IMPORTANCE OF MIGRATORY BIRDS AS A VECTOR IN SPREADING OF SALMONELLA IN EGYPT IN THE PERIOD FROM NOVEMBER 2017 TO MARCH 2018

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ABSTRACT
Migratory birds play a significant role in the ecology, circulation and transmission of zoonotic pathogens specially Salmonella that producing animal and human illnesses in addition to sever economic losses to poultry industry, so that the prevalence of Salmonella was estimated and studied from six species of migratory waterfowl birds along Manzala Lake in Dakahlia and Damietta Governorates, Egypt during the period from November 2017 to March 2018. A total of 100 live birds were collected from hunters and the internal organs of those birds were subjected to bacteriological examinations, antimicrobial susceptibility testing and molecular detection of some virulence genes. Seven Salmonella isolates were isolated with a percentage of 7%, (7 out of 100) from Common Teal, Mallard ducks and Shoveler birds. Four different serotypes across the study sites were identified; S. Typhimurium, S. Bardo, S. Montevideo and S. Kentucky. All Salmonella isolates showed high sensitivity to Streptomycin, Erythromycin, Norfloxacin, Colistin sulphat and Doxycline. Two S. Bardo isolates, S. Typhimurium, S. Kentucky showed multi-drug resistance. S. Montevideo and 2 S. Bardo isolates showed higher sensitivity to most of the used antimicrobial agents. Seven virulence genes (invA, sopB, mgtC, bcfC, spsC, fimH and fimA) were detected using Polymerase Chain Reaction technique (PCR) in all of the examined Salmonella isolates.

Key words: Migratory birds, Salmonella, virulence genes, antimicrobial susceptibility.

INTRODUCTION
Migratory birds travel across national and international borders; they can transfer microorganisms across the world and play a significant role in the ecology and circulation of pathogenic organisms (Georgopoulou and Tsiouris, 2008), those birds considered as long-distance vectors for a wide range of microorganisms (Nuttall, 1997). It can carry zoonotic pathogens, including enteric pathogenic bacteria, either being themselves diseased or being carriers (Abulreesh et al., 2007) and implicated in the transmission of zoonoses and other microbial pathogen by three mechanisms: mechanical carriers, biological carriers and carriers of infected ectoparasites (Jourdain et al., 2007), the mode of transmission can be directly through bird its self or indirectly through arthropods, soil, food and water (Hubalek, 2004). Also it plays a significant role in the epidemiology of enteric zoonotic bacteria including Escherichia coli and Salmonella (Tsiodras et al., 2008). It also involved in the dissemination of Salmonella to human or other domestic animals and birds (Millán et al., 2004).

Several birds are migrating to Egypt in winter season such as Coot (Fulica atra), Teal (Anas crecca), Shoveler (Anas clypeata), Pintail (Anas Acuta), Wigeon (Anas Penelope) and Mallard (Anas Platyrbynchos) (Goodman and Meininger, 1989). Common Coot (Fulica atra) birds are distrusted in whole Eurasia and Northern Africa. Most of those birds stay in central and Western Europe, but some continue as far south as North Africa (Delany et al., 2006), those birds are common breeding resident on larger lakes of Nile Delta (Egypt), also it considered as abundant winter visitor from mid-September, large number of this species present in mid-winter and by mid-March and most Coots leave Egypt (Goodman and Meininger, 1989).

Common Teal (Anas crecca) birds migrated from Western Eurasia toward North Africa (Scott and
In Egypt Teal birds are common migrants and winter visitor to land waters on Nile Delta lakes, Lake Qarun (Egypt) and along the Nile from early September to late April (Goodman and Meininger, 1989).

Shoveler (Anas clypeata) birds migrated from SW-Siberia in autumn toward Egypt (Scott and Rose, 1996), (McClure, 1998) and (Veen et al., 2005) and considered as common passage migrant throughout Egypt from late August to late May but rare in summer. Small flocks have been recorded along north coast of Sinai in September and October (Baha el Din and Salama, 1984) but this species are regular visitor to all inland waters as lakes (Manzala, Burullus and Qarun) (Meininger and Mullie, 1981).

Pintail (Anas Acuta) birds migrated to Egypt from SW-Siberia in winter (McClure, 1998) and (Veen et al., 2005) and move in winter from Russia, western and central Siberia via Egypt toward Netherlands, France, Great Britain North Africa and Senegal delta (Scott and Rose, 1996) and (Wernham et al., 2002), other birds migrated to Egypt from Netherlands (McClure, 1998). In Egypt, Pintail birds are winter visitor and migrate with large number in autumn throughout the country, this autumn passage continues into December along north coast of Sinai, Nile Delta. Wigeon birds also considered as winter visitor to most inland waters as Nile Delta lakes, lake Qarun, lake Bardawil and Nile valley (Goodman and Meininger, 1989).

Wigeon (Anas Penelope) birds migrated in winter from West and Central Siberia toward North-Africa (Bianki and Dobyryina, 1997). In Egypt, Wigeon birds considered as winter visitor to most inland waters as Nile Delta lakes, lake Qarun, lake Bardawil and Nile valley (Goodman and Meininger, 1989).

Mallard (Anas Platyrbynchos) birds distributed in Czechoslovakia, S-European and Russia move in winter to North Africa, Italy and Germany (McClure, 1998).

Bacteria of the genus Salmonella colonize the digestive tract of birds, mammals and reptiles (Silva et al., 2010), producing gastroenteritis in humans leading to economic losses, animal and human illnesses (Hilbert et al., 2012), affecting poultry industry and causing reduced production (Lutful Kabir, 2010). Salmonella commonly found in the intestinal tract of wild birds (Tsiodras et al., 2008) and it can be introduced into poultry houses via free flying wild birds. The infected birds may transmit infection directly or indirectly via infecting pets and food animals (Tizard, 2004).

Salmonella is Gram negative, non- spore forming bacteria, usually motile and belongs to the family of Enterobacteriaceae (Bennasar et al., 2000). It’s a facultative intracellular pathogen causing localized, systemic infections and chronic asymptomatic carrier state (Su et al., 2011). Signs of paratyphoid infection in all species of young poultry include closed eyes, droopy wings, ruffled feathers, anorexia and profuse watery diarrhea with pasted vent (Gast and Beard, 1992). The postmortem findings are enteritis, necrosis in mucosal wall of small intestine, enlarged liver with necrotic foci and cheesy cecal cores (Hoop and Pospischill, 1993).

**Aim of study**

This study was undertaken to estimate the incidence of Salmonella isolated from migratory birds and to identify the role of those birds as a vector in the spreading of drug-resistant Salmonellae during the period from November 2017 to March 2018 in Dakahlia and Damietta Governorates.

**MATERIALS AND METHODS**

**Sampling strategy**

A total of 100 live migratory birds from different locations along Manzala Lake in Dakahlia and Damietta Governorates, Egypt were collected from hunters during the period from mid- November 2017 to late- March 2018. The species scientific names, English names and local names in Egypt according to Goodman and Meininger, (1989) were listed in (Table, 1). All of the collected birds were transported to Reference Laboratory for Veterinary Quality Control on Poultry Production (Gamasa lab.- Dakahlia branch), Animal Health Research Institute, then humanly sacrificed and subjected to postmortem examinations. A total of 500 internal organs form 100 birds (liver, cecum, spleen, lung and heart) were collected aseptically and processed for further examinations.
Salmonella isolation, identification and serotyping
Liver, cecum, spleen, lung and heart from each bird were pooled together as a one sample and then subjected to Salmonella isolation and identification according to ISO 6579 (2017) as follow: Samples were pre-enriched into buffered peptone water with a dilution (1:10) and incubated at 37°C for 18 hours. A total of 0.1 ml of the pre-enriched broth was transferred into a tube containing 10 ml of Rappaport-Vassiliadis medium with soy and incubated at 41.5°C for 24 hours. Another 1 ml of the pre-enrichment broth was transferred into a tube containing 10 ml of Muller-Kauffmann tetrationionate novobiocin broth and incubated at 37°C for 24 hours. A loop-full from each broth was streaked separately onto (Xylose Lysine Deoxycholate, Hektoen Enteric, MacConkey’s and SS) agar plates and incubated at 37°C for 24 hours then checked for growth of typical Salmonella colonies. The isolates that were biochemically identified as Salmonella were serologically identified according to Kauffman- white scheme (Kauffman, 1974) for determining somatic (O) and flagellar (H) antigens (Cruickshank et al., 1975) and (WHO, 2007).

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing was performed using agar disc diffusion method on Muller Hinton agar plates according to Finegold and Martin (1982). In brief, one colony from cultured plates of each Salmonella isolate was suspend into 5 ml Muller Hinton broth and incubated at 37°C for 2-8 hours until turbidity was seen. The turbidity was adjusted by careful dilution to be equivalent to a 0.5 McFarland's standard. A sterile swab was dipped into the Mueller Hinton broth then streaked onto a Mueller Hinton agar plate, and then the antimicrobial discs were arranged by using sterilized forceps at least 15 mm distance from the edge of the plate and apart from each other.

The antimicrobial agents that used were: Ciprofloxacin (5µg), Enrofloxacin (5µg), Norfloxacin (10µg), Levofloxacin (25µg), Ampicillin - sulbactam (20µg), Tetracycline (30µg), Doxycyclin (30µg), Erythromycin (15µg) Streptomycin (10µg), Neomycin (30µg) and Colistin sulphate (25µg). The Mueller Hinton plates were incubated at 37 °C/ overnight. The diameters of the inhibition zones were measured and the antimicrobial agents were categorized into susceptible, intermediate and resistant categories according to (CLSI, 2016).

Molecular detection of Salmonella virulence genes
DNA was extracted from Salmonella isolates using QIAamp DNA Mini kit (Qiagen, Germany, Gmbh) with modifications from the manufacturer’s recommendations. Briefly, 200 µl of samples suspension incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 minutes. Then 200 µl of 100% ethanol was added to the lysate. The samples then washed and centrifuged. Nucleic acid was eluted with 100 µl of elution buffer.

The oligonucleotide primers that used were provided from Metabion (Germany) listed in table (2); Primers were utilized in a 25 µl reaction containing 12.5 µl of Emerald AMP Max PCR master mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water and 6 µl of DNA template. The reaction was performed in a thermal cycler (T3, Biometra).

After that the PCR products were separated by electrophoresis on (1.5%) agarose gel (Applichem, Germany, Gmbh) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. A gelpilot 100 bp DNA Ladder (Qiagen, Germany, Gmbh) and gene ruler 50 bp, 100 bp ladders (Fermentas, Thermo) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data were analyzed through computer software (Automatic image capture software, protein simple formerly cell, Bioscience, UAS).

### Table 1: Number, species and localities of the collected migratory birds.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>English name</th>
<th>Local name in Egypt</th>
<th>Total number (100 birds)</th>
<th>Governorate</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anas clypeata</td>
<td>Shoveler</td>
<td>Kiish</td>
<td>6</td>
<td>Dakahlia</td>
<td>0</td>
</tr>
<tr>
<td>Anas crecca</td>
<td>Common Teal</td>
<td>Sharshiir</td>
<td>60</td>
<td>Dakahlia</td>
<td>55</td>
</tr>
<tr>
<td>Anas acuta</td>
<td>Pintail</td>
<td>Balbuul</td>
<td>11</td>
<td>Dakahlia</td>
<td>0</td>
</tr>
<tr>
<td>Anas platyrhynchos</td>
<td>Mallard</td>
<td>Khudaarri</td>
<td>14</td>
<td>Dakahlia</td>
<td>0</td>
</tr>
<tr>
<td>Fulica atra</td>
<td>Common Coot</td>
<td>Ghurr</td>
<td>4</td>
<td>Dakahlia</td>
<td>2</td>
</tr>
<tr>
<td>Anas penelope</td>
<td>Wigeon</td>
<td>Siwaay</td>
<td>5</td>
<td>Dakahlia</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dakahlia</td>
<td>5</td>
</tr>
</tbody>
</table>

Assiut Veterinary Medical Journal
Table 2: Oligonucleotide primers sequences, target genes, and cycling conditions.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primers sequences</th>
<th>Seg. (bp)</th>
<th>P. dent.</th>
<th>Amplification (35 cycles)</th>
<th>Final ext.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>invA</td>
<td>GTGAAATTA TCGCCACGTT CCGGCAA TCTATCGCAC CTTAAGGG AACCC</td>
<td>284</td>
<td>94°C 5 min.</td>
<td>94°C 30 sec. 55°C 30 sec. 72°C 30 sec.</td>
<td>72°C 10 min.</td>
<td>Oliveira et al., 2003</td>
</tr>
<tr>
<td>sopB</td>
<td>TCA GAA GRC GTC TAA CCA CTC TAC CGT CCT GCA CAC TC</td>
<td>517</td>
<td>94°C 5 min.</td>
<td>94°C 30 sec. 58°C 40 sec. 72°C 45 sec.</td>
<td>72°C 10 min.</td>
<td></td>
</tr>
<tr>
<td>mgtC</td>
<td>TGA CTA TCA ATG CTC CAG TGA AT ATT TAC TGG CCG CTA TGC TGT TG</td>
<td>677</td>
<td>94°C 5 min.</td>
<td>94°C 30 sec. 58°C 40 sec. 72°C 45 sec.</td>
<td>72°C 10 min.</td>
<td>Huehn et al., 2010</td>
</tr>
<tr>
<td>bcfC</td>
<td>ACC AGA GAC ATT GCC TTC C TTC TGC TCG CCG CTA TTC G</td>
<td>467</td>
<td>94°C 5 min.</td>
<td>94°C 30 sec. 53°C 40 sec. 72°C 45 sec.</td>
<td>72°C 10 min.</td>
<td></td>
</tr>
<tr>
<td>spvC</td>
<td>ACCAGAGAC ATTGCTTC C TTCTGATGC CGCTATTC G</td>
<td>467</td>
<td>94°C 5 min.</td>
<td>94°C 30 sec. 58°C 40 sec. 72°C 45 sec.</td>
<td>72°C 10 min.</td>
<td></td>
</tr>
<tr>
<td>fimH</td>
<td>GTGCAATT CCTCTTAAC CT TGGAAATAAT CGTACCGTT GCG</td>
<td>164</td>
<td>94°C 5 min.</td>
<td>94°C 30 sec. 59°C 30 sec. 72°C 30 sec.</td>
<td>72°C 7 min.</td>
<td>Hojati et al., 2013</td>
</tr>
<tr>
<td>fimA</td>
<td>CCT TTC TCC ATC GTC CTG AA TGG TGT TAT CTG CCT GAC CA</td>
<td>85</td>
<td>94°C 5 min.</td>
<td>94°C 30 sec. 50°C 30 sec. 72°C 30 sec.</td>
<td>72°C 7 min.</td>
<td>Cohen et al.1996</td>
</tr>
</tbody>
</table>

Seg. (bp)= amplified segment & P. dent. = primary denaturation & Sec. denat. = secondary denaturation & Extens= extension & Final ext. = final extension

RESULTS

Cultural and biochemical characteristics of the isolated Salmonellae

Cultural and biochemical characteristics of the isolated Salmonellae were similar to that recorded in OIE, (2004). Salmonella fermented dextrose, maltose, and mannitol with acid and gas production. Methyl red, citrate utilization and triple sugar iron tests were positive but Voges-proskauer, urease and indole tests were negative.

Incidence of Salmonella isolation and serotyping results from different migratory birds

A total of 7 Salmonella isolates were reported from 100 migratory birds that collected along Manzala Lake in Dakahlia and Damietta Governorates with an incidence of (7%); 5 Salmonella isolates from Common Teal with a percentage of (5/60) (8.3%), one isolate from Mallard duck with a percentage of (1/14) (7.1%) and one isolate from Shoveler with a percentage of (1/6) (16.6 %). Pintail, Common Coot

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and Wigeon birds were negative for Salmonella detection.

Four different serovars across study sites were reported (S. Typhimurium, S. Bardo, S. Montevideo and S. Kentucky). Along Manzala Lake at Dakahlia Governorates; 4 S. Bardo isolates were recorded from the collected Common Teal birds. However along the lake at Damietta Governorate, 3 Salmonella isolates were recorded; one S. Montevideo from Common Teal birds, one S. Typhimurium from Shoveler birds and one S. Kentucky from Mallard ducks (Table 3).

Table 3: Serotyping of Salmonellae and percentage of isolate per species.

<table>
<thead>
<tr>
<th>Location</th>
<th>Species of bird</th>
<th>No. and % of Salmonella isolates</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dakahlia</td>
<td>Common Teal</td>
<td>4 (4/55) (7.3%)</td>
<td>S. Bardo</td>
</tr>
<tr>
<td>Damietta</td>
<td>Common Teal</td>
<td>1 (1/5) (20%)</td>
<td>S. Montevideo</td>
</tr>
<tr>
<td></td>
<td>Mallard</td>
<td>1 (1/14) (7.1%)</td>
<td>S. Kentucky</td>
</tr>
<tr>
<td></td>
<td>Shoveler</td>
<td>1 (1/6) (16.7%)</td>
<td>S. Typhimurium</td>
</tr>
</tbody>
</table>

Antimicrobial Susceptibility pattern

Characterization of the isolated Salmonellae based on the multiple antimicrobial resistances was an important issue in this study since these results indicated that Salmonella plays an important role as reservoirs of multi-drug resistant bacteria. All Salmonella isolates in this study showed high sensitivity to Streptomycin, Erythromycin, Norfloxacin, Colistin sulphat and Doxycycline. Two S. Bardo isolates, S. Typhimurium, S. Kentucky showed multi-drug resistance. S. Montevideo and 2 S. Bardo isolates showed higher sensitivity to most of the used antimicrobial agents (Table 4 and 5).

Table 4: Antimicrobial Susceptibility pattern of isolated Salmonellae.

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin/sulb.*</td>
<td>-</td>
<td>R</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>R</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>-</td>
<td>R</td>
<td>S</td>
<td>-</td>
<td>I</td>
<td>-</td>
<td>R</td>
</tr>
<tr>
<td>Enerofloxacin</td>
<td>-</td>
<td>R</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>R</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>I</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>Neomycin</td>
<td>-</td>
<td>R</td>
<td>I</td>
<td>-</td>
<td>-</td>
<td>I</td>
<td>-</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>Colistin sulphat</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>-</td>
<td>R</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>S</td>
</tr>
</tbody>
</table>

*Ampicillin/sulb.= Ampicillin/sulbectam. The antimicrobial agents were categorized into susceptible, intermediate and resistant categories according to (CLSI, 2016). 1, 2, 3, 7 = S. Bardo, 4= S. Typhimurium, 5= S. Kentucky, 6= S. Montevideo.

Table 5: Number of sensitive, intermediate and resistant Salmonella isolates against different antimicrobial agents.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Salmonella (7 isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>Ampicillin/sulbectam</td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2</td>
</tr>
<tr>
<td>Enerofloxacin</td>
<td>4</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>6</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>4</td>
</tr>
<tr>
<td>Neomycin</td>
<td>3</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>6</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>7</td>
</tr>
<tr>
<td>Colistin sulphat</td>
<td>6</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>6</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>5</td>
</tr>
</tbody>
</table>

PCR was a good tool for accurate detection of several virulence genes such as \textit{inv}A, \textit{sop}B, \textit{mgf}C, \textit{bcf}C, \textit{spv}C, \textit{fim}H and \textit{fim}A in the examined Salmonella isolates. All of the examined genes were recorded in all isolates (figure 1, 2, 3, 4, 5, 6 and 7).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Agarose gel electrophoresis of PCR products for Salmonella isolates to detect \textit{inv}A gene in genomic DNA. Lane L: 100-1000 bp DNA ladder. Neg: Negative control, Pos: Positive control. Lanes: 1 to 7 were positive samples.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Agarose gel electrophoresis of PCR products for Salmonella isolates to detect \textit{sop}B gene in genomic DNA. Lane L: 100-600 bp DNA ladder. Neg: Negative control, Pos: Positive control. Lanes: 1 to 7 were positive samples.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Agarose gel electrophoresis of PCR products for Salmonella isolates to detect \textit{mgf}C gene in genomic DNA. Lane L: 100-1000 bp DNA ladder. Neg: Negative control, Pos: Positive control. Lanes: 1 to 7 were positive samples.}
\end{figure}
Figure (4): Agarose gel electrophoresis of PCR products for Salmonella isolates to detect *spvC* gene in genomic DNA. Lane L: 100-600 bp DNA ladder. Neg: Negative control, Pos: Positive control. Lanes: 1 to 7 were positive samples.

Figure (5): Agarose gel electrophoresis of PCR products for Salmonella isolates to detect *fimH* gene in genomic DNA. Lane L: 100-600 bp DNA ladder. Neg: Negative control, Pos: Positive control. Lanes: 1 to 7 were positive samples.

Figure (6): Agarose gel electrophoresis of PCR products for Salmonella isolates to detect *fimA* gene in genomic DNA. Lane L: gene ruler 50 bp DNA ladder. Neg: Negative control, Pos: Positive control. Lanes: 1 to 7 were positive samples.
In this study 3 migratory bird species (Common Teal, Mallard and Shoveler) were positive for Salmonella in Dakahlia and Damietta governorates along Lake Manzala. Four Salmonella serotypes were reported. Seven virulence genes were recorded in all isolates. Multidrug resistance was present in 3 Salmonella serotypes meanwhile one isolate (S. Montevideo) showed no resistance (Table, 6).

Table 6: collective data of the positive bird species for Salmonella isolation, resistant antimicrobial agents and Salmonella virulence genes.

<table>
<thead>
<tr>
<th>Bird species</th>
<th>Site of collection</th>
<th>Isolated Serotype</th>
<th>Resistant antimicrobial agents</th>
<th>Salmonella virulence genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common Teal</td>
<td>Dakahlia</td>
<td>S. Bardo</td>
<td>AMP* CIP ENR N E C T DO TE</td>
<td>invA sopB mgtC bcfC spvC fimH fim A</td>
</tr>
<tr>
<td>Common Teal</td>
<td>Damietta</td>
<td>S. Montevideo</td>
<td>-</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>Mallard</td>
<td>Damietta</td>
<td>S. Kentucky</td>
<td>AMP CIP ENR LEV</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>Shoveler</td>
<td>Damietta</td>
<td>S. Typhimurium</td>
<td>AMP CIP ENR NOR TE</td>
<td>+ + + + + + + +</td>
</tr>
</tbody>
</table>

*Ciprofloxacin (CIP), Enrofloxacin (ENR), Norfloxacin (NOR), Levofloxacin (LEV), Ampicillin - sulbactam (AMP), Tetracycline (TE), Doxycyclin (DO), Erythromycin (E), Neomycin (N) and Colistin sulphate (CT).

DISCUSSION

Salmonella is one of the most common zoonotic bacteria that cause public health threat and severe losses to poultry industry (Faruq et al., 2016).

The migratory birds are flying across national and intercontinental borders, they can become long range vectors for Salmonella (Georgopoulou and Tsiouris, 2008), during the migration period, the birds immune system is weakened due to stress of migration that contributing to disease spreading and latent infections can be occurred (Altizer et al., 2011). Many serotypes of the genus Salmonella were able to survive form weeks to months in poultry litter, wild bird feces, soil and dust particles (Berchieri Junior and Freitas Neto, 2009). This explained the obtaining results in this study where Salmonella was isolated from 6 species of 100 migratory birds [Shoveler (6), Common Teal (60), Pintail (11), Mallard (14), Common Coot (4)
and Wigeon (5)] in winter season along Manzala Lake in Dakahlia and Damietta Governorates in Egypt. Seven Salmonellae were isolated with a percentage of (7%); 5 isolates (4 S. Bardo and one S. Montevideo) were reported from Common Teal with a percentage of (8.3%), one S. Kentucky isolate from Mallard with a percentage of (7.1%) and one S. Typhimurium isolate from Shoveler with a percentage of (6.3 %).

The incidence of Salmonella isolation in this study was (7%) and it considered higher from other studies conducted by Fallacara et al. (2004) who reported 8 Salmonella isolates from 450 free living water fowl in Columbus Zoo, Ohio with a percentage of (1.7%), Rodríguez et al. (2018) who isolated 4 Salmonella serovars (S. Typhimurium, S. Schwarzengrund, S. enterica subsp. I [4,12: i -j] and S. enterica subsp. IIb [60: r; e, n, x, z151]) with a percentage of (1%) from cloacal swabs of 599 free-living waterfowl from April 2014 to July 2016, Fallacara et al. (2001) reported a lower incidence of Salmonella (0.2%) from free living water fowl and Foti et al. (2011) who isolated 2 S. bongori isolates from faecal swabs and internal organs of migratory birds in Italy.

Several researchers such as Fallacara et al. (2001), Fallacara et al. (2004), Foti et al. (2011) and Rodríguez et al. (2018) isolated Salmonella from cloacal swabs collected from free living water fowl but in this study Salmonella was isolated from internal organs of these birds with a higher percentage. This might be attributed to the intermittent shedding of Salmonella.

In the present study; Pintail, Common Coot and Wigeon birds were negative for Salmonella isolation and this agreed with Antilles et al. (2015) who didn’t isolate Salmonella from cloacal swabs of the same birds that collected from north-east Spain during the hunting season (October to February) from the end of 2008 to 2011.

In the present study, the examined migratory birds may act as reservoirs for antimicrobial resistant Salmonella pathogen and can be transmitted by direct contact with food-producing animals, human waste and with species that can act as vectors (insects, rodents, and other birds).

Recently the misuse of antimicrobial agents resulted in multidrug resistance of Salmonella particularly in the developing countries (Faruq et al., 2016). Two S. Bardo isolates, S. Typhimurium and S. Kentucky in this study showed multi-drug resistance that raise an alert for the need and importance of a surveillance programs to avoid Salmonella infection. These obtaining results were agreed to a great extent with (Palmgren et al., 1997) who reported multidrug-resistant strains of S. Typhimurium isolated from migratory birds in Sweden.

The obtaining results in the present study showed that S. Montevideo and two S. Bardo isolates showed higher sensitivity to most of the used antimicrobial agents and these nearly agreed with Grigar et al. (2017) who reported that S. Thompson and S. Braenderup isolated from waterfowl along the Texas Gulf coast were susceptible to amoxicillin/clavulanic acid, ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline and trimethoprim/sulfamethoxazole.

Salmonella pathogenesis is controlled by a series of genes that responsible for invasion (Porter et al., 1997), colonization (Thiagarajan et al., 1996), and spread (Libby et al., 1997) within the host. The host adaptation of Salmonella was influenced by the distribution of fimbrial and non fimbrial adhesins (Baumler et al., 1997). The virulence of Salmonella isolates was assessed using PCR technique for the detection of invA, sopB, mgtC, bcfC, spvC, fimH and fimA virulence genes using PCR technique; all genes were recorded in all Salmonella isolates. Some researchers such as Krawiec et al. (2015) reported invA and sopB genes with a percentage of (100%) and (94.45%) respectively in Salmonella isolated from aquatic wild birds and free living birds in Poland, Hudson et al. (2000) isolated Salmonella from non-domestic birds in Southeastern United States and all isolates contained the invasion gene invA but 17 isolates contained the spvC gene.

The findings in this study suggest that migratory birds considered as an important source of Salmonella strains that can contaminate the environment around poultry farms and produce a new endemic area of Salmonellosis that adversely affect poultry industry leading to sever economic losses. Also migratory birds may considered as a source of Salmonella strains that are pathogenic to people.

CONCLUSION

In conclusion: Identification and antimicrobial resistance of Salmonella isolated from migratory birds is necessary to the early detection of zoonotic strains and also to evaluate the emergence of new resistance strains. Salmonella was isolated from two Egyptian Governorates along Manzala Lake with an incidence of (7%); S. Bardo, S. Montevideo, S. Kentucky and S. Typhimurium. Some of the isolated Salmonellae showed multi-drug resistance. Several virulence genes such as invA, sopB, mgtC, bcfC, spvC, fimH and fimA were detected in all of recorded Salmonella isolates.

Further studies and surveillance programs should be conducted to investigate Salmonella strains isolated from humans residing in fishing, backyards and poultry farms to provide an overview about the
transmission processes of Salmonella from migratory birds to human and studying the migration pattern that will be useful in the prediction of future outbreaks due to emerging zoonotic pathogens.

Finally, it’s necessary to apply control measures and biosecurity programs in poultry farms to avoid any risk of Salmonella transmission and other zoonotic diseases via migratory birds. Also people that hunt, cook and eat migratory birds should be aware with the risk involved from such birds.

REFERENCES


أهمية الطيور المهاجرة كعامل لنشر السالمونيلا في مصر في الفترة من نوفمبر 2017 إلى مارس 2018

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لعب الطيور المهاجرة دورا مهما في نقل الأمراض المشتركة خاصة السالمونيلا والتي تسبب حالات مرضية في الإنسان والحيوان بالإضافة إلى خسائر اقتصادية في صناعة الدواجن ولذلك تم تحديد نسبة انتشار السالمونيلا في ستة أنواع من الطيور المهاجرة في امتداد بحيرة المنزلة في محافظتي الدقهلية ودمياط بمصر في الفترة من نوفمبر 2017 إلى مارس 2018. تم تجميع 100 طائر حي من الصيادين حيث تم تجمع الأعضاء الداخلية وإجراء الفحص البكتيري وتحليلات الحساسية. تم إجراء اختبار تفاعل إنزيم البلمرة المتسلسل لبعض جينات الضراوة لميكروب السالمونيلا. تم تسجيل 7 معزولات من السالمونيلا بنسبة (7%) من طيور الشرير، الخضاري. أوثر. تم تسجيل 7 عزرات سالمونيلا في هذه الدراسة (تيفيموريم، باردو، مونتينيفيدو، كنتاكي). أظهرت معزولات السالمونيلا حساسية عالية للإستربتوميسين، نورفلاكسين، فلوكساسين، كلورامينوف، ميكلسبيكين، دوكسي سيكللين بينما اظهرت معزولات سالمونيلا باردو ومعزولة سالمونيلا تيفيموريم ومعزولة سالمونيلا كنتاكي مقاومة عالية لمعظم مضادات الميكروبات. أظهرت سالمونيلا مونتينيفيدو ومعزولات سالمونيلا باردو مقاومة عالية لمعظم مضادات الميكروبات المستخدمة. تم تسجيل توأمة لهذا الميكروب باستخدام اختبار تفاعل إنزيم البلمرة المتسلسل invA, sopB, mgtC, bcfC, spvC, fimH , fimA حيث توجد هذه الجينات في جميع معزولات السالمونيلا بهذه الدراسة.