RESEARCH ARTICLE

Tracing of *Salmonella* Contaminations Throughout an Integrated Broiler Production Chain in Dakahlia Governorate, Egypt

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A B S T R A C T

The data available regarding the production chain particularly in poultry flocks in Egypt are yet to be fully elucidated. The study aimed at providing an insight into the cross-contamination with *Salmonella* spp. at three different stages of an integrated broiler production chain. From 440 collected samples, 152 (34.5%) were positive for *Salmonella* spp. corresponded to samples collected from broiler farms (*n*=90, 40.9%), slaughter houses contact surfaces (*n*=12, 24%) and chicken carcass (n=50, 29.4%) in an integrated broiler supply chain. Isolation and identification of *salmonella* spp. were followed the standard procedures. Genomic bacterial DNA was extracted and confirmed using specific oligonucleotide primers sequences. *Salmonella* strains were classified into 11 serotypes. Nine different serotypes were found to contaminate thebroiler houses and flocks with the most prevalent serotypes *S.* Enteritidis (38.8%), *S.* Kentucky (23.3%) and *S.* Typhimurium (11.11%). The overall frequency of *Salmonella* contamination in the live broiler flocks was 40.9% with prevalence of 37.9, 60, 40, 53.3, 60 and 20% from cloacae swabs, litter, feed, water, farms walls and in the hand swabs from workers, respectively. The PCR products of four isolates, (two isolates per *S.* Typhimurium and *S.* Enteritidis) were identified by DNA sequencing to determine the potential genetic relationships in these isolates. The findings of the present study suggested that different *Salmonella* serotypes can persist along the production chain and that contamination could be linked to the contamination of the final product with a potential health hazard to consumers.

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INTRODUCTION

Poultry industry has seen a remarkable growth globally and represents approximately 45% of the world commerce (Henchion *et al*., 2014). In Egypt, this industry provides an integral part of the country's supply from animal protein and remains trendy among Egyptian consumers across the national income because of its low cost compared to red meat and fish. According to the Organization for Economic Co-operation and Development (OECD) and the Food and Agriculture Organization (FAO) of the United Nations for 2017-2026, ~ 993 thousand tons of chicken are consumed in 2017. Poultry, poultry products and other food of animal's origin represented the main reservoir for different *salmonella (S).* enterica serotypes, and human infection

with salmonellosis is often associated with poultry borne illness (Berghaus *et al*., 2013; Ibrahim *et al*., 2013). The bacterium s*almonella,* is a facilitative anaerobic gram negative, has ubiquitous nature and can be present in a variety of host and non-host environments (Elmonir *et al*., 2017). *Salmonella* infection has long been recognized as one of the most important zoonotic contaminant of food and remains the leading bacterial enteric pathogen responsible for food borne outbreaks in the developed and developing countries (Abd-Elghany *et al*., 2015).

Food borne illness caused by *salmonella* infections results in continuous threat to human and animal health and causes serious economic losses in the society (Antunes *et al*., 2016; Aziz *et al*., 2018). It has been stated that *S.* enterica exists in the intestinal tract and reproductive organs of carrier chickens (El-Sharkawy *et*

al., 2017). Hence, the existence of *salmonella* on live poultry causes its widespread dissemination into the processing chain with a potential contamination of the final product (Antunes *et al.*, 2016; Chao *et al.*, 2007). According to the recent report (WHO, 2015), *S.* enterica causes approximately 180 million illnesses or 9% of the diarrheal illnesses that occur globally each year. Therefore, monitoring the prevalence of *S.* enterica in the whole broiler supply chain is imperative to minimize the hazards of *S.* enterica in poultry. In addition, gaining thorough information about the contribution of risk factors in the integrated broiler production chain is important to implement a successful and cost-effective control program for salmonellosis (EFSA, 2015).

Although there have been extensive documents regarding *salmonella* infections in poultry farms in Egypt, no published reports are found about the occurrence of *salmonella* in an integrated poultry production chain in our country. So, it is of utmost significance to carry out this study to explore the potential occurrence of *salmonella* spp. with special reference to the zoonotic serotypes in broiler poultry farms and to track cross-contamination at broiler farms, slaughter houses and whole chicken carcasses in an integrated production chain and to assess the possible genetic relationships among *Salmonella* isolates by DNA sequencing.

MATERIALS AND METHODS

Sample collection: During summer 2017, four hundred and forty samples were collected from poultry production system including poultry farms (*n*=220), slaughter house contact surfaces (*n*=50) and chicken carcasses (*n*=170) at the end of the slaughter line. The samples were kept in coolers and transported to the laboratory and processed immediately. The study followed the guidelines for Mansoura University and approved by its Ethical Committee.

Sampling procedure
Broiler poultry farms: A total of 220 samples (145 cloacae swabs and 15 samples each of feed, litters, water, farm walls and workers hand swabs) were randomly collected from three broiler poultry farms with birds in pre-slaughter age (35 to 40 days) at Dakahlia governorate. A sterile cotton swabs soaked in Buffered Peptone Water (BPW) (Oxoid, Basingstoke, UK) were used for cloacal and hand swabs. Sampling and screening of feed, litter and farm surfaces (walls) were tested according to the methods described by the International Organization for Standardization, Geneva, Switzerland ISO 6579: 2002. All the examined farms have level 1 of biosecurity measures.

Slaughter houses environmental samples: Fifty samples (20 knives, 20 tables and 10 abattoir walls) were collected from the contact surfaces in the abattoir according to the methods described by the American Public Health Association (1992). The procedure was done by swabbing four 100-cm^2 regions of the sample with sterile sponges pre-moistened with 40 mL of BPW. The sponges set were then transferred to sterile bags containing 160 mL of BPW to yield a final volume of 200 mL, corresponding to 400

cm^2. All samples were thoroughly homogenized before laboratory analyses.

Samples from chicken carcasses: One hundred and seventy samples (120 carcass swabs, 25 fillets and 25 livers) were collected from chicken carcasses at the end of slaughter line. Chicken carcass swabs were collected after evisceration using a whole-carcass swab method (McEvoy *et al.*, 2005). Each swab was inoculated in sterilized BPW; while chicken fillets and livers from each individual sample (~25 g of each) were initially incised with a sterile scalpel and then collected into a sterile plastic stomacher bag according to the methods described by ISO 17604: 2003. The samples were transported immediately under aseptic conditions and processed for *salmonella* isolation.

***Salmonella* isolation and identification:** Each swab was cultured in 9 ml BPW while chicken fillets and livers sample (~25 g of each) were homogenized in 225 ml of BPW and incubated at 37°C for 20 hrs, then the incubated samples were gently shacked and added to Rappaport-Vassiliadis broth (Oxoid, Basingstoke, UK) at 1:100 and incubated at 42°C for further 24 hrs followed by streaking on Xylose Lysine Desoxycolates at 37°C for 24 hrs.The plates were examined after incubation for the presence of typical and suspected colonies. Up to five colonies from each sample were further identified on the basis of biochemical characterization (Indole test, motility, nitrate reduction, methyl red, Voges proskauer, citrate utilization, and urease) according to the guidelines of ISO 6579: 2002. Biochemically typical *salmonella* isolates were serotyped according to Kauffman–White–Le–Minor techniqueusing slide agglutination technique with polyvalent somatic (O) and flagellar (H) antisera (Welcome Diagnostic, UK) which were performed at the Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University Egypt.

DNA extraction: Genomic bacterial DNA was extracted and purified from the identified *S.* Typhimurium and *S.* Enteritidis serovar using the QIAamp® DNA Mini Kit Qiagen according to the manufacturer`s guidelines.

Molecular identification: Purified DNA was confirmed using specific oligonucleotide primers sequences for the amplification of *flic* and *sefA* genes according to Oliveira *et al.* (2003) and Akbarmehr *et al.* (2010).

Amplification, sequencing of *flic* and *sefA* Genes, and Data Analyses: The PCR products of four isolates (including two isolates per *S.* Typhimurium and *S.* Enteritidis that recovered from the farms and two other isolates obtained from chicken carcasses) were identified by DNA sequencing of partially *flic* and *sefA* gene. After electrophoresis on a 1.5% agarose gel, the bands were excised and purified using the QIAquick PCR product extraction kits (Qiagen Inc, Valencia, CA) according to the instructions of the manufacturer. Cycle sequencing of the gene fragments was done with the Big Dye ® Terminator v3.1 Cycle Sequencing Kit (Perkin-Elmer, Foster city, CA) according to the manufacturer's recommendations. Sequencing products were analyzed using applied Biosystems 3130 genetic analyzer (Hitachi, Japan).

RESULTS

Of the total 440 samples screened, *salmonella* was isolated from 152 samples (34.5%) including 90 samples (40.9%) from broiler farms, 12 samples (24%) of slaughter houses contact surfaces and 50 samples (29.4%) collected from the processed chicken. The recovered isolates were assigned to 11 different serovares of *S. enteric* subspecies enteric including *S.* Enteritidis (*n*=41, 26.9%), *S.* Typhimurium (*n*=40, 26.3%), *S.* Kentucky (*n*=29, 19.1%), *S.* Bargny (*n*=12, 7.9%), *S.* Molade (*n*=8, 5.3%), *S.* Takoradi (*n*=6, 3.9%), *S.* Labadi(*n*=5, 3.3%), *S.* Infantis (*n*=3, 1.9%), *S.* Papuana (*n*=3, 1.9%), *S.* Tamale (*n*=3, 1.9%), and *S.* Larochelle (*n*=2, 1.3%).

Distribution of *salmonella* spp. in broilers farms: The overall frequency of *salmonella* contamination in the live broiler flocks was 40.9% (90/220) with a prevalence of 37.9% (55/145) from cloacae swabs, and 60% (9/15) from litter, 40% (6/15) from feed, 53.3 % (8/15) from water, 60% (9/15) from farms walls and 20% (3/15), from the worker's hand swabs (Table 1). The isolated serovares from broilers farms were distributed as follows: *S.* Enteritidis 38.8% (35/90), *S.* Kentucky 23.3% (21/90), *S.* Typhimurium 11.1% (10/90), *S.* Molade 7.8% (7/90), *S.*Takoradi 6.7% (6/90), *S.* Bargny 2.2% (2/90) and 3.3% (3/90) for each of *S.* Papuana, *S.* Tamale, *S.* Infantis.

Distribution of different *Salmonella* serotypes in slaughter houses contact surfaces and processed chicken: The data obtained from the processing environment are outlined in Table 2 and 3. In short, 24% (12/50) among slaughterhouses contact surfaces were contaminated with *salmonella* spp. with a higher contamination rate in abattoir walls (60%, 6/10). In general, twelve different *salmonella* isolates were recovered from the screened slaughter house contact surfaces (six isolates of *S.* Kentucky recovered from

abattoir walls; two isolates of *S.* Enteritidis recovered from tables and four isolates of *S.* Bargny were recovered from Knives swabs). The occurrence of *salmonella* from the whole chicken carcass was 29.4% (50/170); being higher than that from processing surfaces (24%). In addition, the number of positive samples of *salmonella* spp. that recovered from the whole chicken carcasses swabs, liver and filet were 30/120, 10/25 and 10/25, respectively.

Molecular characterization of *S.* Enteritidis and *S.* Typhimurium isolates: Fig. 1 showed the amplification of *fliC* and *sefA* genes in the selected four *salmonella* isolates. The sequencing of the partially *flic* and *sefA* genes demonstrated similar DNA profiles for each of *S.* Typhimurium and *S.* Enteritidis that recovered from the farms and the retail products.

DISCUSSION

The findings of the present study demonstrated the persistence of different *salmonella* serotypes in an integrated broiler supply that alarms for a potential public health hazard. The frequency reported in the present study was similar to that recently reported in Egypt (El-Sharkawy *et al.*, 2017). In that study, the authors identified *salmonella* infection in 17 out of 41 broiler chicken farms (41%) located in Kafr El-Sheikh Governorate in Northern Egypt during 2014–2015. On the other side, higher isolation rate (90.9 and 57.1%) was reported in the USA (Berghaus *et al.*, 2013) and Egypt (Aziz *et al.*, 2018). In the present study, nine different serotypes were found to contaminate the broiler houses and flocks with the most prevalent serotypes *S.* Enteritidis (38.8%), *S.* Kentucky (23.3%) and *S.* Typhimurium (11.11%). These findings went in parallel with that reported by Abd-Elghany *et al.* (2015) who stated that *S.* Typhimurium and *S.* Enteritidis were
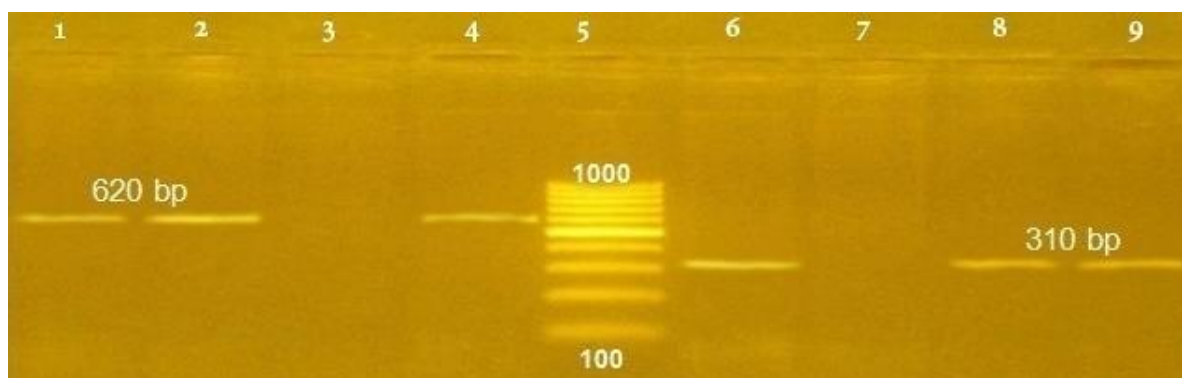

Fig. 1: Agarose gel electrophoresis of PCR amplification of *fli*C and *sef*A of *S.* Typhimurium and *S.* Enteritidis with expected amplicon size ~ 620 and 310 bp, respectively. Lane5 is DNA ladder; lane 4 and 6 are positive controls, while lane 3 and 7 are negative controls.

Table 1: Occurrence and distribution of different *Salmonella* serotypes in broiler farms

Source of samples	No. of samples	No. of positive samples	% of positive	Serotypes
Cloacal swabs	145	55	37.9	*S.* Kentucky (21), *S.* Molade (7), *S.* Enteritidis (27)
Feed	15	6	40	*S.* Takoradi
Litter	15	9	60	*S.* Papuana (3), *S.* Tamale (3), *S.* Enteritidis (1), *S.* Typhimurium (2)
Water	15	8	50	*S.* Enteritidis (4), *S.* Bargny (2), *S.* Typhimurium (2)
Farm walls	15	9	60	*S.* Enteritidis (3), *S.* Typhimurium (6)
Hand swabs from workers	15	3	20	*S.* Infantis
Total	220	90	40.9	*S.* Kentucky (21), *S.* Enteritidis (35), *S.* Typhimurium (10), *S.* Molade (7), *S.* Takoradi (6), *S.* Papuana (3), *S.* Tamale (3), *S.*Infantis (3), *S.* Bargny (2)

Table 2: Occurrence and distribution of different *Salmonella* serotypes from slaughter house environment

Source of samples	No. of samples	No. of positive samples	% of positive	Serotypes
Knives swabs	20	4	20	S. Bargny
Table	20	2	10	S. Enteritidis
Abattoir wall	10	6	60	S. Kentucky
Total	50	12	24	

considered the most predominant serotypes present in Egyptian poultry farms. On the other side, low frequency of *salmonella* infection was reported in broilers chicken flocks in several studies (Ibrahim *et al*., 2013; El-Tawab *et al*., 2015; Ren *et al*., 2016; Elmonir *et al*., 2017; Elkenany *et al*., 2018) corresponded to 16.6, 12.4, 7, 20 and 13.5%, respectively. In an interesting study (Pieskus *et al*., 2008), the occurrence of *salmonella* infection in conventional broiler stocks located in different areas in Italy, Germany, Lithuania and the Netherlands were 20%, 0, 29 and 11%, respectively. Given that litter, farm walls swabs and water samples provoked a consistently higher number of positive samples than individual chickens or chicken feed, these findings suggest that the farm walls, litter and water might be as a potential reservoir for contamination in the examined broiler farms. Several pathways associated with increase flock colonization by *salmonella* include vertical and horizontal transmission, overcrowding, unhygienic farming activities, lack of adequate biosecurity measures, contamination from previous flocks, exposure to potential sources of the bacterium such as neighboring animals on the farm, insects, rodents, environment, housing system and flock size, season of the year, different hygienic measures, inadequate cleaning and disinfection of broiler rearing houses and contamination of feed, litter and drinking water. These factors could play a vital role for the pathogen to be easily disseminated between the flocks.

The information about the occurrence of *salmonella* contamination at slaughter facilities remains scarce despite being the main source of cross-contamination. In this respect, nearly similar incidences of surface contamination for *salmonella* were reported by several researchers (Adzitey *et al*., 2012; Wang *et al*., 2013) in poultry processing environment with a rate of 23.5 and 25%, respectively. On the other side, high positive percentage rate (62.9%, 22/35) was also reported in china slaughter house samples (Ren *et al*., 2016). However, diverse surveys are still reported from several developing countries. For example, high percentages of positive samples (approximately 35%) were reported from Nigeria (Adeyanju and Ishola 2014) and Egypt (Abd-Elghany *et al*., 2015). In South America, the detection rates were ranging between ~13 and 39% (Ribeiro *et al*., 2007; Donado-Godoy *et al*., 2015), while in Asia, it ranged between 35 and 43% (Ta *et al*., 2014; Yang *et al*., 2014).

In China, 54·67% of the 75 retail store raw chicken samples was contaminated with *Salmonella* spp. (Ren *et al*., 2016). Nevertheless, a comparatively low isolation rate (1.56%) was reported from Morocco in poultry processing plan (Cohen *et al*., 2007). Different prevalence rates from retail outlets, retail markets and processing plans were reported worldwide. The discrepancy among the previously reported contamination rates of *salmonella* in chicken meat could possibly reflect the diverse hygienic conditions along the production practices, transportation stress and lack of control measures along the slaughter houses environment. The high contamination rate of whole carcass swab would suggest a contamination during de-feathering in the slaughter house and highlight the need for adequate hygienic procedures. It becomes clear that chicken products could be an important vehicle of *S*. enterica, and hence signify its hygienic hazard at this stage.

Although several serotypes of *Salmonella* were recovered separately during the different stages of production, *S*. Enteritidis and *S*. Kentucky were prevailed at all stages of the production chain and *S*. Typhimurium was found in common between the farms and the retail products. *Salmonella* Typhimurium and *S*. Enteritidis demonstrated similar DNA profiles by sequencing and were considered the most prevalent serovars being isolated from the screened broilers on the farms and also recovered from the whole chicken carcasses within the same area. A similar finding was previously given by Hugas and Beloeil (2014) and reported that *S*. Enteritidis and *S*. Typhimurium were commonly associated with poultry and its products and have great public health significance.

The occurrence of *S*. Kentucky at each stage in the production chain was really surprising. The overall prevalence of *S*. Kentucky in the present study was 19.1%. Data reported from a recent study showed that *S*. Kentucky was detected in poultry as a predominant serotype and could be implicated in human s*almonellosis* (Westrell *et al*., 2014). In that study, 1301 cases *of salmonella* infections were caused by *S*. Kentucky in 12 European countries between 2007 and 2012. The authors added the rationale for increasing the occurrence of *S*. Kentucky could be linked to increase globalization of food/animal trades. Recently, *S*. Kentucky (particularly the epidemic clone ST198-X1) has been recovered from several livestock reservoirs, particularly from poultry farms in Europe, Africa and Asia (Antunes *et al*., 2016). In the present study, *S*. Infantis was recovered from the hand swabs of farm workers at the percentage of 20%. According to the European Food Safety Authority (EFSA, 2015), human infection cases due to *S*. Infantis have been increased between 2011 and 2013 and was being considered the fourth most common serotype reported in broilers and broiler meat.

Table 3: Occurrence and distribution of different Salmonella serotypes from processed broilers

Source of samples	No. of samples	No. of positive samples	% of positive	Serotypes
Carcass	120	30	25.6	S. Typhimurium (21), S. Kentucky (2), S. Molade (1), S.Bargny (6)
Liver	25	10	40	S. Typhimurium (5), S.Labadi(5)
Fillet	25	10	40	S. Typhimurium (4), S. Enteritidis (4), S. Larochelle (2),
Total	170	50	29.4	S. Typhimurium (30), S. Bargny (6),S. Kentucky (2), S. Molade (1), S. Labadi(5), S. Enteritidis (4), S. Larochelle (2)

Taken together, it can be concluded that different *Salmonella* serotypes can persist along the integrated production chain (on-farm, slaughter operations, equipment, chicken carcasses, handlers and retail meat). Such contamination is significantly linked to the contamination of the final product which highlights a serious public health hazard. Additionally, an integrated surveillance (i.e. collaboration between different authorities), containment strategies and continuous monitoring are urgently needed through a farm-to-table approach at every stage of the production chain in order to minimize contamination and also reduce the emergence of potential *Salmonella* serotype along the food chain which is of critical importance for public health.

Authors contribution: All authors contributed equally in study design, samples collection and processing and data interpretation. All authors revised the manuscript and approved the final version before publication.

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