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

Mona Nasser*, Adel El-Gohary** and Amro Mohamed**

*Veterinary medical Directorate, Dakahlia, General Organization of Veterinary Services (GOVS)
**Department of Hygiene and Zoonoses, Faculty of Veterinary Medicine, Mansoura University

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 feacal 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.

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

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Oligonucleotide sequence (5‘ → 3’)</th>
<th>Product size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>stx1</em> (F)</td>
<td>5’ ACACTGGATGATCTCAGTGG ’3</td>
<td>614</td>
<td>Dhanashree and Mallya (2008)</td>
</tr>
<tr>
<td><em>Stx1</em> (R)</td>
<td>5’ CTGAATCCGCCCTCCATTATG ’3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Stx2</em> (F)</td>
<td>5’ CCATGACAACGGACACGCAGTT ’3</td>
<td>779</td>
<td></td>
</tr>
<tr>
<td><em>Stx2</em> (R)</td>
<td>5’ CCTGTCACACTGAGCAAGCAGTGTG ’3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>eaeA</em> (F)</td>
<td>5’ GTGGCGAATACTGGCGAGACT ’3</td>
<td>890</td>
<td>Mazaheri et al. (2014)</td>
</tr>
<tr>
<td><em>eaeA</em> (R)</td>
<td>5’ CCCCATTTTTTTCACCGTCG ’3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

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).
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% agarose 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:

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Oligonucleotide sequence (5’ → 3’)</th>
<th>Product size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>invA</em> (F)</td>
<td>5’ GTGAAATTATCACCAGTTCGGGCA ‘3</td>
<td>284</td>
<td>Shanmugasamy et al. (2011)</td>
</tr>
<tr>
<td><em>invA</em> (R)</td>
<td>5’ TCATCGCACCCTCAAAGGAACC ‘3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>hilA</em> (F)</td>
<td>5’ CTGCCGCAGTGTAAAGGATA ‘3</td>
<td>497</td>
<td>Guo et al. (2000)</td>
</tr>
<tr>
<td><em>9hilA</em> (R)</td>
<td>5’ CTGTGCCTTAATCGCATGT ‘3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>fimH</em> (F)</td>
<td>5’ GGA TCC ATG AAA ATA TAC TC ‘3</td>
<td>1008</td>
<td>Menghistu (2010)</td>
</tr>
<tr>
<td><em>fimH</em> (R)</td>
<td>5’ AAG CTT TTA ATC ATA ATC GAC TC ‘3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Source of samples</th>
<th>No of examined</th>
<th>No of positive</th>
<th>%*</th>
<th>E. coli</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of positive</td>
<td>%</td>
<td>No. of positive</td>
<td>%</td>
<td>No. of positive</td>
</tr>
<tr>
<td>Chicken</td>
<td>70</td>
<td>29</td>
<td>41.4</td>
<td>25</td>
<td>35.7</td>
</tr>
<tr>
<td>Duck</td>
<td>55</td>
<td>17</td>
<td>30.9</td>
<td>14</td>
<td>25.5</td>
</tr>
<tr>
<td>Pigeon</td>
<td>70</td>
<td>37</td>
<td>52.9</td>
<td>34</td>
<td>48.6</td>
</tr>
<tr>
<td>Total</td>
<td>195</td>
<td>83</td>
<td>42.6</td>
<td>73</td>
<td>37.4</td>
</tr>
</tbody>
</table>

*The percentage was calculated from each total bird samples.
Table (4): Occurrence of *E. coli* and *Salmonella* spp. isolated from feather swabs of bird.

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>No of examined</th>
<th>No of positive</th>
<th>%*</th>
<th><em>E. coli</em></th>
<th><em>Salmonella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No of positive</td>
<td>No of positive</td>
</tr>
<tr>
<td>Chicken</td>
<td>70</td>
<td>33</td>
<td>47.1</td>
<td>29</td>
<td>41.4</td>
</tr>
<tr>
<td>Pigeon</td>
<td>70</td>
<td>26</td>
<td>37.1</td>
<td>23</td>
<td>32.9</td>
</tr>
<tr>
<td>Duck</td>
<td>55</td>
<td>23</td>
<td>41.8</td>
<td>21</td>
<td>38.2</td>
</tr>
<tr>
<td>Total</td>
<td>195</td>
<td>82</td>
<td>42.1</td>
<td>73</td>
<td>37.4</td>
</tr>
</tbody>
</table>

* The percentage was calculated from each total bird samples.

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

<table>
<thead>
<tr>
<th>Isolated organism</th>
<th>Total no. of sample</th>
<th>NO. of positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>35</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>35</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Isolated organism</th>
<th>Total no. of sample</th>
<th>No. of positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>25</td>
<td>16</td>
<td>64%</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>25</td>
<td>1</td>
<td>4%</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th><em>E. coli</em> strains</th>
<th><em>stx1</em></th>
<th><em>stx2</em></th>
<th><em>eaeA</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>O1 : H7</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>O2 : H6</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O26 : H11</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>O44 : H18</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O78</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O91 : H21</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>O114 : H4</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>O127 : H6</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>O146 : H21</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O158</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th><em>Salmonella</em> strains</th>
<th><em>invA</em></th>
<th><em>hilA</em></th>
<th><em>fimH</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. Enteritidis</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>S. Typhimurium</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>S. Kentucky</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>S. Inganda</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>S. Takoradi</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*invA*: invasion A gene
*hilA*: hyper-invasive locus gene
*fimH*: fimbrial gene
+
, presence of gene.
-, absence of 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 Hasson and Aml (2014) and Amira et al. (2017). Moreover, lower than the result reported by Halfouai 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, Mohammed 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 6,7 and 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 successed 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 O91and 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, hilA* 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

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دور الطيور المنزلي في نقل الميكروبات القولونى العصوى وأنواع السالمونيلا

كمسببات مرضية مشتركة

ط. ب. متى ناصر محمد، أ. د. عادل حلمي الجوهرى، أ. د. عمرو عبد الفتاح محمد

* مديرية الطب البيطري - محافظة الدقهلية
** قسم الصحة والأمراض المشتركة، جامعة المنصورة

إن الدور الحقيقي للطيور والبيئة المحيطة بها في نقل بعض البكتيريا ضمن عائلة الأنتييريكترية ما زال محل الدراسة. ولذلك فإن الهدف من هذه الدراسة هو تحديد دور الطيور المنزلي كمسر رئيسي في نقل البكتيريا إلى مربي الطيور. ولذا فقد تم تجميع عدد 42 عينة من الطيور بمدينة المنصورة والقرى المحيطة بها، محافظة الدقهلية، جمهورية مصر العربية وشملت هذه العينات التي تم جمعها عدد 191 مسحة من مجمع الطيور مقسمة إلى 70 من الدواجن، 51 من البط و100 من الحمام) كما تم تجميع عدد 191 مسحة من رش نفس الطيور بالإضافة إلى 10 عينة تم تجميعها من مربي الطيور (25 عينة براز و35 عينة من مسحات الآبيدي). تم العثور على عدد كبير من الميكروبات القولونى العصوى في العينات التي تم تجميعها فمثلًا تم العثور على الميكروبات القولونى العصوى في مسحة المجمع من الطيور بنسبة 32.4% والسالمونيلا بنسبة 6.4% كما تم عزل الميكروبات القولونى العصوى بنسبة 37.4% من رش الطيور والسالمونيلا بنسبة 4.1% كما تم عزل الميكروبات القولونى العصوى من مسحات براز الإنسان بنسبة 24% والسالمونيلا بنسبة 4% ومن مسحات الآبيدي تم عزل الميكروبات القولونى العصوى بنسبة 20% والسالمونيلا بنسبة 2% وقد تم اختبار 32 عينة عشوائية ممثلة لجميع الفئات المعزولة من الميكروبات القولونى العصوى وعد 14 عينة السالمونيلا لاختراعهم للالتهابات السيرولوجية ليتم تصنيفها وفي نفس الوقت تم عمل اختبار البلازما المتسلسل للميكروبات القولونى العصبي باستخدام البادئة المشتركة لكل جين من جينات الضروارتين الشبا وهم (stx2, stx1 و eaeA) (وقد تبين وجود جين أو أكثر في عرات الإيشيريشيا كولاريتي) المزعولة. كما أنه تم عمل اختبار PCR لليمكروبات السالمونيلا باستخدام البادئ المتخصص لكل جين من جينات الضروارتين الشبا وهم (وقد تبين وجود واحد أو أكثر من هذه الجينات في) الغيرات المزعولة، ومن نتائج هذه الدراسة يوضح لنا الدور الهام الذي تلعبه الطيور والبيئة المحيطة بها في نقل الميكروبات المشتركة التي تفرز الأنتييريكترية والتي تؤثر تأثيرًا سلبيًا على صحة الإنسان وقد تم مناقشة الأهمية المشتركة للميكروبات المزعولة وتاثيرها على الصحة العامة للإنسان.