et al. 2017**), but were not similar to other authors (**Ashraf and Tadashi, 2012** and **Abdeen et al. 2018**),). The results were lower than the results reported by **Nógrády et al. (2008)** and **Se-Yeoun et al. (2013)**.

Regarding occurrence of *E. coli* in feather samples table (4) illustrate that *E. coli* isolated from feather samples of 70 chickens, 70 pigeons and 55 ducks with respective percentages of 41.4, 32.2 and 38.2. Table (4) show that occurrence of *Salmonella* spp. isolated from poultry feather samples of 70 chickens, 70 pigeons and 55 ducks with respective percentages of 5.7, 4.3 and 3.6.

Table (5) shows that *E. coli* was isolated from 18 out of 35 hand swabs (51.4%). lower results were recorded by **Heba (2003)**. Moreover, **Mohamed et al (2004)** identified *E. coli* from 6 of mother's hands with percentages of 18.8. In the current investigation, results recorded in table (5) show that the percentage of isolated *Salmonella* spp. from hand swabs of poultry handlers was 8.6 (three out of 35). Nearly similar result (8.3%) was recorded by **Mohammed et al. (1999)**. However, **Sadoma (1997)** and **Heba (2003)** isolated *Salmonella* spp. with percentages of 12.7% and 3.1%. From zoonotic point of view, *Salmonella* can be directly transmitted to man through handling of infected birds because their feathers can harbor the infective organisms

Regarding the examinations of 25 human stool samples for the isolation and identification of *Enterobacterial* strains, table (6) shows the overall percentage of *E. coli* isolates was 64 (16 out of 25). Nearly similar

results were obtained by **Taha (1989)** and **Mohamed et al, (2004)** who found that, *E. coli* comprised 52.6, 50%, 64.3, respectively. **Taha (2002)** and **Alizadeh et al. (2007)** had all observed and reported less distribution of *E. coli* among man. However, lower results were obtained by **Bodhidatta et al. (2002)** who isolated *E. coli* from 6% of examined diarrheic cases. The high percentage occurrence of *E. coli* in man may be due to many factors, the most important of which is the fact that man live in contact with poultry; socio-economic level, environmental conditions, and low standard of sanitation and hygienic measurements are also other factors compromised in increasing the occurrence of *E. coli* infection.

Regarding occurrence of *Salmonella spp.* in human stool samples table (6) showed that. *Salmonella spp.* were isolated from 1 (4%) out of 25 humans. This result was nearly to the result previously recorded by **Mohamed et al (2004)**

By serotyping of 36 isolates (12 chicken, 10 ducks, 8 pigeons and 6 humans) for identification of the isolated *E. coli* serotypes. the identified serotypes typed from birds and humans were O78, O91:H21, O2:H6, O1:H7, O158, O26:H11, O114:H4, O44:H18, O146:H21 and O127:H6 with respective percentages of 11, 22.2, 16.7, 5.6, 5.6, 8.3, 8.3, 2.8, 13.9 and 5.6. There are two *E. coli* strains which isolated from hand swabs of poultry handlers serotyped as O91 and O2 with a percentage of 22.2 and 16.7, respectively. Regarding strain characterized of *E. coli* of some representative samples isolated from bird, and humans. Results show that *E. coli* serotypes as O91:H21, with characterized strain EHEC (enterohemorrhagic *E. coli*), O2:H6, O78, O1:H7, O146:H21, O44:H18, O114:H4 and O158 with strain characterization EPEC (enteropathogenic *E. coli*), O127:H6 with strain characterization ETEC (enterotoxigenic *E. coli*).

The identified *Salmonella* serotypes isolated from birds, and humans were *S. Takoradi*, *S. enteritidis*, *S. Inganda*, *S. Typhimurium* and *S. Kentucky* with respective percentages of 7.1, 35.7, 7.1, 35.7 and 14.3. Regarding the *Salmonella* serogroups identified from chicken samples, the results proved that *S. enteritidis*, *S. Typhimurium* and *S. Kentucky* were among the identified serotypes. Similar the results were previously recorded by **Orji et al. (2005)**, **Ashraf and Tadashi (2012)**, **Nagwa et al. (2012)** and **Abdeen et al. (2018)**. Moreover, **Amira et al. (2017)** identified same serotypes from chickens in Egypt including *S. Kentucky* with percentage of 6.7. Regarding the serotyping of three representative *Salmonella* strains isolated from humans, two stool samples and one hand swabs of poultry handlers, results shows that *Salmonella* isolated from stool samples were allocated to *Salmonella Enteritidis* (7.1%) and *Salmonella Typhimurium* (7.1). While the serotype identified from hand swab was *Salmonella Enteritidis* (7.1). These results coincide with results obtained by **Maysa et al (2013)** and **Nagwa et al. (2012)** who isolated *Salmonella Typhimurium* from chicken samples and stool samples of humans. *S. Takoradi* among the isolates belonged to serogroup C2, with antigenic structure (O 8,20 and H i:1,5), *S. Enteritidis* belong to serogroup D1, with antigenic structure (O 1,9,12 and H g,m:-), moreover *S. Inganda* belong to serogroup C1, with antigenic structure (O 6,7and H Z10:1,5), while *S. Typhimurium* belong to serogroup B, with antigenic structure (O 1,4,5,12 and H i:1,2) and *S. Kentucky* belong to serogroup C3, with antigenic structure (O 8,20 and H i:Z6).

Molecular characterization of *E. coli* and *Salmonella* spp. isolated from birds and humans.

In this study 16 representative *E. coli* from birds, feed, water and human table (21) were subjected for further identification by PCR which succeeded for confirmation of identified serotypes and detection of virulence genes at specific band for stx1 at base pair 614, for stx2 at base pair 779 and for eaeA at 890 bp. PCR success the amplification of *E. coli* with ratio (100%) Photograph (1).

Table (7) photograph (1) showed the incidence of virulence genes of *E. coli* strains isolated from some samples isolated from bird, feed, water and humans. O26 showed serotype specific bands of stx2, stx1 and eaeA genes on agarose gel electrophoresis by multiplex PCR, while O91 and O158 showed bands of stx2 and stx1 genes, in addition O2, O44 and O146 showed bands of stx2. Bands of eaeA was showed by O1 and O127. Molecular detection and characterization of shiga toxin producing *E. coli* were previously applied by *Janben et al. (2001)*, *Farooq et al. (2009)*, *Dutta et al. (2011)*.

Regarding the virulence genes of *Salmonella* strains of representative samples isolated from bird, feed and humans are illustrated in table (8) photograph (2). By using m-PCR it was revealed that *S. Enteritidis*, *S. Typhimurium* and *S. Kentucky* have three virulence genes (*invA*, *hlyA* and *fimH* genes), in addition *S. Inganda* have (*invA* and *fimH* genes), while *S. Takoradi* have only *fimH* gene. The characterization of *Salmonella* species by presence of *invA* gene was previously applied by *Cortez et al. (2006)* and *Hu et al (2011)*.

Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper

REFERENCES

- Abdeen, E.; Elmonir, W.; Suelam, I.I.A. and Mousa, W.S. (2018)*: Antibigram and genetic diversity of *Salmonella enterica* with zoonotic potential isolated from morbid native chickens and pigeons in Egypt.
- Adzitey, F.; Liew, C.Y.; Aronal, A.P. and Huda, N. (2012)*: Isolation of *Escherichia coli* from Ducks and Duck Related Samples., 7: 351-355.
- Alizadeh, A.H.; Behrouz, N.; Salmanzadeh, S.; Ranjbar, M.; Azimian, M.H.; Jaafari, F.; Zolfagharian, K. and Zali, M.R. (2007)*: *Escherichia coli*, *Shigella* and *Salmonella* spp. in acute diarrhea in Hamedan, Islamic Republic of Iran. East Mediterr. Health J., 13(2): 243-249.
- Alvarez, J.; Sota, M.; Vivanco, A.; Perales, A.; Ramón Cisterna, R.; Rementeria, A. and Garaizar, J. (2004)*: Development of a Multiplex PCR Technique for Detection and Epidemiological Typing of *Salmonella* in Human Clinical Samples., 42(4):1734-8
- Amira, M.; Helmut, H.; Omnia, A.; Herbert, H.; Heinrich, N.; Hafez, M. and Hosny, E. (2017)*: Occurrence of *Salmonella enterica* and *Escherichia coli* in raw chicken and beef meat in northern Egypt and dissemination of

their antibiotic resistance markers., 9-57.

- Ashraf, A. and Tadashi, S. (2012):** Genetic analysis of multiple antimicrobial resistance in *Salmonella* isolated from disease broiler in Egypt., 56 (4): 254–261.
- Ashraf, H.M.; Ibrahim, A.I.; Amal, A.M.; Eid, M.; Julie, S.; Ganwu, L.; Nolan, K. and Logue, C. (2013):** Molecular and Phenotypic Characterization of *Escherichia coli* Isolated from Broiler Chicken Flocks in Egypt., 57(3):602-611.
- Bai, L.; Lan, R.; Zhang, X.; Cui, S.; Xu, J.; Guo, Y., Li, F. and Zhang, D. (2015):** Prevalence of *Salmonella* Isolates from Chicken and Pig Slaughter houses and Emergence of Ciprofloxacin and Cefotaxime Co-Resistant *S. enterica* Serovar Indiana in Henan, China., 10(12).
- Bodhidata, L.; Vithayasai, N.; Eimpokalarp, B.; Pitarangsi, C.; Serichantalergs, O.; and Isenbarder, D.W. (2002):** Bacterial enteric pathogens in children with acute dysentery in Thailand: increasing importance of quinolone-resistant *Campylobacter*. South East Asian J. Trop. Med. Puplic Health., 33(4): 752-757.
- Braden, C.R. (2006):** *Salmonella enterica* serotype Enteritidis and eggs: A national epidemic in the United States. Clin. Infect. Dis., 43:512–517.
- Cortez, A.L.L.; Carvalho, A.C.F.B.; Ikunob, A.A.; Bürger, K.P. and Vidal-Martinsa, A.M.C. (2006):** Identification of *Salmonella* spp. isolates from chicken abattoirs by multiplex-PCR., 81(3): 340-344.
- Cruickshank, R.; Duguid, J.; Marmion, B. and Swain, R.H. (1975):** Medical Microbiology. 12th Ed., Edinburg, London and New York.
- Dhanashree, B. and Mallya, S. (2008):** Detection of shiga-toxicogenic *Escherichia coli* (STEC) in diarrhoeagenic stool and meat samples in Mangalore, India. Indian J. Med. Res., 128: 271-277.
- Dutta, T.K.; Roychoudhury, P.; Bandyopadhyay, S.; Wani, S.A. and Hussain, I. (2011):** Detection & characterization of Shiga toxin producing *Escherichia coli*(STEC) & enteropathogenic *Escherichia coli* (EPEC) in poultry birds with diarrhea., 133(5): 541–545.
- Fagan, P.; Hornitzky, M.; Bettelheim, K. and Djordjevic, S. (1999):** Detection of Shiga-Like Toxin (*stx1* and *stx2*), Intimin (*eaeA*), and Enterohemorrhagic *Escherichia coli* (EHEC) Hemolysin (EHEC *hlyA*) Genes in Animal Feces by Multiplex PCR. Appl. Environ. Microbiol., 65 (2): 868–872.
- Farooq, I.; Hussain, M.A.; Mir, M.A.; Bhat, M.A.; and Wani, S.A. (2009):** Isolation of atypical enteropathogenic *Escherichia coli* and Shiga toxin 1 and 2f-producing *Escherichia coli* from avian species in India., 48(6): 692–697.
- Guo, X.; Chen, J.; Beuchat, L. and Brackett, R. (2000):** PCR detection of *Salmonella enterica* serotype Montevideo in and on raw tomatoes using primers derived from *hilA*. Appl. Environ. Microbial., 66: 5248-5252.
- Halfaoui, Z.; Menoueri, N. and Bendali, L. (2017):** Serogrouping and antibiotic resistance of *Escherichia coli* isolated from broiler chicken with colibacillosis in center of Algeria., 10(7): 830–835.

- Hanan, M. and Gharabibeh, R. (2004)** Rapid and Simultaneous Identification of Two *Salmonella enterica* Serotypes, Typhimurium from Chicken and Meat Products by Multiplex PCR., 3(1): 44-48.
- Hassan, H.K.H. and Aml, A.M. (2014):** Characterization of *Escherichia coli* strains isolated from infected pigeons in Assiut province., 60 (142).
- Heba, A.A. (2003):** Tracing some sources of infection of some zoonotic bacteria among family Enterobacteriaceae. M.V.SC (Zoonoses), Fac. Of Vet. Med., Zagazig univ. (2003).
- Hu, Q.; Tu, J.; Han, X.; Zhu, Y.; Ding, C. and Yu, S. (2011)** Development of multiplex PCR assay for rapid detection of *Riemerella anatipestifer*, *Escherichia coli*, and *Salmonella enterica* simultaneously from ducks., 87(1): 64-69.
- Janben, T.; Schwarz, C.; Preikschat, P.; Voss, M.; Philipp, H.C. and Wieler, L.H. (2001):** Virulence-associated genes in avian pathogenic *Escherichia coli* (APEC) isolated from internal organs of poultry having died from colibacillosis., 291(5): 371-378.
- Kauffman, G. (1974):** Kauffmann white scheme. J. Acta. Path. Microbiol. Sci., 61:385.
- Kok, T.; Worswich, D. and Gowans, E. (1996):** Some serological techniques for microbial and viral infections. In Practical Medical Microbiology (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A., eds.), 14th ed., Edinburgh, Churchill Livingstone, UK.
- Kreig, N. and Holt, J. (1984):** Bergey's Manual of systemic bacteriology Vol.1. William and Wilkins, Baltimore, M.D.21202, USA.
- Lutful, S.M. (2010):** Avian Colibacillosis and Salmonellosis: A Closer Look at Epidemiology, Pathogenesis, Diagnosis, Control and Public Health Concerns., 7(1): 89-114.
- MacFaddin, J.F. (2000):** Biochemical tests for identification medical bacteria. Waryery Press Inc, Baltimore, Md. 21202 USA.
- Maysa, A.; Merwad, A.M. and Rehab, E.M. (2013):** Prevalence of Zoonotic *Escherichia coli* and salmonellae in Wild Birds and Humans in Egypt with Emphasis on RAPD-PCR Fingerprinting of *E. coli*., 11 (6): 781-788.
- Mazaheri, S.; Ahrabi, S. and Aslani, M. (2014):** Shiga toxin-producing *Escherichia coli* isolated from lettuce samples in Tehran, Iran Jundishapur J. Microbiol., 7 (11): 1-6.
- Menghistu, H. (2010):** Studies on molecular heterogeneity among *Salmonella Gallinarum* isolates of poultry origin. M.V.Sc. Thesis, Deemed Univ., IVRI, Izatnagar, Bareilly.
- Mohamed, A.A.; Nasser, M.M.; Mowafy, L.E.; Magda, A.A. and Heba, A.A. (2004):** Tracing some sources of infection of some zoonotic disease due to some bacteria among family Enterobacteriaceae. Zag. Vet. J., 32(1): 1-14.
- Mohammad, L.N.; Samaha, H.A.; Draz, A.A. and Haggad, Y.N. (1999):** Salmonellae among birds and human beings., 15 (1): 147-154.
- Mondal, T; Khan, M.S.R.; Alam, M.; Purakayastha, M.; Das, M and Siddique, M.P. (2008):** Isolation,

identification and characterization of *Salmonella* from duck., 6 (1): 07–12

- Nagwa, S.R.; Nashwa, O.K.; Mervat, E.I. and Jehan S.A. (2012):** Epidemiological and Molecular Studies of *Salmonella* Isolates from Chicken, Chicken Meat and Human in Toukh, Egypt., 8 (2): 128-132.
- Nógrády, I.N.; Kardos, G.; Bistyák, A.; Turcsányi, I.; Mészáros, J.; Galántai, Zs.; Juhász, Á.; Samu, P.; Kaszanyitzky, J.É.; Pászti, J. and Kiss, I. (2008)** Prevalence and characterization of *Salmonella infantis* isolates originating from different points of the broiler chicken–human food chain in Hungary., 127(1–2):162-167.
- Orji, M.U.; Onuigbo, H.C.; Mbata, T.I. (2005):** Isolation of *Salmonella* from poultry droppings and other environmental sources in Awka, Nigeria., 9(2): 86-89.
- Quinn, P.J.; Carter, M.E.; Markey, B.K. and Carer, G.R. (1994):** Clinical Veterinary Microbiology. Mosby Londen. WCTH, 9LB, England.
- Sadoma, A.M. (1997):** Salmonella in chicken in connection with human Infection. M.V.Se Thesis. Fac.of Vet.Med. Tanta University.
- Se-Yeoun, C; MinKang, Y.; Choi-Kyu, P.; Oun-young, M.; Hyung-K.(2013) :** Prevalence and antimicrobial susceptibility of *Salmonella* isolates in Pekin ducks from South Korea., 36(5): 473-479.
- Scallan, E.; Hoekstra R.M.; Angulo F.J.; Tauxe, R.V.; Widdowson M.A.; Roy, S L.; Jones, J.L. and Griffin, P.M. (2011):** Foodborne illness acquired in the United States—Major pathogens. Emerg. Infect. Dis., 1:1–21.
- Shah, D.; Shringi, S.; Besser, T. and Call, D. (2009):** Molecular detection of foodborne pathogens, Boca Raton: CRC Press, In Liu, D. (Ed) Taylor & Francis group, Florida, USA, Pp., 369-389.
- Shanmugasamy, M.; Velayutham, T. and Rajeswar, J. (2011):** InvA gene specific PCR for detection of *Salmonella* from broilers. Vet. World., 4 (12): 562-564.
- Singh, A., Yadav, S., Singh, S. and Bharti, P. (2010):** Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. Food Res. Inter., 43: 2027-2030.
- Taha, N.A.A. (2002):** Zoonotic importance of entero pathogenic *Escherichia coli* (EPEC). Ph.D. Thesis, Fac. of Vet. Med. zag .Univ.

المخلص العربي

دور الطيور المنزلية فى نقل الميكروب القولونى العصى وأنواع السالمونيلا كمسببات مرضية مشتركة

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ان الدور الحقيقى للطيور والبيئة المحيطة بها فى نقل بعض البكتريا ضمن عائلة الانتيروبيكترياسى ما زال محل الدراسة . ولذلك فان الهدف من هذه الدراسة هو تحديد دور الطيور المنزلية كمصدر رئيسى فى نقل البكتريا الى مربي الطيور. ولهذا فقد تم تجميع عدد ٤٤٢ عينة من الطيور بمدينة المنصورة والقرى المحيطة بها، محافظة الدقهلية، جمهورية مصر العربية وشملت هذه العينات التى تم جمعها عدد ١٩١ مسحات من مجمع الطيور مقسمة الى (٧٠ من الدواجن، ٥١ من البط و ٧٠ من الحمام) كما تم تجميع عدد ١٩١ مسحات من ريش نفس الطيور بالاضافة الى ٦٠ عينة تم تجميعها من مربي الطيور (٢٥ عينة براز و ٣٥ عينة من مسحات الايدي)، تم العثور على عدد كبير من الميكروب القولونى العصى فى العينات التى تم تجميعها فمثلا تم العثور على الميكروب القولونى العصى فى مسحات المجمع من الطيور بنسبة ٣٧,٤% والسالمونيلا بنسبة ٥,١% كما انه تم عزل الميكروب القولونى العصى بنسبة ٣٧,٤% من ريش الطيور والسالمونيلا بنسبة ٤,٦% كما تم عزل الميكروب القولونى العصى من مسحات براز الانسان بنسبة ٦٤% والسالمونيلا بنسبة ٤% ومن مسحات الايدي تم عزل الميكروب القولونى العصى بنسبة ٢٠% والسالمونيلا بنسبة ٢% وقد تم اختيار ٣٦ عينة عشوانيا ممثله لجميع الفئات المعزولة من الميكروب القولونى العصى وعدد ١٤ عينة للسالمونيلا لاختبارهم للاختبارات السيرولوجية ليتم تصنيفها وفى نفس الوقت تم عمل اختبار البلمرة المتسلسل للميكروب القولونى العصى باستخدام البادى المتخصص لكل جين من جينات الضراوة الاكثر شيوعا وهم (stx2, stx1 and eaeA) وقد تبين وجود جين او اكثر فى عترات الايشيريشيا كولاي المعزولة. كما انه تم عمل اختبار PCR لميكروب السالمونيلا باستخدام البادى المتخصص لكل جين من جينات الضراوة الاكثر شيوعا وهم (invA, hilA and fimH genes) وقد تبين وجود واحد او اكثر من هذه الجينات فى العترات المعزولة.ومن نتائج هذه الدراسة يتضح لنا الدور الهام الذى تلعبه الطيور والبيئة المحيطة بها فى نقل الميكروبات المشتركة التى تفرز الانتيروتوكسين والتي تؤثر تأثيرا سلبيا على صحة الانسان وقد تم مناقشة الأهمية المشتركة للميكروبات المعزولة وتأثيرها على الصحة العامة للانسان.