

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 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. Seven virulence genes (*invA*, *sopB*, *mgtC*, *bcfC*, *spvC*, *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 Platyrhynchos*) (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

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Rose, 1996). 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 Dobrynina, 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 from 100 birds (liver, cecum, spleen, lung and heart) were collected aseptically and processed for further examinations.

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

Species			Total number (100 birds)	Localities and No. of birds	
Scientific name	English name	Local name in Egypt		Governorate	Number
<i>Anas clypeata</i>	Shoveler	Kiish	6	Dakahlia	0
				Damietta	6
<i>Anas crecca</i>	Common Teal	Sharshiir	60	Dakahlia	55
				Damietta	5
<i>Anas acuta</i>	Pintail	Balbuul	11	Dakahlia	0
				Damietta	11
<i>Anas platyrhynchos</i>	Mallard	Khudaarii	14	Dakahlia	0
				Damietta	14
<i>Fulica atra</i>	Common Coot	Ghurr	4	Dakahlia	2
				Damietta	2
<i>Anas penelope</i>	Wigeon	Siwwaay	5	Dakahlia	0
				Damietta	5

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 soya 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 tetrathionate 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 S S) 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 Mueller 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 (Appllichem, 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 2: Oligonucleotide primers sequences, target genes, and cycling conditions.

Target gene	Primers sequences	Seg. (bp)	P. dent.	Amplification (35 cycles)			Final ext.	Reference
				Sec. dent.	Annealing	Ext.		
<i>invA</i>	GTGAAATTA TCGCCACGTT CGGGCAA TCATCGCAC CGTCAAAGG AACC	284	94°C 5 min.	94°C 30 sec.	55°C 30 sec.	72°C 30 sec.	72°C 10 min.	Oliveira et al., 2003
<i>sopB</i>	TCA GAA GRC GTC TAA CCA CTC TAC CGT CCT CAT GCA CAC TC	517	94°C 5 min.	94°C 30 sec.	58°C 40 sec.	72°C 45 sec.	72°C 10 min.	
<i>mgfC</i>	TGA CTA TCA ATG CTC CAG TGA AT ATT TAC TGG CCG CTA TGC TGT TG	677	94°C 5 min.	94°C 30 sec.	58°C 40 sec.	72°C 45 sec.	72°C 10 min.	Huehn et al., 2010
<i>bcfC</i>	ACC AGA GAC ATT GCC TTC C TTC TGC TCG CCG CTA TTC G	467	94°C 5 min.	94°C 30 sec.	53°C 40 sec.	72°C 45 sec.	72°C 10 min.	
<i>spvC</i>	ACCAGAGAC ATTGCCTTC C TTCTGATCGC CGCTATTC G	467	94°C 5 min.	94°C 30 sec.	58°C 40 sec.	72°C 45 sec.	72°C 10 min.	
<i>fimH</i>	GTGCCAATT CCTCTTACCG TT TGGAATAAT CGTACCGTT GCG	164	94°C 5 min.	94°C 30 sec.	59°C 30 sec.	72°C 30 sec.	72°C 7 min.	Hojati et al., 2013
<i>fimA</i>	CCT TTC TCC ATC GTC CTG AA TGG TGT TAT CTG CCT GAC CA	85	94°C 5 min.	94°C 30 sec.	50°C 30 sec.	72°C 30 sec.	72°C 7 min.	Cohen et al.1996

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

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.

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

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.

Antimicrobial Agent	1			2			3			4			5			6			7		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ampicillin/sulb.*	-	-	R	S	-	-	S	-	-	-	-	R	-	-	R	S	-	-	-	-	S
Ciprofloxacin	-	-	R	S	-	-	-	I	-	-	-	R	-	-	R	-	I	-	S	-	-
Enerofloxacin	-	-	R	S	-	-	S	-	-	-	-	R	-	-	R	S	-	-	S	-	-
Norfloxacin	S	-	-	S	-	-	S	-	-	-	-	R	S	-	-	S	-	-	S	-	-
Levofloxacin	-	I	-	S	-	-	S	-	-	-	I	-	-	-	R	S	-	-	S	-	-
Neomycin	-	-	R	-	I	-	S	-	-	S	-	-	-	I	-	S	-	-	-	-	R
Erythromycin	S	-	-	S	-	-	-	-	R	S	-	-	S	-	-	S	-	-	S	-	-
Streptomycin	S	-	-	S	-	-	S	-	-	S	-	-	S	-	-	S	-	-	S	-	-
Colistin sulphat	S	-	-	S	-	-	S	-	-	S	-	-	S	-	-	S	-	-	-	-	R
Doxycycline	S	-	-	S	-	-	S	-	-	S	-	-	S	-	-	S	-	-	-	-	R
Tetracycline	-	-	R	S	-	-	S	-	-	-	-	R	S	-	-	S	-	-	S	-	-

*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.

Antimicrobial agent	Salmonella (7 isolates)		
	Sensitive	Intermediate	Resistant
Ampicillin/sulbectam	4	0	3
Ciprofloxacin	2	2	3
Enerofloxacin	4	0	3
Norfloxacin	6	0	1
Levofloxacin	4	2	1
Neomycin	3	2	2
Erythromycin	6	0	1
Streptomycin	7	0	0
Colistin sulphat	6	0	1
Doxycycline	6	0	1
Tetracycline	5	0	2

PCR technique results for detection of *invA*, *sopB*, *mgtC*, *bcfC*, *spvC*, *fimH* and *fimA* virulence genes in the examined Salmonella isolates.

PCR was a good tool for accurate detection of several virulence genes such as *invA*, *sopB*, *mgtC*, *bcfC*,

spvC, *fimH* and *fimA* in the examined Salmonella isolates. All of the examined genes were recorded in all isolates (figure 1, 2, 3, 4, 5, 6 and 7).

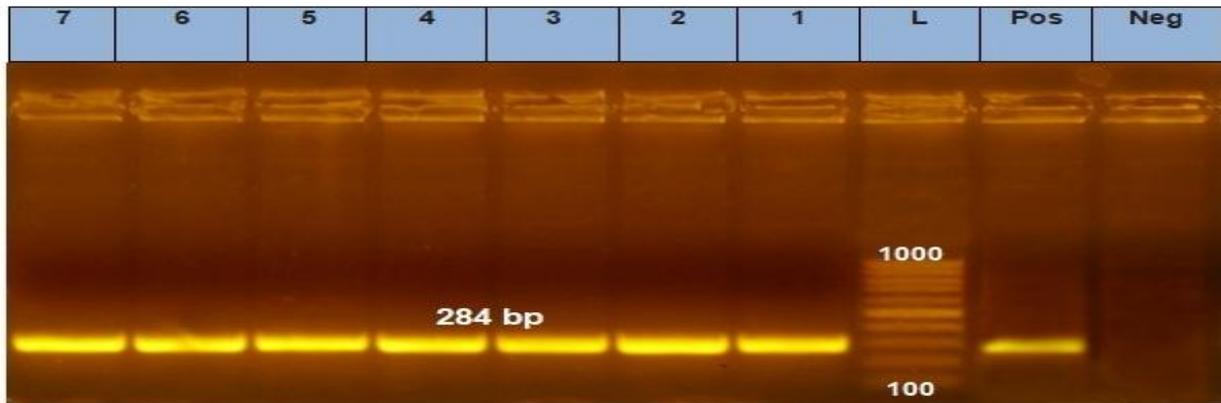


Figure (1): Agarose gel electrophoresis of PCR products for Salmonella isolates to detect *invA* gene in genomic DNA. Lane L: 100-1000 bp DNA ladder. Neg: Negative control, Pos: Positive control. Lanes: 1 to 7 were positive samples.

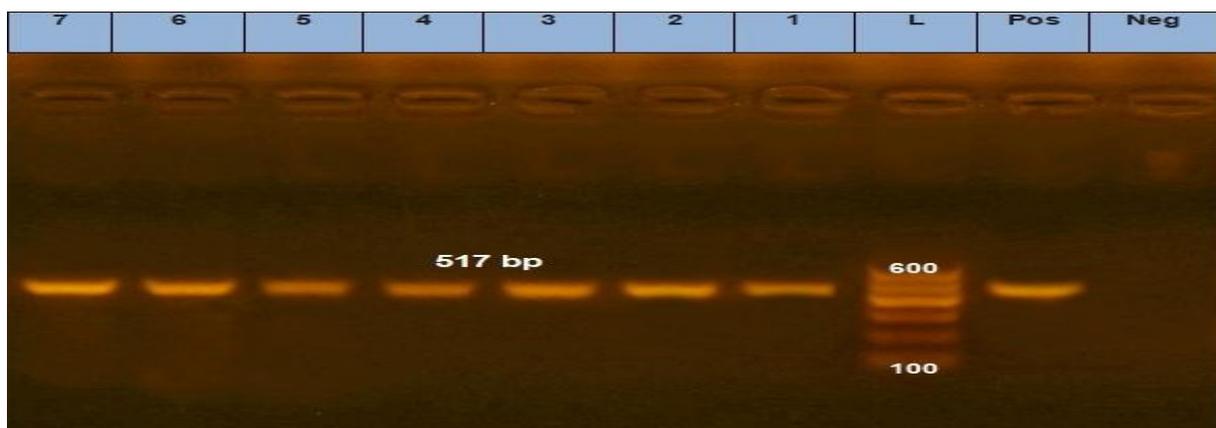


Figure (2): Agarose gel electrophoresis of PCR products for Salmonella isolates to detect *sopB* gene in genomic DNA. Lane L: 100-600 bp DNA ladder. Neg: Negative control, Pos: Positive control. Lanes: 1 to 7 were positive samples.

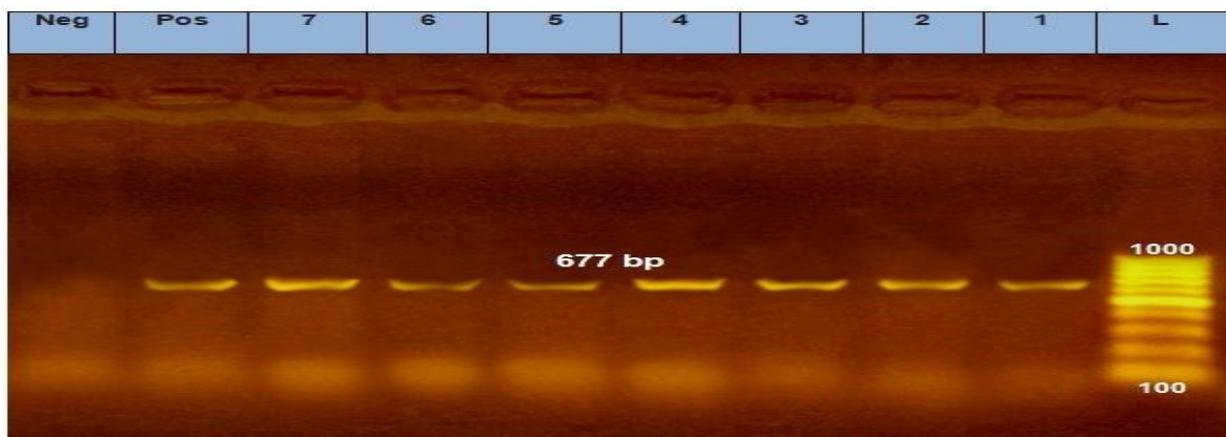


Figure (3): Agarose gel electrophoresis of PCR products for Salmonella isolates to detect *mgtC* gene in genomic DNA. Lane L: 100-1000 bp DNA ladder. Neg: Negative control, Pos: Positive control. Lanes: 1 to 7 were positive samples.

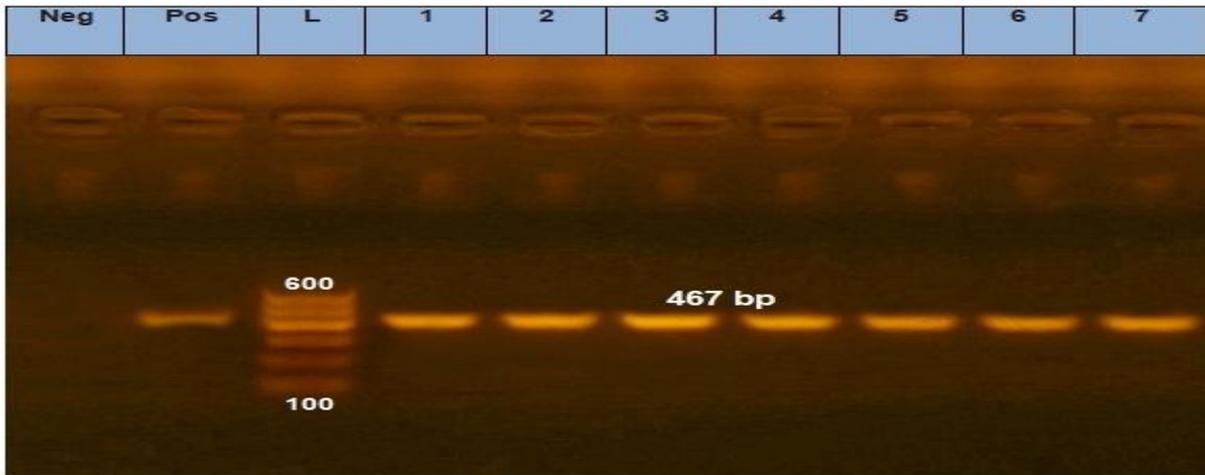


Figure (4): Agarose gel electrophoresis of PCR products for Salmonella isolates to detect *spvC* gene in genomic DNA. Lane L: 100-600 bp DNA ladder. Neg: Negative control, Pos: Positive control. Lanes: 1 to 7 were positive samples.

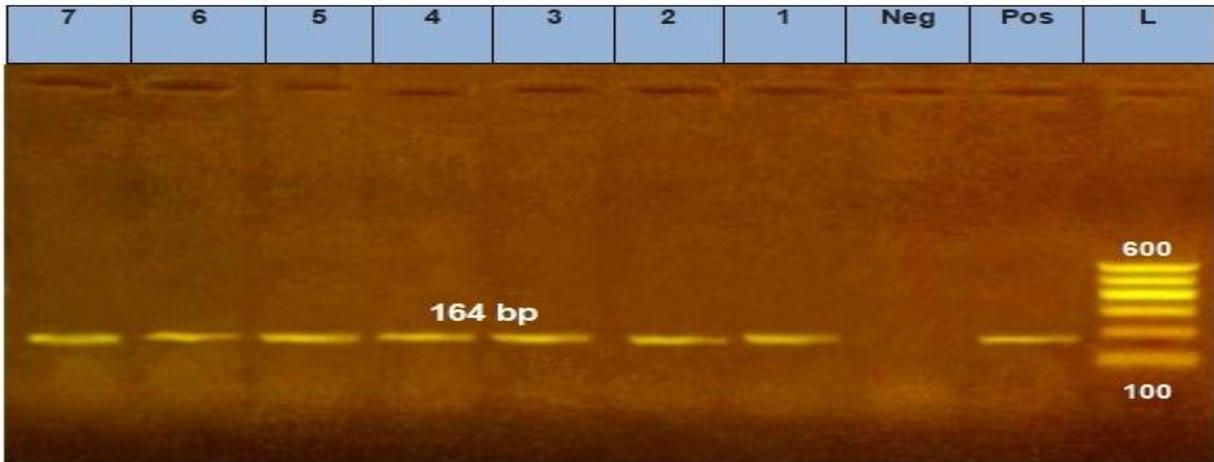


Figure (5): Agarose gel electrophoresis of PCR products for Salmonella isolates to detect *fimH* gene in genomic DNA. Lane L: 100-600 bp DNA ladder. Neg: Negative control, Pos: Positive control. Lanes: 1 to 7 were positive samples.

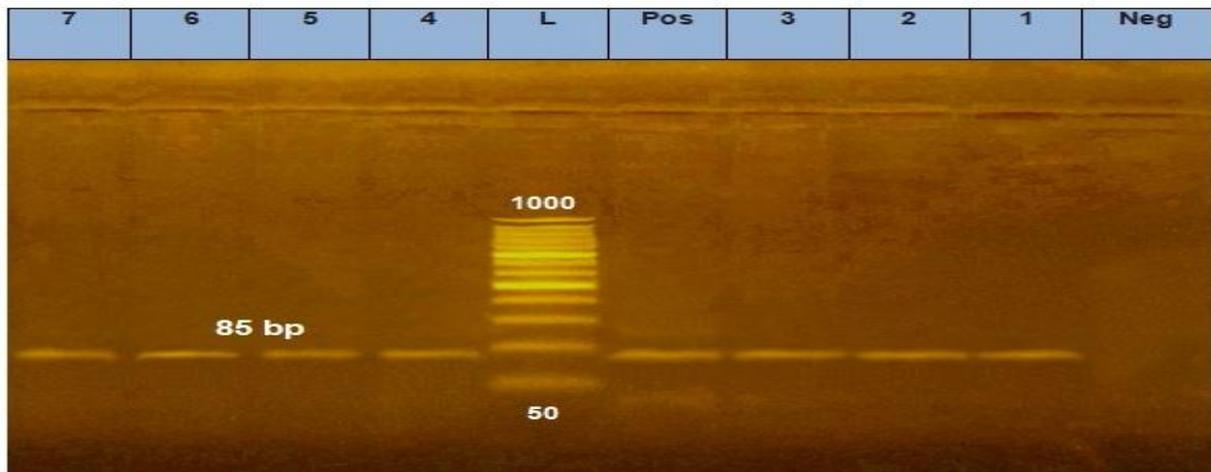


Figure (6): Agarose gel electrophoresis of PCR products for Salmonella isolates to detect *fimA* gene in genomic DNA. Lane L: gene ruler 50 bp DNA ladder. Neg: Negative control, Pos: Positive control. Lanes: 1 to 7 were positive samples.

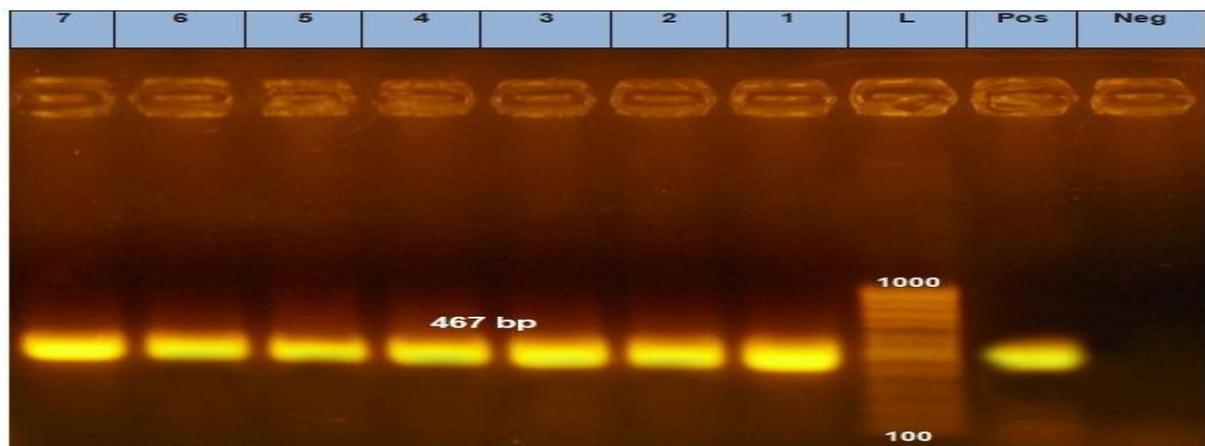


Figure (7): Agarose gel electrophoresis of PCR products for Salmonella isolates to detect *bcfC* gene in genomic DNA. Lane L: 100-1000 bp DNA ladder. Neg: Negative control, Pos: Positive control. Lanes: 1 to 7 were positive samples.

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 mean while 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.

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

*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 sever 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: -] and *S. enterica* subsp. IIIb [60: r: e, n, x, z15]) 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 birds 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*, *mgfC*, *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*, *mgfC*, *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.

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أهمية الطيور المهاجرة كعائل لنشر السالمونيلا في مصر في الفترة من نوفمبر ٢٠١٧ الي مارس ٢٠١٨

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تلعب الطيور المهاجرة دورا مهما في نقل الامراض المشتركة خاصة السالمونيلا والتي تسبب حالات مرضية في الانسان والحيوان بالاضافة الي خسائر اقتصادية في صناعة الدواجن ولذلك تم تحديد نسبة انتشار السالمونيلا في ستة انواع من الطيور المهاجرة علي امتداد بحيرة المنزلة في محافظتي الدقهلية ودمياط بمصر في الفترة من نوفمبر ٢٠١٧ الي مارس ٢٠١٨. تم تجميع ١٠٠ طائر حي من الصيادين حيث تم تجميع الاعضاء الداخلية و اجراء الفحص البكتيريولوجي واختبارات الحساسية. تم اجراء اختبار تفاعل انزيم البلمرة المتسلسل لبعض جينات الضراوة لميكروب السالمونيلا. تم تسجيل ٧ معزولات من السالمونيلا بنسبة (٧%) من طيور الشرشير- الخضاري- الكيش. تم تسجيل ٤ عترات سالمونيلا في هذه الدراسة (تيفيموريم- باردو- مونتيفيدو- كنتاكي). اظهرت معزولات السالمونيلا حساسية عالية للاستربتوميسين- اريثرومايسين- نورفلوكساسين- كوليستين سلفات- دوكسي سيكلين بينما اظهرت معزولاتان سالمونيلا باردو ومعزولة سالمونيلا تيفيموريم ومعزولة سالمونيلا كنتاكي مقاومة متعددة لمضادات الميكروبات. اظهرت سالمونيلا مونتيفيدو ومعزولتان سالمونيلا باردو حساسية عالية لمعظم مضادات الميكروبات المستخدمة. تم تسجيل تواجد ٧ جينات ضراوة *invA*, *sopB*, *mgtC*, *bcfC*, *spvC*, *fimH*, *fimA* لهذا الميكروب باستخدام اختبار تفاعل انزيم البلمرة المتسلسل حيث تواجدت هذه الجينات في جميع معزولات السالمونيلا بهذه الدراسة.