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Phenotypic and Genotypic Characterization of *Salmonella* enterica Serovars Isolated from Imported Poultry

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Abstract | Monitoring of imported 1-day-old poultry is mandated in Egypt to prevent the possible introduction of new *Salmonella* serovars into the country's poultry industry. Such serovars are considered to be a major public health threat. We examined 391 imported poultry flocks for the presence of salmonellae (231 duckling, 84 chick, and 76 turkey poult), serotyped all isolated salmonellae, and performed antimicrobial susceptibility testing. Molecular profiles were also constructed based on results of conventional polymerase chain reaction assays to detect virulence genes (*stn*, *avrA*, and *sopB*) and antibiotic resistance genes (*blaTEM*, *tetA(A)*, and *qnrS*) in the *Salmonella* isolates. Thirty *Salmonella* strains were isolated from the 391 samples (7.7%). By poultry type, salmonellae were isolated from 21 of 231 (9.1%) duckling samples, 6 of 84 (7.1%) of chick samples, and 3 of 76 (3.9%) turkey poult samples. Serotyping of the isolates identified 16 different serovars: *S.* Enteritidis, *S.* Typhimurium, *S.* Sinstorf, *S.* Muenster, *S.* Vejle, *S.* Cuckmere, *S.* Indiana, *S.* Infantis, *S.* Koenigstuhl, *S.* Macallen, *S.* Nchanga, *S.* Neftenbach, *S.* Newlands, *S.* Nigeria, *S.* Nyborg, and *S.* Regent. The isolates showed variable degrees of antibiotic resistance across species. All tested *Salmonella* strains harbored the virulence and antibiotic resistance genes, with the exception of *qnrS*, which was found in only 50% of the isolates. In conclusion, examination of imported poultry is a critical point of control to prevent poultry from becoming a reservoir for human health hazards, including salmonellae with antimicrobial resistance phenotypes.

Keywords | Imported poultry, *Salmonella* serovars, sensitivity, virulence genes, antibiotic genes.

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INTRODUCTION

In addition to its role in food-borne illness all over the world, *Salmonella* is an important bacterial pathogen in poultry (Osman et al., 2010; Foley et al., 2011). Several serovars have been isolated from both poultry and humans, and poultry can transmit *Salmonella* to humans (Osman et al., 2010; European Food Safety Authority [EFSA], 2019). The international trade of poultry is one of the factors affecting the spread of salmonellosis (EFSA, 2019) by acting as a portable reservoir for *Salmonella* (Osman et al., 2010), transmitting infection through the food production chain (Velhner et al., 2018), and vertically through the infected parents (Foley et al., 2011; Osman et al., 2014b), by transmission of multidrug-resistant (MDR) *Salmonella* in the Mediterranean region (Le Hello et al., 2013).

EFSA (2019) classified salmonellosis as the second human zoonotic disease underlying gastrointestinal illness and (Ezzeldeen et al., 2013) mentioned that the salmonellosis can cause multi-organ systemic infection. In the European Union (EU), salmonellae are estimated to cause

almost 92,000 illnesses annually (EFSA, 2019). These illnesses are primarily caused by two serovars, *Salmonella* Enteritidis and *Salmonella* Typhimurium (Sodagari et al., 2019). However, non-typhoidal *Salmonella* (NTS) can also cause mild to severe illness and sometimes act as a means of transmission of antibiotic resistance to other *Salmonella* importance in poultry and human health (Osman et al., 2014b; Sodagari et al., 2019).

Uncontrolled and indiscriminate use of antibiotics for growth promotion or prophylaxis, especially in low- and middle-income countries, can lead to the emergence of antibiotic resistance to fluoroquinolones and extended-spectrum beta-lactam antibiotics in the poultry sector (Badr et al., 2015; Velhner et al., 2018). MDR salmonellae are significant for both human and animal health because they can lead to illness that is unresponsive to antibiotic treatment (Chen et al., 2013).

Antibiotic resistance is transmitted between bacteria through mobile genetic elements such as plasmids, transposons, and integrons. Such transmission results in healthy animals becoming carriers for antibiotic-resistant bacteria, which may subsequently cause human infections that are difficult to treat (Mthembu et al., 2019).

Bacterial self-defense against antibiotics involves developing modifications such as decreased cell permeability, alteration or replacement of the target, and enzyme inactivation that serve as mechanisms enabling antibiotic resistance (Frye and Jackson, 2013). For example, bacteria that are resistant to beta-lactam antibiotics produce beta-lactamase, an enzyme that can destroy the beta-lactam ring and thus inactivate the antibiotics (Mthembu et al., 2019). In salmonellae, many extended-spectrum beta-lactamases (ESBLs) exist, with *tem*, *shv*, and *ctx-M* genes being the most effective and frequent encoding genes (Jin and Ling, 2006).

In gram-negative bacteria, *tetA* is the most frequent antibiotic-resistance gene, and it may be present on either chromosomes or plasmids (Pezella et al., 2004). This gene enables tetracycline resistance in salmonellae that infect humans and animals (Threlfall, 2002). Qnr proteins are a group of penta peptide repeat proteins that allow bacteria to evade the effects of quinolones and develop resistance to these antibiotics. *Qnr* genes prevent or limit ciprofloxacin from disabling bacterial DNA gyrase and topoisomerase IV, enzymes that are necessary for bacterial DNA synthesis and thus growth (Tran and Jacoby, 2002).

Various virulence genes are important for *Salmonella* pathogenesis, such as *sopB* and *avrA*, which encode T3SS proteins that are involved in the Salmonella-induced inflammatory response and have anti-apoptotic roles. Together, the two proteins delay apoptosis of epithelial cells, which aids salmonellae in avoiding host adaptive immune response and in constructing a stable intracellular niche (Wu et al., 2011). *Salmonella* enterotoxin (stn) is an important virulence factor that causes diarrhea (Chopra et al., 1999). Some authors have reported that *stn* is not a virulence factor because they did not find any decrease in virulence phenotypes when the *stn* gene was deleted in tested mutant (Nakano et al., 2012). In contrast, earlier research (Chopra et al., 1999) found that inactivation of the *stn* gene reduced the ability of *Salmonella* Typhimurium ability to induce intestinal fluid accumulation.

The present study was conducted to determine the presence of salmonellae in chicks, ducklings, and poults being imported into Egypt. The isolates were studied with regard to their phenotypic characteristics and identified by serovar. In addition, their antimicrobial resistance profiles and genotypic profiles were defined based on important virulence genes (*stn*, *avrA*, and *sopB*) and antibiotic resistance genes (*blaTEM*, *tetA(A)*, and *qnrS*).

MATERIALS AND METHODS

Ethics approval

Birds were handled in accordance with the regulations for collecting samples from live animals, and this study was approved by the animal care committee of the Animal Health Research Institute.

Sampling

We obtained 391 samples from imported poultry flocks (231 ducklings, 84 chicks, and 76 turkey poults) from January to December 2019, by collecting the paper-lined the boxes used to transport the birds. Each box contained 30 young birds. The collected samples represented the pooled meconium samples from these 30 birds/box.

Isolation and characterization of salmonellae

Salmonella spp. were isolated and identified according to ISO 6579-1: 2017. The paper -lined the box (average 15-20gm) was placed in pre-enrichment medium 1:10 dilution (buffered peptone water; Oxoid, UK), which was subsequently incubated at 37°C for 16–18 h. Next, 0.1 mL of the pre-enrichment medium was transferred to modified semisolid Rappaport-Vassiliadis medium (MSRV, LabM, UK) and incubated at 41.5°C for 24 h. In addition, 1 mL of the pre-enrichment medium was transferred to MKTTn broth (LabM, UK) and incubated aerobically 37°C for 24 h, and then streaked onto XLD (LabM, UK) and SS (Oxoid, UK) agar plates, which were subsequently incubated at 37°C for 24 h aerobically. Selected colonies were then identified by biochemical tests (urea agar, triple sugar iron,

and lysin iron) (Oxoid, UK).

Serotyping of *Salmonella* isolates

Next, the isolated *Salmonella* species were serotyped according to ISO 6579-3: 2014, and the serotypes were classified according to the Kauffman–White scheme (Grimont and Weill, 2007) using Salmonella antiserum (Sifin Co., Japan).

Antimicrobial susceptibility test

The antimicrobial sensitivity of each *Salmonella* strain was then tested. An antibiogram of the *Salmonella* isolates was created based on the results of disc-diffusion tests, conducted according to Koneman et al. (1997), using 13 antibiotics (ampicillin, apramycin, cefotaxime, ciprofloxacin, clindamycin, colistin sulphate, levofloxacin, lincomycin, nalidixic acid, norfloxacin, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole; Oxoid). The results were interpreted according to CLSI/NCCLS (2017).

Molecular identification

A conventional polymerase chain reaction (PCR) assay was used to detect virulence and antibiotic-resistance genes in *Salmonella* isolates. DNA extraction was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH), with modifications of the manufacturer's recommendations. Briefly, 200 µL of the sample suspension was incubated with 10 µL of proteinase K and 200 µL of lysis buffer at 56°C for 10 min. After incubation, 200 µL of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µL of elution buffer provided in the kit. The oligonucleotide primers used were supplied from metabion international AG (Germany) and are listed in Table 1. Each 25-µL reaction mixture contained 12.5 µL of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µL of each primer (20 pmol concentrations), 5.5 µL of water, and 5 µL of DNA template. The reaction was performed in an Applied Biosystems 2720 thermal cycler.

The PCR products were separated by electrophoresis on 1.5% agarose gel (AppliChem, GmbH, Germany) in 1× TBE buffer at room temperature using gradients of 5 V/cm. For gel analysis, 20 µL of conventional PCR products were loaded into each gel slot. GeneRuler 100 bp DNA Ladder (Fermentas, Thermo, Germany) was used to determine the fragment sizes. The gel was photographed with a gel documentation system (Alpha Innotech, Biometra, Germany), and the data were analyzed through computer software.

RESULTS

Prevalence of *Salmonella*

Among the 391 imported poultry flock samples (231 duckling, 84 chick, and 76 turkey poult) collected, 30 (7.7%) were positive for *Salmonella*. Based on species, 21 of 231 (9.1%) duckling samples, 6 of 84 (7.1%) chick samples, and 3 of 76 (3.9%) poult samples yielded *Salmonella* isolates as shown in Table (2).

Table 2: Number of *Salmonella* isolated from different imported poultry samples

Samples	Number of tested samples	Positive Salmonella isolation *	
		No.	(%)*
Duckling	231	21	(9.1%)
Chicks	84	6	(7.1%)
Poults	76	3	(3.9%)
Total	391	30	**(7.7%)**

(%)*: Number of positive related to number of tested samples.

Serotyping of the *Salmonella* strains isolated from the three different bird species revealed a broad range of serovars. Poults had three different serotypes (*S.* Enteritidis, *S.* Muenster, and *S.* Cuckmere), and chicks had two strains for both *S.* Typhimurium and *S.* Vejle and one each for *S.* Enteritidis and *S.* Sinstorf. Ducklings had four *S.* Enteritidis strains, three *S.* Vejle strains, and two *S.* Nyborg strains, as well as one strain for each of the following serotypes: *S.* Typhimurium, *S.* Sinstorf, *S.* Muenster, *S.* Indiana, *S.* Infantis, *S.* Koenigstuhl, *S.* Macallen, *S.* Nchanga, *S.* Neftenbach, *S.* Newlands, *S.* Nigeria, *S.* Nyborg, and *S.* Regent as shown in Table (3).

Antimicrobial susceptibility

All strains showed variable degrees of antibiotic resistance as shown in Table 4. The most frequent resistance phenotypes involved clindamycin and lincomycin, with all species being resistant to these antibiotics. Nalidixic acid resistance was found in 57.2% of the *Salmonella* isolates from ducklings and 16.7% of those from chicks. A High level of trimethoprim-sulfamethoxazole resistance was found in isolates from chicks and poults, with 50% and 66.7% of isolates being resistant, respectively. Resistance to ampicillin was found for 38% of isolates from ducklings and 33.3% of those from both chicks and poults. Tetracycline resistance was observed for 38.1% of isolates from ducklings and 33.3% of those from chicks. Streptomycin resistance was associated with 38.1% of isolates from ducklings, 16.7% from chicks, and 66.7% from poults. Lower levels of resistance were found for cefotaxime, with 33.3% of duckling isolates showing resistance, and for ciprofloxacin, with 28.6% of duckling isolates and 33.3% of both chick and

Table 1: Primers sequences, target genes, amplicon sizes and cycling conditions.

Target genes	Primers sequences	Amplified segment (bp)	Annealing	Reference
stn	TTGTGTCGCTATCACTGGCAA CC	617	59°C 40 sec.	Murugkar et al., 2003
	ATTCGTAACCCGCTCTCG TCC			
avrA	CCT GTA TTG TTG AGC GTC TGG	422	58°C 40 sec.	Huehn et al. 2010
	AGA AGA GCT TCG TTG AAT GTC C			
sopB	TCAGAAGRCGTCTAACCACTC	517	58°C 40 sec.	Thung et al., 2018
	TACCGTCCTCATGCACACTC			
qnrS	ACGACATTCGTCAACTGCAA	417	55°C 40sec.	Robicsek et al., 2006
	TAAATTGGCACCCTGTAGGC			
tetA (A)	GGTTCACTCGAACGACGTCA	576	50°C 40 sec.	Randall et al. 2004
	CTGTCCGACAAGTTGCATGA			
bla TEM	ATCAGCAATAAACCAGC	516	54°C 40 sec.	Colom et al., 2003
	CCCCGAAGAACGTTTTC			

Thermo cycler program: Primary denaturation 94°C/5 min. then Amplification (35 cycles) {Secondary denaturation 94°C/ 30 sec., Annealing differ from each primer, Extension 72°C/ 45 sec.} then final extension72°C/ 10 min.

Table 3: *Salmonella* serovars with antigenic formula in different samples

Serotypes	Ducklings (21)	Chicks(6)	Poults (3)
S. Enteritidis "1,9,12;g,m;--"	4	1	1
*S.*Typhimurium "1,4,5,12;i;1,2"	1	2	-
S. Sinstorf "3,10;l,v;1,5"	1	1	-
S. Muenster "3,10;e,h;1,5"	1	-	1
S. Vejle "3,10;e,h;1,2"	3	2	-
S. Cuckmere "3,10;i;1,2"	-	-	1
S. Indiana "1,4,12;z;1,7"	1	-	-
S. Infantis "6,7,14;r;1,5"	1	-	-
S. Koenigstuhl "1,4,[5],12;z;e,n,z_{15}"	1	-	-
S. Macallen "3,10;z_{36},-"	1	-	-
S. Nchanga "3,10,15;l,v;1,2"	1	-	-
S. Neftenbach "4,12;z;e,n,x"	1	-	-
S. Newlands "3,10;e,h;e,n,x"	1	-	-
S. Nigeria "6,7;r;1,6"	1	-	-
S. Nyborg ""3,10;e,h;1,7"	2	-	-
S. Regent "3,10;f,g,s;1,6"	1	-	-

Table 4: Interpretation of antibiotic sensitivity test for *Salmonella* isolates

Antibiotics	Ducklings N=21(%)			Chicks N=6 (%)			Poults N=3 (%)		
	R	I	S	R	I	S	R	I	S
AMP (10 µg)	8 (38)	-	13 (62)	2 (33.3)	-	4 (66.7)	1 (33.3)	-	2 (66.7)
APR (15 µg	4 (19)	9 (43)	8 (38)	-	1 (16.7)	5 (83.3)	-	2 (66.7)	1 (33.3)
CTX (30 µg)	7 (33.3)	5 (23.7)	9 (43)	-	2 (33.3)	4 (66.7)	-	1 (33.3)	2 (66.7)
CIP (5 µg)	6 (28.6)	14 (66.6)	1 (4.8)	2 (33.3)	3 (50)	1 (16.7)	1 (33.3)	2 (66.7)	-
DA (2 µg)	21 (100)	-	-	6 (100)	-	-	3 (100)	-	-
CT (10 µg)	1 (4.8)	-	20 (95.2)	-	-	6 (100)	1 (33.3)	-	2 (66.7)

LEV (5 µg)	3 (14.3)	2 (9.5)	16 (76.2)	-	-	6 (100)	-	-	3 (100)
MY (10 µg)	21 (100)	-	-	6 (100)	-	-	3 (100)	-	-
NA (30 µg)	12 (57.2)	2 (9.5)	7 (33.3)	1 (16.7)	1 (16.7)	4 (66.7)	-	1 (33.3)	2 (66.7)
NOR (10 µg)	1 (4.8)	2 (9.5)	18 (85.7)	-	1 (16.7)	5 (83.3)	-	-	3 (100)
S (10 µg)	8 (38.1)	11 (52.4)	2 (9.5)	1 (16.7)	3(50)	2 (33.3)	2 (66.7)	1 (33.3)	-
TE (30 µg)	8 (38.1)	1 (4.8)	12 (57.1)	2 (33.3)	-	4 (66.7)	-	-	3 (100)
SXT (1.25/23.75 µg)	3 (14.3)	-	18 (85.7)	3 (50)	-	3 (50)	2 (66.7)	-	1 (33.3)

R: Resistance, I: Intermediate, S: Sensitive

AMP= Ampicillin, **APR**= Apramycin, **CTX**= Cefotaxime, **CIP**= Ciprofloxacin, **DA**= Clindamycin, **CT**= Colistin sulphate, **LEV**= Levofloxacin, **MY**= Lincomycin, **NA**= Nalidixic acid, **NOR**= Norfloxacin, **S**= Streptomycin, **TE**= Tetracycline, **SXT**= Trimethoprim-sulfamethoxazole.

Table 5: Identification of antibiotic resistance and virulence genes in *Salmonella* isolates

Sample	blaTEM	tetA(A)	qnrS	stn	avrA	*sop*B
Duckling (21 isolates)	21/21 (100%)	21/21 (100%)	10/21 (47.6%)	21/21 (100%)	21/21 (100%)	21/21 (100%)
Chicks (6 isolates)	6/6 (100%)	6/6 (100%)	3/6 (50%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
Poults (3 isolates)	3/3 (100%)	3/3 (100%)	2/3 (66.6%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Total	30/30 (100%)	30/30 (100%)	15/30 (50%)	30/30 (100%)	30/30 (100%)	30/30 (100%)

Table 6: Virulence and antibiotic resistance genes and antibiotic resistance profiles of *Salmonella* Serovars:

Code No.	Sample species	Salmonella Serovars	Antibiotic resistance genes	Virulence genes	Antibiotic resistance profile/ resistance (intermediate)
1	Duckling	Enteritidis	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	DA, MY (CTX, S, CIP)
2	Duckling	Macallen	*blaTEM* , *tetA(A), qnrS*	*stn, avrA, sopB*	CTX, DA, MY (S, APR, CIP)
3	Duckling	Enteritidis	*blaTEM* ,*tetA(A), qnrS*	*stn, avrA, sopB*	DA, MY (CTX, CIP)
4	Chicks	Sinstorf	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	DA, MY (CIP)
5	Duckling	Typhymurium	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	DA, MY, NA (S, CIP)
6	Chicks	Typhymurium	*blaTEM* , *tetA(A), qnrS*	*stn, avrA, sopB*	DA, SXT, MY (S, CIP)
7	Duckling	Regent	*blaTEM* , *tetA(A), qnrS*	*stn, avrA, sopB*	DA, APR, MY, NA (CTX, TE, S, CIP)
8	Duckling	Kenigstuhl	*blaTEM* , *tetA(A), qnrS*	*stn, avrA, sopB*	DA, MY, NA (S, CIP)
9	Poults	Enteritidis	*blaTEM* , *tetA(A), qnrS*	*stn, avrA, sopB*	DA, SXT, MY, CIP (S, APR, NA)
10	Duckling	Enteritidis	*blaTEM* , *tetA(A), qnrS*	*stn, avrA, sopB*	AMP, CTX, DA, TE, S, MY, NA, CIP (NOR, LEV)
11	Duckling	Nyborg	*blaTEM* , *TetA(A)*	*stn, avrA, sopB*	S, CTX, DA, TE, S, MY, NA, CIP (LEV)
12	Chicks	Vejle	*blaTEM* , *tetA(A), qnrS*	*stn, avrA, sopB*	DA, MY, CIP (CTX, S, APR)
13	Poults	Cuckmere	*blaTEM* , *tetA(A), qnrS*	*stn, avrA, sopB*	AMP, DA, SXT, S, MY (APR, CIP)
14	Chicks	Vejle	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	DA, SXT, MY (S)

15	Duckling	Nchanga	*blaTEM* , *tetA(A)*, *qnrS*	*stn, avrA, sopB*	CTX, DA, MY (S, APR, NA, CIP)
16	Duckling	Vejle	*blaTEM* , *tetA(A)*, *qnrS*	*stn, avrA, sopB*	AMP, DA, NOR, SXT, TE, S, LEV, CT, APR, MY, NA, CIP
17	Duckling	Neftenbach	*blaTEM* , *tetA(A)*, *qnrS*	*stn, avrA, sopB*	DA, S, APR, MY, NA (CIP)
18	Duckling	Sinstorf	*blaTEM* , *tetA(A)*, *qnrS*	*stn, avrA, sopB*	DA, SXT, MY (S, APR)
19	Duckling	Muenster	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	DA, MY (S, APR, CIP)
20	Duckling	Vejle	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	AMP, DA, TE, MY (APR, NA, CIP)
21	Duckling	Enteritidis	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	AMP, CTX, DAM TE, S, LEV, MY, NA, CIP (APR)
22	Poults	Muenster	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	DA, S, CT, MY (CTX, CIP)
23	Duckling	Newlands	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	AMP, CTX, DA, TE, S, MY (CIP)
24	Duckling	Vejle	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	DA, MY (S, CIP)
25	Duckling	Indiana	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	DA, MY, NA (CTX, S, CIP)
26	Chicks	Enteritidis	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	AMP, DA, TE, MY, NA, CIP (CTX, NOR)
27	Duckling	Infantis	*blaTEM* , *tetA(A)*, *qnrS*	*stn, avrA, sopB*	AMP, CTX, DA, TE, S, LEV, APR, MY, NA, CIP (NOR)
28	Chicks	Typhymurium	*blaTEM* , *tetA(A)*, *qnrS*	*stn, avrA, sopB*	AMP, DA, SXT, TE, S, MY (NA, CIP)
29	Duckling	Nyborg	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	AMP, DA, SXT, TE, S, MY, NA, CIP (CTX)
30	Duckling	Nigeria	*blaTEM* , *tetA(A)*	*stn, avrA, sopB*	DA, MY, NA (S, CIP)

poult isolates showing resistance. Colistin sulphate resistance was detected in 33.3% of isolates from poults. On the other hands, each strain separately identified their antibiotic resistance on Table 5.

Molecular identification

To determine the virulence and antibiotic resistance profiles for all of the isolated *Salmonella* spp. (30 isolates), PCR was performed for the related genes. We found *stn*, *avrA*, and *sopB* virulence genes in all tested samples. With regard to antibiotic resistance, all tested strains carried *blaTEM* and *tetA(A)* genes, while the *qnrS* gene was reported in 50% of the isolates (Table 5) and Fig. (1, 2, 3, 4, 5 & 6).

On association relationship between species, resistance patterns, related antibiotic resistance genes and the virulence genes individually with its serotype as shown on Table (6); clarify the significance of each isolate and throw light about the multidrug resistance profile.

DISCUSSION

Poultry plays an important role in *Salmonella* transmission because the animals serve as a reservoir. Although the birds may appear to be healthy, they intermittently shed *Salmonella*, and many reports have documented true- and/or pseudo-vertical transmission of *Salmonella* from chickens (EFSA, 2012). Examination of imported poultry flocks in the current study revealed that the incidence of *Salmonella* from 1-day-old poultry of different species was 7.7%, overall; by species, *Salmonella* was isolated from 9.1% of duckling samples, 7.1% of chick samples, and 3.9% of turkey poult samples. Each type of poultry carried multiple serotypes of *Salmonella*. Ducklings had the most serotypes (*S.* Enteritidis, *S.* Typhimurium, *S.* Sinstorf, *S.* Muenster, *S.* Vejle, *S.* Indiana, *S.* Infantis, *S.* Koenigstuhl, *S.* Macallen, *S.* Nchanga, *S.* Neftenbach, *S.* Newlands, *S.* Nigeria, *S.* Nyborg, and *S.* Regent), while chicks carried *S.* Enteritidis, *S.* Typhimurium, *S.* Sinstorf, and *S.* Vejle, and poults carried *S.* Enteritidis, *S.* Muenster, and *S.* Cuckmere. Jodas and Hafez (2002) reported similar results, isolating *S.* Enteritidis from 7 of 231 (3%) of meconium samples from poults. In addition, rates of 4.3% and 4% were reported by Abdallaha et al. (2015) and Osman et al. (2014b), respectively, for poults imported into Egypt, while Sorour et al. (2016) and Osman et al. (2010) isolated *Salmonella* from 11.3% (6/53) and 12.6% of imported poults to Egypt, respectively.

The prevalence of *Salmonella* in ducklings in our study was substantially similar to the 2.1% found by Wang et al. (2020) isolated from China and the 6.45% reported by Badr et al. (2015) for imported ducklings into Egypt; se

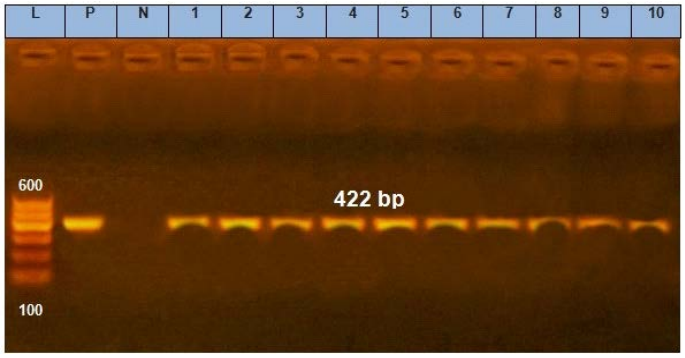

Figure 1: Agarose gel electrophoresis showing amplification of 422 bp fragments of avrA gene.
Lane (1 to 10) shows the positive amplification of ten representing isolates. L: Ladder (100-600). P: Positive control and N: Negative control.

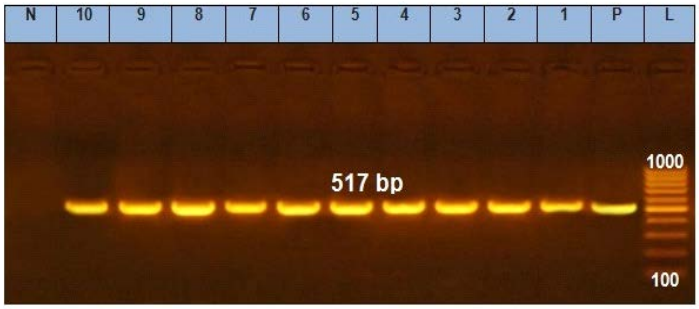

Figure 2: Agarose gel electrophoresis showing amplification of 517 bp fragments of sopB gene.
Lane (1 to 10) shows the positive amplification of ten representing isolates. L: Ladder (100-1000). P: Positive control and N: Negative control.

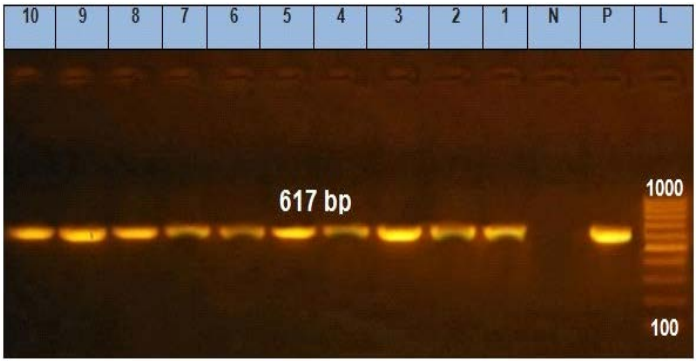

Figure (3): Agarose gel electrophoresis showing amplification of 617 bp fragments of *stn* gene.
Lane (1 to 10) shows the positive amplification of ten representing isolates. L: Ladder (100-1000). P: Positive control and N: Negative control.

rotyped as *S.* Derby, *S.* Newport, *S.* Togo and *S.* Ball. Yang et al. (2019) reported a higher percentage of samples with *Salmonella* (12.2 %) from China, and Osman et al. (2010) and Osman et al. (2014c) similarly reported high percentages of 19.3% and 18.5% (25/135), respectively, among ducklings imported into Egypt. Ezzeldeen et al. (2013) documented a prevalence of 36% among duckling import

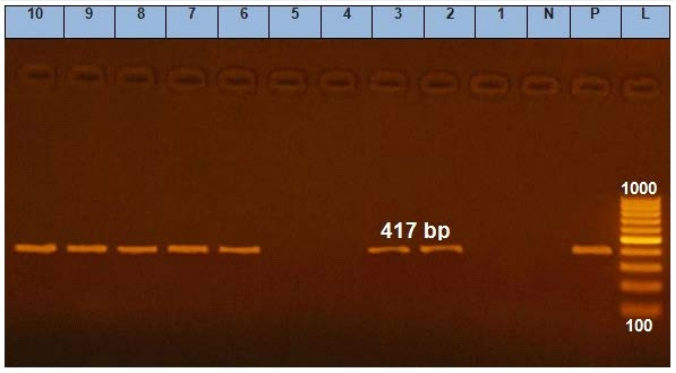

Figure 4: Agarose gel electrophoresis showing amplification of 417 bp fragments of qnrS gene.
Lane (1 to 10) shows the amplification result of ten representing isolates. L: Ladder (100-1000). P: Positive control and N: Negative control.

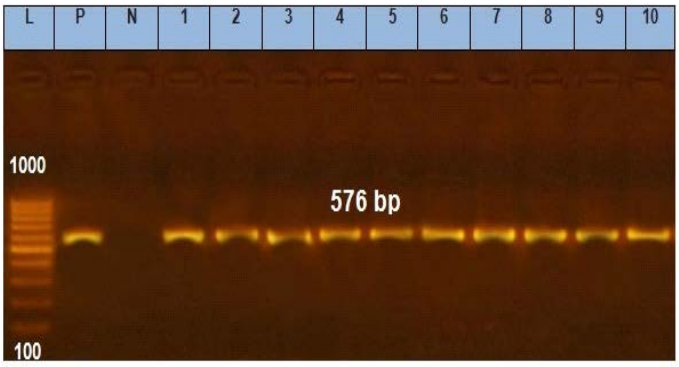

Figure 5: Agarose gel electrophoresis showing amplification of 576 bp fragments of tetA(A) gene.
Lane (1 to 10) shows the positive amplification of ten representing isolates. L: Ladder (100-1000). P: Positive control and N: Negative control.

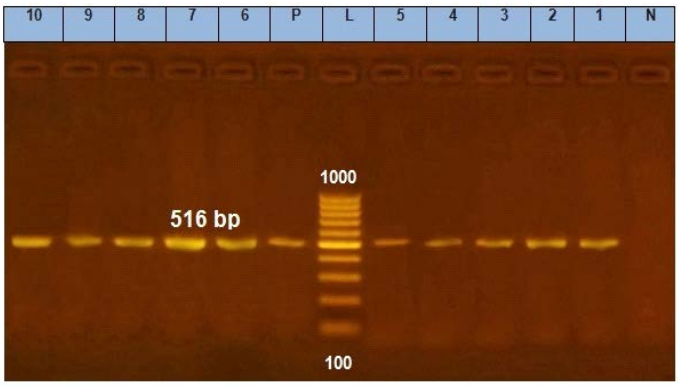

Figure 6: Agarose gel electrophoresis showing amplification of 516 bp fragments of *blaTEM* gene.
Lane (1 to 10) shows the positive amplification of ten representing isolates. L: Ladder (100-1000). P: Positive control and N: Negative control.

ed into Egypt, and they identified the serotypes as *S.* Enteritidis, *S.* Kentucky, *S.* Newport, *S.* Brenderup, *S.* Tishiongwe, and *S.* Shubra. Habing et al. (2015) analyzed duckling samples into USA and showed that 30% (9/30)

contained four serovars, including *S.* Typhimurium, *S.* Mbandaka, *S.* Infantis, and *S.* Lille, which were associated with outbreaks in 2013. In Brazil, Ribeiro et al. (2006) found 26 of 40 (65.0%) imported flocks were positive for *Salmonella*, which included the serovars *S.* Indiana, *S.* Newport, *S.* Saintpaul, *S.* Kottbus, *S.* Agona, *S.* London, *S.* Chester, and *S.* Kentucky. Wang et al. (2020) reported serovars *S.* Anatum, *S.* Apeyeme, and *S.* Montevideo.

Similar results were reported by Wang et al. (2020) who found a *Salmonella* prevalence of 4.2% (19/450) in 1-day-old chicks, Abdel Rahman et al. (2014) who reported 5.2% in imported chicks into Egypt, and Shehata et al. (2019) who demonstrated 7% prevalence in broiler chicks in Egypt. In contrast, Osman et al. (2014a) found a higher prevalence rate, with *Salmonella* isolated from 11.8% of imported chicks into Egypt in their study (13/110). The serotypes were *S.* Enteritidis, *S.* Typhimurium, *S.* Dublin, *S.* Shagoua, *S.* Hindmarch, and *S.* Inganda, with one isolate being untypeable. Habing et al. (2015) also found a high prevalence rate in their study that examined *Salmonella* from hatchling shipment boxes that contained chicks. That study showed a prevalence of 27.1% (44/162).

Sharma et al. (2018) isolated *Salmonella* from shipping boxes of hatchling poultry into U.S and identified multiple serovars, including *S.* Indiana, *S.* Muenster, *S.* Typhimurium, *S.* Enteritidis, *S.* Infantis, and 11 others. Shehata et al. (2019) isolated *Salmonella* from 7%, 12.5%, and 15% of samples for broilers, dead chicken embryos, and dead duck embryos; these isolated included *S.* Typhimurium, *S.* Enteritidis, *S.* Vejle, and *S.* Infantis, among others. In addition, Abdallaha et al. (2015) isolated *Salmonella* from chicks, ducklings, and poults into Egypt, and serotyping revealed 17 serovars, including *S.* Enteritidis, *S.* Newlands, and *S.* Nigeria.

Recently, antibiotic resistance has been increasing in *Salmonella* species isolated from animal origin with increasing emergence of drug-resistant strains. The common use of antibiotics to promote growth and to treat diseases in the poultry industry adds to the potential risk of the dissemination of MDR salmonellae (Abdallaha et al., 2015). In particular, antibiotic resistance of *Salmonella* associated with hatchlings is due to dipping eggs in an antibiotic solution or inoculation of hatching eggs with antimicrobial agents (Kabir, 2010).

In the present study, salmonellae isolated from ducklings showed high resistance to clindamycin and lincomycin (100%) but less resistance to nalidexic acid (57.2%) and ampicillin, streptomycin, and tetracycline (38%). The isolates had even less resistance to cefotaxime (33.3%) and ciprofloxacin (28.6%), followed by trimethoprim-sulfamethoxazole and levofloxacin (14.3%). Badr et al. (2015) reported isolates with high resistance to ciprofloxacin and nalidixic acid (reaching 75%), while Abdallaha et al. (2015) observed maximum resistance to trimethoprim (70.5%) followed by penicillin (41%), streptomycin (29.5%), nalidixic acid (23.5%), and ciprofloxacin (5.8%) in *Salmonella* strains isolated from ducklings, chicks, and poults.

In the current study, isolates from chicks showed high resistance to clindamycin and lincomycin 100%, then trimethoprim-sulfamethoxazole (50%) followed by tetracycline, ampicillin, and ciprofloxacin (33%). Similar results were reported for isolates from chicks by Abdel Rahman et al. (2014), who found resistance of 100%, 75%, 50%, 50%, and 50% to streptomycin, nalidixic acid, ciprofloxacin, tetracycline, and trimethoprim-sulfamethoxazole, respectively. Meanwhile, Osman et al. (2014a) documented resistance to lincomycin and susceptibility to ciprofloxacin and colistin sulfate in all *Salmonella* serotypes isolated from chicks, and Shehata et al. (2019) reported resistance to ampicillin, neomycin, trimethoprim-sulfamethoxazole, and streptomycin.

The results of this study showed that isolates from poults had high antimicrobial resistance to clindamycin and lincomycin (100%), followed by trimethoprim-sulfamethoxazole and streptomycin (66.7%) and colistin sulphate, ampicillin, and ciprofloxacin (33.3%). In comparison, Sorour et al. (2016) reported high resistance among *Salmonella* isolates turkey poults to lincomycin and ampicillin (100%), less resistance to ciprofloxacin and nalidixic acid (83.5%), and very low resistance to streptomycin (16.6%).

Wang et al. (2020) reported that the most frequent resistance phenotypes in samples from chicks and ducklings were for nalidixic acid (100%), streptomycin (92.7%), and ampicillin (92.7%), followed by tetracycline (40%) and ciprofloxacin (22.33%). Yildirim et al. (2011) found that *Salmonella* isolates were highly resistant to lincomycin, ampicillin, ciprofloxacin, and nalidixic acid. In contrast, Habing et al. (2015) reported that antimicrobial resistance was uncommon in *Salmonella* isolated from poultry hatchling; however, 19% (11/59) of the isolates were resistant to more than one class of antimicrobials.

With regard to virulence, some researchers (Wallis et al., 1999) have argued that the *stn* gene cannot be considered as a *Salmonella* virulence factor and it is not related to *Salmonella* enterotoxicity, while other research groups have reported that the *stn* gene is specific for all *Salmonella* serotypes (Lee et al., 2009). This finding highlights the *stn* gene as a reliable marker for *Salmonella* screening (Chopra et al., 1999). In the current study, the *stn* gene was detected in all tested samples, indicating that all isolates had the potential to produce the heat-labile exotoxin that is one of the main sharing agents in diarrhea (Van Asten and Van

Dijk, 2005).

The 100% prevalence of *sopB* and *avrA* genes in the tested samples strengthened the virulence profiles of the isolated samples and was in agreement with different earlier research studies in poultry (Osman et al., 2014c; ElSheikh et al., 2019) and in humans (Astolfi-Ferreira et al., 2017).

Although, 100% of the tested isolates were positive for *blaTEM* gene by PCR, not all of them showed a resistance profile for one of beta-lactamases that inhibit antibiotics. This finding may have been related to poor expression of a functional TEM-1 enzyme due to promoter region mutation, or due to the presence of a mutant TEM enzyme that is inactive (Bradford et al., 2001). The discrepancy between the phenotypic resistance profile for quinolones and the *qnrS* gene PCR result is acceptable because quinolone resistance is mediated in three ways, including plasmid-mediated resistance genes (*qnr* genes), mutations in the quinolone resistance determining regions (QRDRs), and overexpression of efflux pumps mediated by *qepA* genes (Lunn et al., 2010). In this order, Egyptian regulations concerning the *Salmonella* in the imported live birds was condamination of the infected flocks with *Salmonella* specially *S.*Typhimurium and *S.*Enteritidis

CONCLUSION

Imported animals may circulate *S. enterica* serovars with the potential for multi-drug resistance and international spread, leading to a public health hazard. Isolation of different *Salmonella* serovars in this study suggested the introduction of new serotypes into Egypt and illustrated the ability of poultry to be a common reservoir for various *Salmonella* serovars in addition to the most dominant two (*S.* Enteritidis and *S.* Typhimurium). These results indicate the importance of applied risk assessment of *Salmonella* in Egypt and provide a guideline for control and continuous survey for identifying circulating *Salmonella* species in field.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

AUTHORS CONTRIBUTION

HB designed the study. HB, HR, HKS, MAA, MFA and AME performed the research and drafted the manuscript. HB and AME analyzed the data. HB, HR, HKS, MAA, MFA and AME write, revised and finalized the manuscript for submission. All authors read and approved the final manuscript.

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