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Serotypes Diversity, Virulence, and Antimicrobial Resistance of Non-Typhoidal *Salmonella* Isolates in Commercial and Backyard Egg Production Systems in Egypt

Walid Elmonir[1]*, Dalia Abdeltawab[1], Hanem El-Sharkawy[2] and Rasha N. Zahran[3]

[1]*Department of Hygiene and Preventive Medicine (Zoonoses), Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt.*
[2]*Department of Poultry and Rabbit Diseases, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt.*
[3]*Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, University of Sadat City, Sadat, Egypt.*

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Key words
***Salmonella*, Virulence, Antimicrobial resistance, Layers, Egypt**

ABSTRACT

Multi-drug resistant (MDR) non-typhoidal *Salmonella* in poultry food chain are emerging worldwide. This study aimed to estimate the serotypes diversity, virulence and antimicrobial resistance traits of non-typhoidal *Salmonella* in commercial and backyard egg production systems in Kafrelsheikh Governorate, Egypt. A total of 610 samples (200 egg shells, 200 egg contents, and 210 environmental samples) were collected from 10 commercial layers farms and 170 samples (50 egg shells, 50 egg contents, and 70 environmental samples) were collected from 10 backyard layers flocks. The overall prevalence of *Salmonella* was 4.1% for each production system. Six commercial flocks (60%) and three backyard layers flocks (30%) harbored at least one *Salmonella* isolate. The prevalence rates of *Salmonella* spp. in eggs and environmental samples from commercial layers flocks were 2.5% (10/400) and 7.1% (15/210), respectively. The prevalence rates of the same samples in backyard production were 6% (6/100) and 1.4% (1/70), respectively. The egg shell contamination positively associated with *Salmonella* detection in egg cartoon trays in commercial layers farms (Pearson's R= 0.8, *P*= 0.004). The serotype distribution was as follows: *S.* Typhimurium 1.3%, *S.* Enteritidis 1.03%, *S.* Kentucky 0.8%, *S.* Infantis 0.3%, *S.* Molade 0.3%, *S.* Tamale 0.3%, *S.* Labadi 0.1%, and *S.* Papuana 0.1%. The *fimH, hilA, stn,* and *sopA* virulence genes were detected in 46.8%, 37.5%, 31.3%, and 40.6% of isolates, respectively. Around two thirds (59.4%) of *Salmonella* strains were resistant to at least one antibiotic and 12 isolates (37.5%) were MDR. The study highlighted the wide-spread of MDR *Salmonella* in egg production systems in study region. Extended surveillance and veterinary monitoring of antibiotics use in this sector are mandatory for public safety.

INTRODUCTION

Food-borne bacterial diseases (FBD) originated from poultry products are a leading cause of high morbidity and mortality worldwide (Li *et al*., 2020; Wessels *et al*., 2021). The continuous increase of poultry share in the food chains is expected to maximize the health concern of the FBD (Wessels *et al*., 2021). The non-typhoidal *Salmonella* infections are one of the major FBD in developed and developing countries (Majowicz *et al*., 2010). These pathogens cause more than 90 million cases and over 150 thousand deaths worldwide annually (Majowicz *et al*., 2010); most of these human illnesses incriminate egg and other poultry products (Li *et al*., 2020).

There is a global increase in incidence of antimicrobial resistant non-typhoidal *Salmonella* strains in poultry food chain (Castro-Vargas *et al*., 2020). This progressive emergence of antimicrobial resistant *Salmonella* is caused by antimicrobial agents overuse in poultry production for non-therapeutic purposes especially in developing countries (Castro-Vargas *et al*., 2020). Additionally, antimicrobial resistant non-typhoidal *Salmonella* strains are more likely associated with invasive diseases and hospitalization than drug susceptible strains (Varma *et al*.,

* Corresponding author: walid.elmonir@gmail.com
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2005) thus increasing the burden of the disease especially in developing countries with limited resources.

Salmonella causes a varied number of illnesses that is associated with acquisition of several clusters of virulence genes (Zhang *et al*., 2002; Elkenany *et al*., 2019). These genes are responsible for host adaptation, host cell invasion, inflammation induction, colonization, and immunity evasion (Bäumler *et al*., 1998; Zhang *et al*., 2002). However, type and rate of acquisition of these genes differ according to several factors including serotype, source and geographical location of the isolates. This necessitate frequent monitoring for better understanding of *Salmonella* pathogenicity evolution.

In Egypt, the table egg is an important source of animal protein, and essential ingredient in Egyptian cuisine. Egypt produces around 10 billons table eggs annually, which make it one of the top ten producers in Africa (FAOSTAT, 2018). Majority of table egg production is from commercial exotic breed layers. However, around one third of market share are attributed to native layers reared in household backyard sector (Hosny, 2006). The high popularity and higher selling price of egg from native breed compared to exotic breed highlight the importance of backyard egg production in Egypt (Hosny, 2006). *Salmonella* detection in table egg is a major health threat for public consumers in Egypt; therefore, this study was designed aiming to determine the prevalence of various *Salmonella* serovars in table egg and their environment from commercial and backyard production systems in Kafrelsheikh Governorate, Egypt. Additionally, the antibiogram profile, and virulence determinants of isolates were studied to assess their potential risk for public safety in study region.

MATERIALS AND METHODS

Sampling

Two chicken egg production systems (commercial intensive and the backyard systems) were investigated for the prevalence of *Salmonella* in Al-Riyad district (31.2365°N 30.9453°E) of Kafrelsheikh Governorate in northern Delta of Egypt during the period March–July 2017. For commercial intensive production system, 10 small scale commercial layers farms with a deep litter system were randomly selected. The flock size ranged from 2000 to 6000 layers per farm. For backyard system, 10 households were randomly selected. The flock size ranged from 50 to 80 layers per household. Chicken in this system roam out in the backyard most of the day, however feed, water and egg nests are located in a shed where they are confined at nighttime. The number and type of samples collected per each production system are listed in Table I. All samples were shipped in icebox at 4°C to lab for bacteriological analysis within 6 h.

Salmonella *isolation and identification*

Samples processing and Salmonella *isolation*

After egg shell swab samples collection, the egg shell was disinfected by immersion in ethyl alcohol 70% for 1 min, allowed to air dry, and then flamed. The egg shell was cracked and the content was poured in a sterile container. A total of 5 mL of mixed egg content was collected per each egg. Feed, water, and litter samples were mixed and 25 (g or mL) were collected per each sample. All samples were pre-enriched in buffered peptone water (BPW; Oxoid, Hampshire, UK) at 1:10 rate (by volume) and were incubated at 37°C for 18 h. For selective enrichment, 0.1 mL of pre-enriched culture was transferred to 10 mL of Rappaport–Vassiliadis broth (RV; Oxoid, Hampshire, UK), and the inoculated broth was incubated at 42°C for 24 h. Then, a loopful of broth culture was streaked over Xylose Lysine Desoxycholate agar plates (XLD; Oxoid, Hampshire, UK). The XLD plates were incubated at 37°C for 24 h.

Salmonella *identification and serotyping*

Presumptive *Salmonella* isolates recovered from XLD plates were identified biochemically by API-20E (bioMérieux, Marcy-l'Etoile, France). The PCR confirmation for biochemically positive *Salmonella* isolate was performed using *invA* primers as previously described (Cocolin *et al*., 1998). PCR confirmed isolates were further serotyped based on White-Kauffmann scheme. The serotyping was conducted by a slide agglutination test using somatic (O) and flagellar (H) *Salmonella* antisera sets (Denka Seiken Co., Tokyo, Japan) according to the manufacturer's instructions.

Molecular detection of Salmonella fimH, hilA, stn *and* sopA *virulence genes*

Overnight incubated PBS cultures of confirmed *Salmonella* isolates were used for DNA extraction using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Multiplex PCR was used for detection of *fimH*, *hilA*, and *stn* virulence genes. The PCR mixture consisted of 5 µL of DNA template (~50 ng), 1 µL (10 pmoL) of each primer, 25 µL of the master mix EmeraldAmp MAX PCR (Takara Bio, Kusatsu, Japan), and sterile distilled water up to a final volume of 50 µL. The PCR cycling was conducted using the Applied Biosystem 2720 thermal cycler (Applied Biosystem, Foster City, CA, USA). The PCR amplification was initiated by one cycle of denaturation at 94°C for 7 min followed by 35 cycles of 94°C for 1 min, 64°C for 1 min, and 72°C for 2 min, and a final extension at 72°C for 10 min.

Table I. Diversity and frequency distribution of *Salmonella* serovars in commercial and backyard production systems.

Location	Egg		Environment					Total
	ES	EC	L	F	W	H	ET	
Commercial production								
n	**200**	**200**	**30**	**30**	**30**	**20**	**100**	**610**
Flock 1	0	0	0	0	0	0	0	0 (0)
Flock 2	0	0	0	0	0	0	0	0 (0)
Flock 3	0	0	0	0	0	0	0	0 (0)
Flock 4	STy	*STy*	STy	0	0	0	STy, SMo, SLa	0.9 (6)
Flock 5	STa	0	0	0	0	0	STa, SEn	0.5 (3)
Flock 6	STy	STy	0	0	0	0	STy	0.5 (3)
Flock 7	SKe	SKe, SIn	0	0	0	*SKe*	SKe, SIn	0.9 (6)
Flock 8	0	0	0	0	0	0	SPa	0.2 (1)
Flock 9	SEn	SEn	*SEn*	0	SEn	0	SEn, SMo	0.9 (6)
Flock 10	0	0	0	0	0	0	0	0 (0)
Total	2.5 (5)	2.5 (5)	6.7 (2)	0 (0)	3.3 (1)	5 (1)	11 (11)	4.1 (25)
	Egg		**Environment**				**Total**	
	ES	**EC**	**L**	**F**	**W**	**H**		
Backyard production								
n	**50**	**50**	**20**	**20**	**20**	**10**		***170**
Flock 1	0	0		0	0	0		0 (0)
Flock 2	0	0	0	0	0	0		0 (0)
Flock 3	STy	STy	STy	0	0	0		1.8 (3)
Flock 4	SEn	SEn	0	0	0	0		1.2 (2)
Flock 5	0	0	0	0	0	0		0 (0)
Flock 6	0	0	0	0	0	0		0 (0)
Flock 7	0	0	0	0	0	0		0 (0)
Flock 8	0	0	0	0	0	0		0 (0)
Flock 9	SKe	SKe	0	0	0	0		1.2 (2)
Flock 10	0	0	0	0	0	0		0 (0)
Total	6 (3)	6 (3)	5 (1)	0 (0)	0 (0)	0 (0)		4.1 (7)

ES, Egg shell; EC, Egg content; L, Litter; F, Feed; W, Water; H, worker hand swabs; ET, Egg cartoon trays; *STy*, *S.* Typhimurium; *Sen*, *S.* Enteritidis; *SKe*, *S.* Kentucky; *Sin*, *S.* Infantis; *SMo*, *S.* Molade; *Sta*, *S.* Tamale; *Sla*, *S.* Labadi; *Spa*, *S.* Papuana; Brackets, Number of isolates; *, Number of samples.

For detection of *sopA* gene, a 25 µL mixture was used and it contained 12.5 µL of the EmeraldAmp MAX PCR master mix, 1 µL (20 pmol) of each primer, 5 µL of DNA template, and 5.5 µL of water. The cycling conditions were the same as mentioned before for multiplex PCR except for annealing at 58°C for 1 min. The *S.* Typhimurium ATCC 14028 strain was used as a positive control in all reactions, and sterile distilled water was used as a negative control. The primers used for virulence genes detection (Metabion, Steinkirchen, Germany), are shown in Supplementary Table I.

Antimicrobial sensitivity test of detected Salmonella *isolates*

Antimicrobial sensitivity of *Salmonella* isolates was tested by a disk diffusion technique following the instructions of the Clinical and Laboratory Standards Institute (CLSI, 2016). The antimicrobial discs used in this study included: Ampicillin (AMP, 10 µg), cephazolin (KZ, 30 µg), cefotaxime (CTX, 30 µg), imipenem (IMP, 10 µg), nalidixic acid (NA, 30 µg), ciprofloxacin (CIP, 5 µg), amikacin (AK, 30 µg), gentamicin (CN, 10 µg), doxycycline (DO, 30 µg), tetracycline (TE, 30 µg), sulfamethoxazole/

trimethoprim (SXT, 25 µg), and chloramphenicol (C, 30 µg). Isolates that showed resistance to 3rd generation cephalosporins (CTX) were tested by double disk synergy tests for Extended Spectrum Beta-Lactamase (ESBL) production as previously described methods (Drieux *et al*., 2008). In all antimicrobial susceptibility tests, the *E. coli* ATCC 25922 strain was used as a quality control, and *E. coli* NCTC 13353 (for ESBL), and *E. coli* ATCC BAA-2469 (for carbapenemase) strains were used as positive controls. Isolates that showed resistance to three or more classes of antibiotics were considered as multiple drug resistant (MDR).

Statistical analysis

The regression and Pearson's R correlation analysis were applied using SPSS v19 (IBM, Armonk, NY, USA). The significance was recorded at $P \leq 0.05$.

RESULTS

Frequency distribution and diversity of Salmonella *spp. from commercial and household's layers farms*

The prevalence rates of *Salmonella* spp. in samples collected from commercial and backyard layers flocks were 2.5% (10/400) and 6% (6/100) for eggs samples and 7.1% (15/210) and 1.4% (1/70) for environmental samples, respectively (Tables I, II). The overall prevalence of *Salmonella* in collected samples was 4.1% for both production systems. The odds of *Salmonella* detection in commercial layers system was higher for environmental samples (OR 5.3) but lower for eggs samples (OR 0.4) compared to backyard layers system, however these associations were not significant (P= 0.09 – 0.1; Table II). Six out of 10 examined commercial flock (60%) harbored at least one *Salmonella* isolate, while it was detected in three of backyard layers flocks (30%) (Table I). There was a positive correlation between egg shell contamination and *Salmonella* detection in egg cartoon trays in commercial layers farms (Pearson's R= 0.8, P= 0.004). No other association was found with rest of environmental samples in commercial farms. Also, no association was detected between shell contamination and environmental samples in backyard flocks.

Salmonella Typhimurium was the most detected serotype; 10 isolates were recovered from two commercial farms (2/10; 20%) and 1 backyard flock (1/10; 10%). It was also the prevalent strain in eggs (6/500; 1.2%) and environmental samples (4/280; 1.4%) from both production systems (Table I). On the other hand, 8 strains of *S.* Enteritidis, the second most prevalent serovar, were isolated from 2 commercial layers farms (2/20; 20%) and one backyard layers farm (1/10; 10%). The *S.* Kentucky was isolated from one commercial flock (1/10; 10%) and one (1/10; 10%) backyard flock (Table I). The results showed that *S.* Infantis, *S.* Molade, *S.* Tamale, *S.* Labadi, and *S.* Papuana were only isolated from commercial layers farms (Table I).

Table II. Univariate logistic regression model for the associations between production systems and *Salmonella* prevalence in various samples.

Source	Production system	Positive	P value	Odds	CI 95 %
Egg	Backyard	6% (6/100)	-	-	-
	Commercial	2.5% (10/400)	0.09	0.4	0.1-1.1
Environment	Backyard	1.4% (1/70)	-	-	-
	Commercial	7.1% (15/210)	0.1	5.3	0.7-40.9
All samples	Backyard	4.1% (7/170)	-	-	-
	Commercial	4.1% (25/610)	0.9	0.9	0.4-2.3

Virulence factors and antimicrobial resistance pattern of isolated Salmonella *spp.*

Our results showed that 80% of *S.* Typhimurium isolates possesses *fimH* gene and 70% have *hilA* and *sopA* genes, while only 60% contained *stn* gene (Table III, Fig. 1). The same virulence genes were detected in *S.* Enteritidis at rates of 62.5% (*fimH*), 50% (*sopA*), 37.5% (*hilA*), and 25% (*stn*) (Table III, Fig. 1). *S.* Kentucky strains harbored *fimH, stn* and *sopA* (33.3% for each) and *hilA* (16.7%). The *hilA* gene was the only virulence factor present in one strain of *S.* Infantis (Table III, Fig. 1). None of the tested virulence factors were detected in *S.* Molade, *S.* Tamale, *S.* Labadi, and *S.* Papuana (Table III).

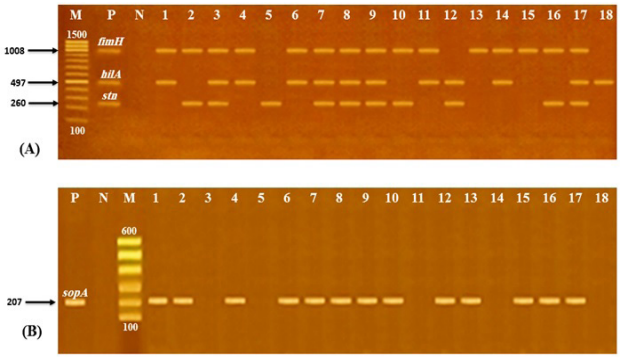

Fig. 1. Frequency distribution of virulence genes among detected *Salmonella* isolates in this study.
M, DNA marker; P, Positive control; N, Negative control; Lanes 1-9, *S.* Typhimurium; Lanes 10-14, *S.* Enteritidis; Lanes 15-17, *S.* Kentucky; Lanes 18, *S.* Infantis.

Table III. The virulence-antibiotic resistance patterns of detected *Salmonella* serovars.

Serotype	No.	P	Virulence profile	Antibiotic resistance profile	
				Antibiotics	MAR
S. Typhimurium (10)	2	P1	*fimH, hilA, stn, sopA*	AMP, KZ, CTX, NA, CIP, AK, CN, DO, TE, SXT, C	0.92*
	1	P2	*fimH, hilA, stn, sopA*	AMP, KZ, CTX, NA, CIP, DO, TE, SXT, C	0.75*
	1	P3	*fimH, hilA, stn*	AMP, NA, TE, SXT	0.33*
	2	P4	*fimH, hilA,, sopA*	NA, TE, C	0.25*
	1	P5	*fimH, stn, sopA*	AMP, NA	0.17
	1	P6	*fimH, hilA, sopA*	NA	0.08
	1	P7	*stn*	-	0
	1	P8	-	-	0
S. Enteritidis (8)	1	P1	*fimH, stn, sopA*	AMP, KZ, CTX, NA, AK, DO, TE, SXT	0.67*
	1	P2	*hilA, stn, sopA*	AMP, KZ, NA, CIP, DO, TE, SXT, C	0.67*
	1	P3	*fimH, hilA*	AMP, KZ, CTX, NA, DO, TE, C	0.58*
	1	P4	*fimH, hilA*	AMP, NA, SXT, C	0.33*
	2	P5	*fimH, sopA*	AMP, KZ	0.17
	1	P6	-	AMP	0.08
	1	P7	-	-	0
S. Kentucky (6)	1	P1	*fimH, hilA, stn, sopA*	AMP, KZ, NA, TE, SXT	0.42*
	1	P2	*fimH, stn, sopA*	AMP, KZ, NA	0.25*
	4	P3	-	-	0
S. Infantis (2)	1	P1	-	NA	0.08
	1	P2	*hilA*	-	0
S. Molade (2)	1	P1	-	AMP	0.08
	1	P2	-	-	0

P, Virulence-Resistance Pattern per isolate; MAR, Multiple antibiotic resistance index; AMP, Ampicillin; KZ, Cefazolin; CTX, Cefotaxime; IPM, Imipenem; NA, Nalidixic acid; CIP, Ciprofloxacin; AK, Amikacin; CN, Gentamicin; Do, Doxycycline; TE, Tetracycline; SXT, Sulfamethoxazole/ Trimethoprim; C, Chloramphenicol; *, MDR isolates. The *S.* Tamale (2 isolates), *S.* Labadi (1 isolate), and *S.* Papuana (1 isolate) were sensitive to all tested antibiotics and also lacked all the examined virulence genes.

The overall prevalence of *Salmonella* strains resistant to at least one antibiotic were 59.4%, and prevalence per serovar was as follows: *S.* Enteritidis (87.5%; 7/8), *S.* Typhimurium (80%; 8/10), *S.* Infantis (50%; 1/2), *S.* Molade (50%; 1/2), and *S.* Kentucky (33.3%; 2/6). A total of 12 isolates (37.5%) showed MDR to three or more classes of antibiotics and these isolates belonged to three serovars: *S.* Typhimurium (60%; 6/10), *S.* Enteritidis (50%; 4/8), and *S.* Kentucky (33.3%; 2/6). The highest resistance rates for individual antibiotics were AMP (49.9%), NA (46.9%), KZ (31.3%), TE (31.3%), C (28.1%), and SXT (25%), while lowest resistance rates were for CN (6.3%), AK (9.4%), CIP (12.5%), CTX (15.6%), and DO (18.8%). None of the isolates were resistant for imipenem (0%).

The patterns of virulence and antimicrobial resistance of detected isolates showed high diversity between isolates especially for *S.* Typhimurium (10 isolates; 8 patterns), and *S.* Enteritidis (8 isolates; 7 patterns) (Table III). These isolates also showed the highest MAR values: 0.92 for *S.* Typhimurium and 0.67 for *S.* Enteritidis (Table III). Additionally, the results recorded a positive correlation between the presence of virulence factors and resistance to any (Pearson's R= 0.6, *P*= 0.002) or multiple antibiotics (Pearson's R= 0.7, *P*= >0.001).

DISCUSSION

The overall prevalence of *Salmonella* was the same in commercial and backyard production systems (4.1% per each). This result is different from a recent study reported that a low rate (2%) of *Salmonella* in backyard production (McDonagh *et al*., 2019). However, other previous studies agreed with our findings and showed no difference in *Salmonella* prevalence between different

housing/production systems (Dewulf *et al*., 2011). These studies concluded that differences in the prevalence may be attributed to other factors rather than type of production system as flock size, flock age differences, building condition, biosecurity level, and previous *Salmonella* infections on the farm (Dewulf *et al*., 2011).

The prevalence rates in eggs were 2.5% and 6% and in environmental samples were 7.1 and 1.4% for commercial and backyard layers flocks, respectively. Another study in China showed higher rate of *Salmonella* in farm eggs (Li *et al*., 2020) of this study showed that the risk of *Salmonella* detection in commercial layers system was lower for eggs samples (OR 0.4), but higher for environmental samples (OR 5.3) compared to backyard layers system, yet these differences were not significant ($P = 0.09 – 0.1$). This means that *Salmonella* are common in both production systems in the study region despite some difference in its distribution within the farm depending on nature of the production system. Higher odds for environmental contamination in commercial production may be attributed to a closed system with higher number of layers which increases chances for within farm environmental contamination unlike the opened backyard system. Higher prevalence in eggs in backyard system may be attributed to higher infection rate in native layers compared to exotic breed reared in commercial production. Despite the known higher resistance of native breed reared in households compared to exotic breed (Hosny, 2006), yet the free access to other animals, vectors and environment makes them prone to multiple sources of infection. This is supported by the findings of Abdeen *et al*. (2018) who reported the recent emergence of MDR *Salmonella* in native breed in Egypt.

One major difference between commercial and backyard production system in our study was the great variation of *Salmonella* serovars isolated from commercial flocks (8 serovars) in this study compared to backyards flocks (3 serovars; *S.* Typhimurium, *S.* Enteritidis, and *S.* Kentucky). This finding agreed with Dewulf *et al*. (2011) who reported that layers that have access to outdoor facilities are less likely to become infected with *Salmonella* serovars other than *S.* Enteritidis and *S.* Typhimurium. This could be attributed to the higher rates of *S.* Enteritidis and *S.* Typhimurium serovars in wildlife and animals in outdoors which increase the chances of backyard layers infection with these two serovars than others (Dewulf *et al*., 2011). On the other hand, the reuse of cartoon egg trays in commercial farms could be attributed to the high serovars diversity in this production system. This study recorded the isolation of multiple serovars from egg trays in the same farm and there was a positive correlation between egg shell contamination and *Salmonella* detection in egg trays ($P = 0.004$). Personal communication with workers in these farms showed that instead of single use, cartoon egg trays were reused several times in different production cycles and sometimes egg trays were exchanged between different farms or between farms and egg collectors' stores. These practices may have contributed to the contamination of egg trays with multiple *Salmonella* serovars and then the reuse of these contaminated trays may introduce new serovars to the farm. In sum, egg trays may play an important role in introduction and persistence of *Salmonella* infection in commercial layers farms. The role of reusable egg trays in *Salmonella* contamination of eggs within layers farm was previously recorded (Utrarachkij *et al*., 2012).

A total of 32 *Salmonella* isolates belonged to 8 different serotypes were recovered in this study. *Salmonella* Typhimurium and *S.* Enteritidis were the predominant serovars from both flock types, which agreed with reports from Egypt (Elkenany *et al*., 2019) and India (Singh *et al*., 2010). This was in line with the WHO report which stated that *S.* Typhimurium and *S.* Enteritidis are the globally most common clinically significant *Salmonella* serovars (Hosny, 2006) and highlight the public health risk associated with consumption of eggs from both production systems in the studied region.

The *Salmonella* virulence genes are responsible for *Salmonella* pathogenicity through participating in the adhesion and invasion of the pathogen to the host or help in the pathogen survival within the host. In the current study, the occurrence of *fimH, hilA, stn* and *sopA* virulence genes were investigated. During the process of *Salmonella* colonization and invasion of host tissue, bacterial adhesion depends on the adhesive property of FimH protein (Kuzminska-Bajor *et al*., 2015). In the current study, *fimH* gene was detected in 80% of *S.* Typhimurium isolates, 62.5% of *S.* Enteritidis and 33% of *S.* Kentucky, while being absent in all other serovars. The *hilA* gene encodes an OmpR/ToxR transcriptional regulator, which plays a central role in the coordinated environmental regulatory effects of invasion genes in *Salmonella* pathogenicity (Bajaj *et al*., 1996). In the current study, the presence of *hilA* virulence gene was confirmed in 70% of *S.* Typhimurium isolates, 37% of *S.* Enteritidis, 38% of *S.* Kentucky and 50% of *S.* Infantis, while being absent in the reset of other *Salmonella* serovars giving an overall 37% detection rate. Comparable rate was reported in *S.* Typhimurium isolates in another study in Egypt (Ammar *et al*., 2016). The *Salmonella* enterotoxin (*stn)* gene contribute is key factor in *Salmonella*-induced gastroenteritis and diarrhea (Chopra *et al*., 1994). In the current study, the *stn* gene was detected in *S.* Typhimurium (60%), S. Enteritidis (25%) and *S.* Kentucky (33%), with an overall 31% detection rate, which was in line with another report from Egypt (Elkenany *et al*., 2019). The *sopA* gene is required for

significant induction of inflammation (Zhang *et al*., 2002). In the current study, *sopA* gene was detected in 70% of *S.* Typhimurium isolates, 50% of *S*. Enteritidis and 33 % of *S.* Kentucky, while being absent in the reset of other *Salmonella* serovars. The acquisition of the aforementioned virulence genes by *Salmonella* isolates in this study may be attributed to the evolution of these isolates for host adaption as previously recorded (Bäumler *et al*., 1998).

The overall *Salmonella* resistance rates to any and multiple antibiotics were 59.4% and 37.5%, respectively. The MAR values ranged between 0.0-0.92 with the highest values were recorded by *S.* Typhimurium (0.92), and *S.* Enteritidis (0.67) isolates. High resistance rates were recorded for β-Lactams (AMP 46.9%, and KZ 31.3%), and other classes including NA (46.9%), TE (31.3%). This was comparable to a previous report in Thailand (Utrarachkij *et al*., 2012); Majority of isolates were sensitive to ciprofloxacin and gentamicin, which agreed with other reports in India (Singh *et al*., 2010), and in Egypt (Ammar *et al*., 2016). None of the isolates were resistant to imipenem which agreed with a previous record in Egypt (Elkenany *et al*., 2019). The pattern of antibiotic resistance recorded in our study is probably reflecting the over or misuse of antibiotics in treating or preventing poultry diseases in study region by breeders without veterinary supervising. Antibiotics overuse in the animal production systems is known to develop MDR bacteria (McEwen and Fedorka-Cray, 2002).

Salmonella isolates in this study showed high diversity in virulence-antibiotic resistance patterns, which may highlight several sources of infection introduction in both production systems. However, identical patterns were recorded by two isolates of *S.* Typhimurium (P1, MAR 0.92), and two isolates of *S.* Enteritidis (P5, MAR 0.17). These identical isolates of each species belonged to the same farm (two *S.* Typhimurium from flock 4; two *S.* Enteritidis from flock 9). Hence, these isolates may share same source or were established over subsequent production cycles in the same farm. There was a significant correlation between acquisition of virulence genes and resistance to any (P= 0.002) or multiple antibiotics (P= <0.001). A similar trend was observed by Elkenany *et al.* (2019) who reported that *Salmonella* strains with virulence genes had higher drug resistance capabilities. This could be attributed to the antibiotic selective pressure among virulent strains by subsequent therapeutic intervention over time.

CONCLUSION

This study concluded that non-typhoidal *Salmonella* is common in eggs and their environment in commercial and backyard layers production systems in Egypt. *S.* Typhimurium and *S.* Enteritidis were the predominant serovars and most of these isolates were also MDR. This implies an alarming public health risk associated with eggs from both production systems in studied region. The study also highlighted the role of reusable egg cartoon trays in the introduction and persistence of *Salmonella* infection in commercial layers farms.

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IRB approval

The protocol of this study was approved by the institutional Animal welfare, Hygiene and Zoonoses committee at Kafrelsheikh University, Egypt (KFS-2017/2).

Ethical statement

The informed consents were obtained from the owners of commercial layer farms and backyard layer farms for the participation in this study and the publication of any relevant data.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20220802160857

Statement of conflict of interest

The authors have declared no conflict of interests.

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