Epidemiological, molecular characterization and antibiotic resistance of *Salmonella enterica* serovars isolated from chicken farms in Egypt

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**Abstract**

**Background:** *Salmonella* is one of major causes of foodborne outbreaks globally. This study was conducted to estimate the prevalence, typing and antibiotic susceptibilities of *Salmonella enterica* serovars isolated from 41 broiler chicken farms located in Kafr El-Sheikh Province in Northern Egypt during 2014–2015. The clinical signs and mortalities were observed.

**Results:** In total 615 clinical samples were collected from broiler flocks from different organs (liver, intestinal content and gall bladder). *Salmonella* infection was identified in 17 (41%) broiler chicken flocks and 67 *Salmonella* isolates were collected. Recovered isolates were serotyped as 58 (86.6%) *S. enterica* serovar Typhimurium, 6 (9%) *S. enterica* serovar Enteritidis and 3 (4.5%) were non-typable. The significant high mortality rate was observed only in 1-week-old chicks. *sopE* gene was detected in 92.5% of the isolates which indicating their ability to infect humans. All *S. enterica* serovar Enteritidis isolates were susceptible to all tested antimicrobials. The phenotypically resistant *S. enterica* serovar Typhimurium isolates against ampicillin, tetracycline, sulphamethoxazole and chloramphenicol were harbouring *Bla*TEM, (*tetA* and *tetC*), (*sul*1 and *sul*3) and (*cat1* and *floR*), respectively. The sensitivity rate of *S. enterica* serovar Typhimurium to gentamycin, trimethoprim/sulphamethoxazole and streptomycin were 100, 94.8, 89.7%, respectively. The silent streptomycin antimicrobial cassettes were detected in all *Salmonella* serovars. A class one integron (*dfrA12*, *orfF* and *aadA2*) was identified in three of *S. enterica* serovar Typhimurium strains.

**Conclusions:** To the best of our knowledge, this study considered first report discussing the prevalence, genotyping, antibiotic susceptibility and public health significance of *S. enterica* serovars in broilers farms of different ages in Delta Egypt. Further studies are mandatory to verify the location of some resistance genes that are within or associated with the class one integron.

**Keywords:** *Salmonella*, Broiler, Epidemiology, Antimicrobial, Integron

**Background**

In spite of significant improvement in technology and hygienic practices at all stages of poultry production accompanied with advanced improvement in public sanitation, salmonellosis and *Salmonella* infections remains a persistent threat to human and animal health. In many countries high incidence of salmonellosis in man appears to be caused by infection derived from contaminated eggs, poultry meat and meat-products. The contaminated products cause disease as a result of inadequate cooking or cross contamination of working surfaces in kitchen environment [1–3].
The genus *Salmonella* of the family *Enterobacteriaceae* includes more than 3000 distinct serovars that have many host species and cause different diseases; most of which show little specificity for their host species [4–7]. The genus *Salmonella* can roughly be classified into three categories or groups: Group 1, highly host-adapted and invasive serovars: this group includes species restricted and invasive *Salmonella* such as *S. Pullorum*, *S. Gallinarum* in poultry and *S. Typhi* in humans. Group 2, non-host-adapted and invasive serovars: this group consists of approximately 10–20 serovars that are able to cause an invasive infection in poultry and may be capable of infecting humans. Currently, the most important serovars are *S. Enteritidis*, *S. Typhimurium*, *S. Hadar*, *S. Heidelberg*, *S. Saintpaul* and *S. Infantis*. Group 3, non-host-adapted and non-invasive serovars: most serovars of the genus *Salmonella* belong to this group and may cause disease in humans and other animals [8–14].

Although the acute enteritis caused by *Salmonella* species in humans is usually self-limiting, salmonellosis may be complicated especially in younger and older ages by severe systemic sequelae depending on serotype and on host-specific factors [15–17].

*Salmonella enterica* serovar *Typhimurium* and *S. enterica* serovar *Enteritidis* have been identified as the predominant serotypes present in Egyptian poultry farms [18].

*Salmonella enterica* serovar *Enteritidis* has been associated with disease in broiler breeding stock and can be transmitted vertically to their progeny [19]. Infection of adult chickens with *S. enterica* serovar *Typhimurium* is usually without clinical manifestation [20]. *S. enterica* serovar *Enteritidis* can inhabit the intestinal tract of several bird species such as chickens, turkeys and game birds and has the ability to survive outside of the host for over 1 year. *S. enterica* serovar *Enteritidis* infection in adult poultry is usually asymptomatic and infected bird will become a chronic carrier [21, 22]. In chickens up to 6 weeks of age *S. enterica* serovar *Enteritidis* may produce clinical symptoms including depression, disinclination to move, and diarrhea, with high mortality especially in chicks less than 1 week of age [23], while older chicks may show uneven growth and stunting. Laying hens sometimes produce *S. enterica* serovar *Enteritidis* contaminated eggs leading to public health concerns [19]. The diseased birds may show lesions of pericarditis, perihepatitis and septicaemia. The mortality and morbidity vary and has been found to depend upon the dosage and phage type of the *S. enterica* serovar *Enteritidis* infection [24, 25].

Antimicrobial resistance is increasingly becoming an issue with salmonellosis infections in both animals and humans [26]. Understanding the key mechanisms involved in the evolution of antibiotics resistance in bacteria may aid scientific innovations aimed at controlling antimicrobial resistance [27, 28]. Bacteria can acquire resistance genes through mobile elements such as plasmids, which provide flexibility to host bacteria and help in the spread and distribution of these genes across diverse bacterial populations [29].

The inappropriate use of antibiotics in chicken farms in developing countries, including Egypt, is thought to be one of the main reasons for the increase in multidrug resistant bacteria [30]. These multidrug resistant bacteria including both *S. enterica* serovar *Typhimurium*, and *S. enterica* serovar *Enteritidis* that have the potential to infect humans and with a consequent failure of treatment can lead to systemic infection and death [31].

In this study, the incidence and antimicrobial resistance of *S. enterica* serovars *Typhimurium* and *Enteritidis* isolated from broiler chicken farms in Kafr El-Sheikh Province, Northern Egypt was reported. Determination of genes associated with antimicrobial resistance was investigated by examining the distribution of mobile integrons that carry the multidrug resistance cassettes within the genome of the isolated strains.

**Methods**

**Sampling strategy and *Salmonella* isolation**

This study was conducted in 41 broiler flocks located in Kafr El-Sheikh Province in Delta Egypt. Twenty flocks of 1-week-old birds and 21 flocks of 5-week-old birds were investigated. The observed clinical symptoms were observed and recorded (Table 1). Five living morbivid birds from each flock were randomly selected and humanly sacrificed. At necropsy, sections of liver and intestinal wall plus contents were collected aseptically and processed for *Salmonella* isolation. From the same bird bile was aspirated from the gall bladder. Wetted cotton swabs in bacteriological transport media were used to collect samples from each specimen. Collected swabs and tissue samples were immediately frozen on ice and stored at −20 °C for further investigation within 5 h. Each tissue sample and swabs were inoculated in 10 ml selenite F broth (Oxoid, UK) and incubated at 37 °C overnight. A loopful of inoculated broth was streaked on selective *Salmonella Shigella* (SS) agar (Oxoid, UK) and incubated at 37 °C overnight. The suspected colony was sub-cultured on Xylose lysine deoxycholate (XLD) agar (Oxoid, UK) and on brilliant green (BG) agar (Oxoid, UK) and incubated at 37 °C for 16–18 h. The suspected colonies were collected for further biochemical identification using API 20E (BioMérieux, Marcy-l’Étoile, France).
| Flock no. | No. of birds | Age/day | Clinical signs | Mortality, n (%) | Isolation results |
|----------|--------------|---------|----------------|------------------|------------------|
| 1        | 10,000       | 1       | Pasty diarrhea, blindness, lameness and high mortality | 850 (8.5) | S. Enteritidis |
| 2        | 10,000       | 2       | Inappetence and respiratory manifestation | 110 (1.1) | Negative |
| 3        | 15,000       | 3       | Pasty diarrhea, conjunctivitis, lowering in body weight and high mortalities | 975 (6.5) | S. Enteritidis |
| 4        | 15,000       | 4       | Inappetence, ruffling feather and nervous signs | 360 (2.4) | Negative |
| 5        | 25,000       | 5       | Lowering body rate and respiratory signs | 550 (2.2) | Negative |
| 6        | 2000         | 7       | Pasty diarrhea, loss of appetite, ruffling feather and high mortalities | 190 (9.5) | S. Typhimurium |
| 7        | 5000         | 7       | Decreased body weight, diarrhea, dehydration and high mortalities | 415 (8.3) | S. Typhimurium |
| 8        | 20,000       | 4       | Decreased body weight | 500 (2.5) | Negative |
| 9        | 10,000       | 6       | Whitish diarrhea, high mortalities, and decreased body weight | 1160 (11.6) | S. Typhimurium |
| 10       | 12,000       | 7       | Inappetence, diarrhea and lowering body weight | 540 (4.5) | Negative |
| 11       | 25,000       | 7       | Inability to move and nervous signs | 850 (3.4) | Negative |
| 12       | 30,000       | 5       | Diarrhea, drop in feed intake and high mortalities | 2610 (8.7) | S. Typhimurium |
| 13       | 5000         | 4       | Respiratory signs and decreased body weight | 225 (4.5) | Negative |
| 14       | 30,000       | 3       | Inappetence, lowering growth rate | 840 (2.8) | Negative |
| 15       | 15,000       | 6       | Whitish diarrhea, conjunctivitis and decreased body weight | 945 (6.3) | S. Typhimurium |
| 16       | 10,000       | 4       | Diarrhea and decrease in body weight and respiratory signs | 350 (3.5) | Negative |
| 17       | 10,000       | 5       | Inappetence, mortalities, lameness and diarrhea | 550 (5.5) | S. Typhimurium |
| 18       | 20,000       | 6       | Decreased body weight and respiratory signs | 640 (3.2) | Negative |
| 19       | 12,000       | 5       | Diarrhea, blindness and high mortality | 648 (5.4) | S. Typhimurium |
| 20       | 10,000       | 5       | Respiratory and nervous signs | 420 (4.2) | Negative |
| 21       | 15,000       | 33      | Inappetence and respiratory manifestation | 375 (2.5) | S. Typhimurium |
| 22       | 20,000       | 32      | Decreased body weight | 280 (1.4) | Negative |
| 23       | 30,000       | 33      | Mortalities | 660 (2.2) | Negative |
| 24       | 25,000       | 29      | Nervous signs | 625 (2.5) | Negative |
| 25       | 15,000       | 34      | Decreased body weight | 345 (2.3) | S. Typhimurium |
| 26       | 20,000       | 29      | Decreased body weight | 460 (2.3) | Negative |
| 27       | 30,000       | 30      | Decreased body weight | 780 (2.6) | Negative |
| 28       | 10,000       | 31      | Respiratory signs and high mortality | 330 (3.3) | S. Typhimurium |
| 29       | 5000         | 28      | Respiratory signs and mortalities | 165 (3.3) | Negative |
| 30       | 15,000       | 33      | Inappetence and mortalities | 480 (3.2) | Negative |
| 31       | 20,000       | 33      | Respiratory signs and high mortality | 600 (3.0) | S. Typhimurium |
| 32       | 20,000       | 32      | Inappetence and respiratory manifestation | 700 (3.5) | S. Typhimurium |
| 33       | 10,000       | 28      | Mortalities | 200 (2.0) | Negative |
| 34       | 20,000       | 29      | Mortalities | 480 (2.4) | Non typable Salmonella (three isolates) |
| 35       | 10,000       | 33      | Respiratory signs and mortalities | 290 (2.9) | S. Typhimurium |
| 36       | 5000         | 33      | Lower body weight and respiratory signs | 135 (2.7) | Negative |
| 37       | 15,000       | 32      | Nervous signs | 345 (2.3) | Negative |
| 38       | 20,000       | 33      | Inappetence and mortalities | 540 (2.7) | Negative |
| 39       | 10,000       | 31      | Respiratory signs and mortalities | 320 (3.2) | S. Enteritidis |
| 40       | 5000         | 29      | Nervous manifestations and inappetence | 140 (2.8) | Negative |
| 41       | 25,000       | 35      | Opisthosomas and ruffled feather | 825 (3.3) | Negative |
Genomic DNA extraction and purification
The identified bacterial cultures were cultivated on SS agar and inoculated on Luria–Bertani (LB) broth (Oxoid, UK) and incubated at 37 °C overnight. The DNA was extracted from bacterial cultures on broth using QiaGen DNA extraction kit (Qiagen, UK) according to the manufacturer’s instructions.

Molecular biological identification and differentiation of Salmonella serovars
In order to make a rapid and definite diagnosis of Salmonella, PCR was conducted using primers to detect the gene marker for S. enterica invA [32], sdfI primers specific for detection of S. enterica serovars Enteritidis [33], and Typh, Sal and fliC specific primers for serovar S. Typhimurium [34, 35] (Table 2).

invA positive strains were tested for the presence of the sefA gene, which encodes for SEF14 fimbriae that can be detected in S. enterica serovar Enteritidis strains and will also be present in the poultry-associated serotype S. Gallinarum.

In order to detect the zoonotic potential of our isolated strains of S. enterica serovar Enteritidis and S. enterica serovar Typhimurium we screened for the presence of the sopE gene [36].

The PCR reaction was geared to a previously described protocol for Salmonella [32–36]. Conserved forward and reverse primers (Eurofins, Japan) were used to generate the target amplicon (Table 1). The PCR cycling conditions were carried out as the following: initial denaturation at 94 °C for 5 min. Thirty cycles of amplification were run for 5 s, at 94 °C, 10 s at 68 °C and 20 s at 72 °C, with the final extension continuing at 72 °C for 7 min. Different annealing temperatures were used as described in Table 1. Five microliter aliquots of reaction mixture were electrophoresed through 1.5% agarose gels (Nipppongene, Japan).

Determination and sequencing of class 1 integrons
The class one integrons PCR fragments were purified from the agarose gel using Nucleospin Gel Extraction Kit (Macherey–Nagel, Germany) and sequenced (Genome centre—Gifu University, Japan). The sequencing results were analysed using BLAST webpage (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Antimicrobial susceptibility testing
The antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion test [37] at the Clinical Veterinary Microbiology Laboratory of the Royal Dick School of Veterinary Study, University of Edinburgh. Briefly, one colony from the SS agar plate of each strain was picked up and streaked onto Mueller–Hinton blood agar (Oxoid, UK) and incubated at 37 °C overnight. Bacterial colonies were suspended in 0.9% NaCl to obtain a McFarland turbidity of 0.5 (Dr. Lange, photometer CADAS 30, Berlin, Germany) that containing about 1–2 × 10⁸ colony forming units (CFU)/ml of Escherichia coli strain American Type Culture Collection (ATCC) 25922. Approximately, 300 μl of the saline suspension was spread onto the surface of a Mueller–Hinton agar plate (Oxoid, UK) using a sterile swab.

Table 2 Primer sequences and their corresponding genes used for the detection of S. enterica serovar Enteritidis and S. enterica serovar Typhimurium

| Gene | Oligonucleotide sequence (5’–3’) | Annealing (°C) | Amplicon size (bp) | Reference |
|------|---------------------------------|----------------|-------------------|-----------|
| invA-F | GCT GCG CGC GAA CGG CGA AG | 62 | 389 | [32] |
| invA-R | TCC CGG CAG AGT TCC CAT T | | | |
| SdfI-F | TGTTGTATTATGTGAAAGAGG | 58 | 293 | [33] |
| SdfI-R | CGTCTTGCTAGCTACGAGTAC | | | |
| SdfII-F | GCGAATATCAGAGTACGAT | 58 | 450 | [33] |
| SdfII-R | GATGTCATACCGTTGAGA | | | |
| SdfIII-F | GCTGACCTACACAGGAATCG | 58 | 350 | [33] |
| SdfIII-R | TCTGATAAGACTGCGTTCCTACT | | | |
| SefA-F | GCC GTA CAC GAG CTT ATA GA | 55 | 250 | [33] |
| SefA-R | ACC TAC AGG GGC ACA ATA AC | | | |
| SfI-fI-C-F | CCCCCGTCCAGCGTGGACTAC | 62 | 433 | [35] |
| SfI-fI-C-R | AGCGGGTTTCCGTTGTTGT | | | |
| SopE-F | ACA CAC TTT CCA CGA GGA AGC G | 55 | 398 | [36] |
| SopE-R | GGA TGC CTT CTG ATG TTG ACT GG | | | |
| Typh-F | TTGTTCACTTTTTACCCCTGA A | 55 | 401 | [34] |
| Typh-R | CCCTGACAGCCGTAGATATT | | | |
The antimicrobial discs (Oxoid, UK) of six clinically used antibiotics that are used in the Egyptian poultry production (tetracycline 30 μg, ampicillin 10 μg, sulfa-famethoxazole/trimethoprim 25 μg, gentamicin 10 μg, streptomycin 25 μg and chloramphenicol 30 μg) were distributed onto the surface of the Mueller–Hinton agar plates using a Multi-disc dispenser (Oxoid, UK). The plates were incubated at 37 °C overnight. The diameters of the inhibited zones were measured using sliding callipers and interpreted using standard break points according to the method described by The European Committee on Antimicrobial Susceptibility Testing [38] (Table 3).

The gene associated with antibiotic resistance was tested in isolated Salmonella strains. Isolates were screened for the presence of 18 genes known to be associated with resistance to the seven tested antibiotics (Table 4).

**Statistical analysis**
The mortality rate associated with Salmonella infection and the rate of S. enterica serovar Typhimurium isolation from internal organs were analysed by the student t test [39].

**Results**

**Clinical signs, mortality and incidence of Salmonella isolation from broiler flocks**

Clinical symptoms of Salmonella infection observed in the 1-week-old broiler chicks included pasty diarrhea, inappetence, dehydration, growth retardation, blindness and lameness. The main gross lesions were hepatomegaly with necrotic foci, splenomegaly, pericarditis, panophthalmitis, and arthritis (Table 1).

In total 615 samples collected from intestine, liver and gallbladder from 41 broiler flocks, 67 (10.9%) Salmonella strains were isolated. In all, 45% of the sampled 1-week-old broiler flocks (9/20) and 38% of the screened 5-week-old broiler flocks (8/21) tested positive for Salmonella (Table 1).

The mean mortality rate (5.23% ± 2.85) of the 1-week-old flocks was significantly higher (P < 0.01) than the mean mortality rate (2.68% ± 0.52) in the 5-week-old flocks. When grouped by Salmonella infection status, the mortality rate observed in the 1-week-old birds was significantly higher (P < 0.001) in the Salmonella positive flocks (7.8% ± 2.07) compared to negative flocks (3.1% ± 0.45) (Table 1). While, there was no significant difference in mortality rate between the infected and non-infected 5-week-old flocks (P = 0.15, Table 1).

**Molecular biological identification of Salmonella serovars and public health significance**

Both S. Enteritidis and S. Typhimurium serovars were isolated and identified from both the 1- and 5-week old sacrificed chicks (Table 5). Three of the collected 67 isolates were Salmonella positive but un-typable serovars.

All 67 recovered isolates were harboured invA gene (Fig. 1a). Out of 67 invA positive Salmonella strains, 6 (9.0%) strains were positive for sefA, sdfI, sdfII and sdfIII genes (Fig. 1a) indicating S. enterica serovar Enteritidis and 58 (86.6%) strains were positive for Typh, sdfI and flIC marker (Fig. 1a) indicating S. enterica serovar Typhimurium. Three Salmonella strains (4.47%) were untypable and were positive for invA and sdfII (Fig. 1a) (Table 5).

The isolation rate of Salmonella serovars from different organs were demonstrated in Table 5. Briefly, there was highly significant difference (P < 0.001) of S. enterica serovars Typhimurium isolated from the gallbladder (14.63%) and liver (9.76%) (P < 0.05) compared to those isolated from the intestine (3.9%) (Table 5). There was no significance difference (P = 0.28) between the isolation rate of S. enterica serovars Enteritidis form liver, intestine and gallbladder. The three un-typable serovars were found only at one farm and were isolated only from the intestinal samples (Table 5).

| Antimicrobial agents | Conc. (µg) | Diameter of inhibition zone (mm) | S. Typhimurium (58) | S. Enteritidis (6) | Non typable (3) |
|---------------------|-----------|---------------------------------|---------------------|-------------------|----------------|
|                     |           |                                 | R      | I      | S      | R      | I      | S      | R      | I      | S      | R      | I      | S      |
| Ampicillin          | 10        | ≤11                               | 14–16  | ≥17    | 58 (100%) | –      | –      | 6 (100%) | 3 (100%) | –      | –      | –      | –      | –      | –      |
| Chloramphenicol     | 30        | ≤11                               | 12–14  | ≥15    | 58 (100%) | –      | –      | 6 (100%) | 3 (100%) | –      | –      | –      | –      | –      | –      |
| Gentamicin          | 10        | ≤11                               | 12–14  | ≥15    | 58 (100%) | –      | –      | 6 (100%) | 3 (100%) | –      | –      | –      | –      | –      | –      |
| Streptomycin        | 25        | ≤11                               | 12–14  | ≥15    | 58 (100%) | –      | –      | 6 (100%) | 3 (100%) | –      | –      | –      | –      | –      | –      |
| Tetracycline        | 30        | ≤11                               | 12–14  | ≥15    | 58 (100%) | –      | –      | 6 (100%) | 3 (100%) | –      | –      | –      | –      | –      | –      |
| Trimethoprim/sulphamethoxazole | 25        | ≤10                               | 11–15  | ≥16    | 58 (100%) | –      | –      | 6 (100%) | 3 (100%) | –      | –      | –      | –      | –      | –      |

S sensitive, I intermediate, R resistance
### Table 4 Primer sequences and their corresponding genes used for detection of antimicrobial resistant genes for *S. enterica* serovars

| Gene | Primer | Nucleotide sequence (5′–3′) | Annealing (°C) | Amplicon size (bp) | Reference |
|------|--------|-----------------------------|----------------|-------------------|-----------|
| *aadA1* | F | TATCAGAGGTAGTTGGCGTCAT | 54 | 484 | [62] |
| R | GTTCCATAGGCTTAAGGTTTCAAT |
| *aadA2* | F | TGTTGTTACGTGGCCTGTGA | 62 | 622 | [62] |
| R | GATCTGCGCTTTTACAAAGC |
| *aadB* | F | GAGCGAAATCTGCCGCTTGGG | 61 | 319 | [62] |
| R | CTGTACAAGGACCTGCGCCGC |
| *aacC* | F | GGCAGGATCAAGGAGATTATCCGA | 58 | 488 | [28] |
| R | CCATTCCGATGCCAGAAAGGACAT |
| *blaTEM* | F | CATTTCCGTCGCGCCCTTAT | 55 | 793 | [62] |
| R | TCCATAGGCTGAATATCC |
| *cat1* | F | CCTTGGGCTGATGATATAAT | 53 | 508 | [27] |
| R | ATCCCAATGCACTCGAAAG |
| *cat2* | F | CCGGATGGACCTGAATACCT | 56 | 572 | [62] |
| R | TCACATACTGCATGATGAAC |
| *dfrI* | F | GTGAAACTATCACTAATGGTAGCT | 54 | 470 | [62] |
| R | ACCCTTTGCGACATTATTGGTAATC |
| *floR* | F | AACCGCCCTCTTGTAACAGTCAA | 60 | 548 | [62] |
| R | CAAATCGAGGGCGCAACGTGAT |
| *strA* | F | AGCGAGGGCCGGCTCCCATC | 59 | 684 | [62] |
| R | CCAAGGCCCCACTCCACAGC |
| *strB* | F | ATGCTGCAAGGATGGAAACC | 49 | 509 | [63] |
| R | GGATGCTGACATATGTTG |
| *sul1* | F | TCACCCAGGATCCTTCCCT | 60 | 316 | [62] |
| R | AATATCGGGATAGGAGGACA |
| *sul2* | F | CGGTGCGCGACATCCGACGCAATCC | 64 | 441 | [62] |
| R | CGAGAACCCAGGGCGCCCG |
| *sul3* | F | GACGCGAGTATTTTGGAATTCC | 51 | 864 | [63] |
| R | CATCTGCGACTAACTGAGGGCTTTGALA |
| *tetA* | F | GCTCATCCTGCTGGCTTTC | 55 | 210 | [64] |
| R | CATAGATCGCGTGAAAGG |
| *tetB* | F | TTGGTATTGGGCAAGTTTTG | 53 | 659 | [64] |
| R | GTAATGCGCCATACACAG |
| *tetC* | F | CTTGAGAGCCCTTCAACCCAG | 56 | 418 | [64] |
| R | ATGGTCGACCTTACCTTCCG |
| *intI* | F | 5′GGCAGTCACGAGGACCCGACGACTGTGTA | 55 | 2000 | [65] |

### Table 5 The rate of *S. enterica* serovars isolation from tissue organs collected from 41 broiler chicken flocks in Kafr El-Sheikh Province in Northern Egypt

| Organs | Liver | Intestine | Gallbladder | Total |
|--------|-------|-----------|-------------|-------|
| No. of collected samples | 205 | 205 | 205 | 615 |
| No. of isolates | 22 (10.74%) | 12 (5.85%) | 33 (16.09%) | 67 (10.9%) |
| *S.* *Enteritidis* | 2 (0.98%) | 1 (0.49%) | 3 (1.46%) | 6 (0.98%) |
| *S.* *Typhimurium* | 20 (9.76%) | 8 (3.9%) | 30 (14.63%) | 58 (9.43%) |
| Un-typable *Salmonella* | 0 | 3 (1.46%) | 0 | 3 (0.49%) |
| Total | 22 (10.74%) | 12 (5.85%) | 33 (16.09%) | 67 (10.9%) |
The *sopE* gene was amplified in 62 (92.5%) *Salmonella* isolates, indicating zoonotic and public health significance of isolated strains (Fig. 1b).

**Phenotypic and genotypic antimicrobial resistance**

All *Salmonella* serovars isolated in this study were sensitive to gentamicin. Fifty-two (89.7%) *S. enterica* serovars Typhimurium isolates were susceptible to streptomycin, while six isolates (10.3%) were intermediate. Fifty-five (94.8%) *S. enterica* serovars Typhimurium isolates were sensitive to trimethoprim/sulphamethoxazole, while 3 (5.2%) isolates were resistant. All non-typhoidal *Salmonella* strains were sensitive to trimethoprim/sulphamethoxazole and streptomycin. All *S. enterica* serovars Typhimurium and non-typhoidal *Salmonella* strains isolated were resistant to ampicillin, chloramphenicol, and tetracycline. However, all *S. enterica* serovars Enteritidis isolates were sensitive to all tested antimicrobial agents (Table 3).

Ten of 18 screened resistance associated genes were amplified in the *S. enterica* serovars Typhimurium isolates (Table 6). All isolates harboured cat1 associated with chloramphenicol resistance. While, 98.3, 96.6 and 94.8% of *S. enterica* serovars Typhimurium isolates were possessed *sul3* (sulphamethoxazole resistance), *tetC* (tetracycline resistance) and *aadA2* (streptomyacin resistance), respectively. Moreover, 65.5, 84.5, 56.9, 62.1 and 79.3% of *S. enterica* serovars Typhimurium were harboured ampicillin (*Bla*TEM), tetracycline (*tetA*), sulphamethoxazole (*sul1*), streptomyacin (*strA*) and chloramphenicol (*floR*) resistance associated genes, respectively (Table 6; Fig. 1c). Eight of the 18 screened resistance genes were amplified in the *S. enterica* serovars Enteritidis isolates; these were tetracycline resistance *tetA* (50%), and *tetC* (33.3%); sulphamethoxazole resistance *sul1* (16.7%); streptomyacin resistance *aadA1* (50%) and *strA* (33.3%); chloramphenicol resistance *cat1* (33.3%) and *floR* (16.7%). The un-typable *Salmonella* isolates were only positive for two genes; 100% for *tetA* (tetracycline resistance) and 33.3% for *cat1* (chloramphenicol resistance). Only four of the screened genes *tetB* (tetracycline resistance), *sul2* (sulphamethoxazole resistance) and *aadB* and *aacC* (gentamycin resistance) were not amplified in all screened isolates.

The amplicons of *intI* integrons were identified with size of 2 kbp in three *S. enterica* serovar Typhimurium strains (Table 6). The sequencing data indicated that these integrons contain *dfrA12-orfF-aadA2*.

**Discussion**

*Salmonella enterica* serovars Typhimurium is known to be able to cause high rates of mortality in early ages of broiler chickens [20]. The InvA protein is a putative inner membrane component of the *Salmonella* pathogenicity island 1 (SPI-1) type 3 secretion system (TTSS) [40]. It has been reported that *invA* is present only in *Salmo­nella* species and therefore is used as a golden marker in genetic diagnosis of *Salmonella* species [35]. In this study 17 broiler flocks were positive and 67 *Salmonella* strains were isolated. The overall rate of incidence of *Salmonella* was (41%) in the screened broiler chicken flocks which was considerably higher than the infection rates that reported in the UK (10.7%), Lithuania (29%), Italy (20%), Netherlands (11%) and Germany (27.5% in chickens and 33.3% in turkeys) [41–45]. The higher infection rate found in this study compared to that of Abd El-Ghany et al. [18] shows the increased sensitivity of the use of the *invA* gene marker for diagnosis compared to isolation through culture on specific agar.
Although the *S. enterica* serovars Enteritidis is closely related to other pathogenic *S. enterica* serovars, this serovar has some characteristics that appear to discriminate it from others serovars. As *S. enterica* is known to contain the *Salmonella* difference fragments (*sdf*), a group of chromosomally encoded genes, which to date are of unknown function. *sdf* was reported by Agron et al. [33] to be found only in *S. enterica* serovars Enteritidis strains and considered to be a strong marker for this *Salmonella* serovar. *sdf* was used as a target for phylotyping of the serotype-specific *S. enterica* serovars Enteritidis. In this study, *sdf* was present in 6 of the 67 invA positive isolates. These *sdf* positive strains were isolated from three of 41 screened farms. Our findings indicated that the *sdf*II gene marker was associated with the *sdf* positive strains. Interestingly, *sdf*II was detected in all 67 strains isolated in this study of different serovars. This indicates that there is some degree of diversity within serovars that can be detected by the primers which in agreement with previous observation [33, 35].

Bacteria use the fimbriae in the adherence to one another and to the host cells and in some instance to inanimate objects. Sef14 fimbriae have been shown to consist of a repeating major subunit of the 14.3 kDa protein SefA, encoded for by the *sefA* gene and are required for macrophage uptake and survival in intraperitoneal infections [46]. The *sefA* gene is known to be specific to the poultry-associated *Salmonella* serotypes Gallinarum and Enteritidis. It is also detected in serotype Dublin, although this serotype is more commonly associated with cattle [47]. In the present study, *sefA* was detected in all isolates of *S. enterica* serovar Enteritidis. In the current study same six *S. enterica* serovar Enteritidis isolates positive for *sdf* and *sdf*III markers were also positive for the *sefA* gene; these six strains came from three *Salmonella* infected chicken farms that were isolated from 41 screened farms.

In this study the *S. enteric* serovar Typhimurium serotype specific virulent flagella genes *Typ* and *fliC* were used for phenotyping as recommended previously [34, 35]. Flagella are multi-functional organelles that play different roles in the biology of bacteria. The motility functions of flagella help bacteria to acquire nutrients, move away from toxic materials, and move to specific colonization sites within hosts and to disperse in the environment during the course of transmission between hosts [48]. The flagellum also primes the host immune system through activation of TLR5 receptors [49].

*sop*E is a translocated effector protein that plays an important part in the systemic phase of salmonellosis infection; *sop*E has been shown to be involved in actin cytoskeletal rearrangements and membrane ruffling [36]. As a virulence factor that is frequently transferred by bacteriophages, the *sop*E gene is encoded in the SPI-1, and has been identified in isolates involved in major

| Table 6 Prevalence of antibiotic resistant associated genes detected in *S. enterica* serovars |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Resistance markers              | Prevalence of resistance genes in screened *Salmonella* isolates | Antimicrobial agent                                    |
|                                 | *S. Typhimurium* n = 58 | *S. Enteritidis* n = 6 | Non-typable n = 3 |
| BlaTEM                          | 38               | 0               | 0               | Ampicillin       |
| tetA                            | 49               | 3               | 3               | Tetracycline     |
| tetB                            | 0                | 0               | 0               | Tetracycline     |
| tetC                            | 56               | 2               | 0               | Tetracycline     |
| sul1                            | 33               | 1               | 0               | Sulphamethoxazole |
| sul2                            | 0                | 0               | 0               | Sulphamethoxazole |
| sul3                            | 57               | 0               | 0               | Sulphamethoxazole |
| aadA1                           | 24               | 3               | 0               | Streptomycin     |
| aadA2                           | 55               | 2               | 0               | Streptomycin     |
| strA                            | 36               | 2               | 0               | Streptomycin     |
| strB                            | 0                | 0               | 0               | Streptomycin     |
| aadB                            | 0                | 0               | 0               | Gentamycin       |
| aacC                            | 0                | 0               | 0               | Gentamycin       |
| cat1                            | 58               | 2               | 1               | Chloramphenicol  |
| cat2                            | 0                | 0               | 0               | Chloramphenicol  |
| floR                            | 46               | 1               | 0               | Chloramphenicol  |
| dfrI                            | 0                | 0               | 0               | Trimethoprim     |
| intI                            | 3                | 0               | 0               | Class 1 integron |
epidemics; sopE has therefore been identified as playing a key role in the emergence of epidemic strains [50].

In study conducted by Rahman et al. [51] indicated that sopE gene appeared to be distributed and conserved among only a few serovars of Salmonella (Enteritidis, Gallinarum and Virchow) irrespective of their source of isolation and the presence of sopE gene in Salmonella provides an important pathogenic means to invade epithelial cells [51]. Moreover Prager et al. [52] identified sopE in all isolates of S. enterica serovar Enteritidis and carrying of sopE in S. Enteritidis may contribute to their epidemiological success [52]. In another study, all Salmonella Enteritidis isolated from human, chicken, and egg houses tested positive for sopE which may indicate its importance in pathogenesis [53].

In this study 92.5% of the Salmonella stains were harboured sopE gene that suggested that these strains could have zoonotic potential as previously reported [50–53]. There was a significant difference in mortality rate between Salmonella infected and non-infected flocks at the 1st week of life, however, there was no difference in mortality between Salmonella infected and non-infected flocks at the 5th week of age; a similar finding was previously reported [20, 23]. According to previous study, the results suggest that the age at infection plays an important role in the persistence of S. enteritidis infection in chickens and may cause severe infections and high mortality in young chickens [54]. Unfortunately, in this study we did not investigate other possible causes of mortality which may act as co-factors.

There was a higher rate of Salmonella isolation from the sampled internal organs, in the gall bladder and liver samples compared to the intestine samples indicating the ability of Salmonella to cause systemic infection which in agreement with previous study [55].

In this study all S. enterica serovars Typhimurium and non-typable Salmonella strains isolated in this study were resistance to ampicillin, chloramphenicol, and tetracycline.

All isolates were sensitive to gentamicin. The susceptibility of S. enterica serovars Typhimurium to streptomycin and trimethoprim/sulphamethoxazole were 89.7 and 94.8%, respectively. In addition 10.3% had intermediate sensitivity to streptomycin while all non-typable Salmonella strains were sensitive to trimethoprim/sulphamethoxazole and streptomycin. However, all S. enterica serovars Enteritidis isolates were sensitive to all tested antimicrobial agents. In contrast Salmonella isolates from South African chickens exhibited resistance to tetracycline (93%), trimethoprim–sulfamethoxazole (84%), gentamicin (48%), ampicillin (47%), chloramphenicol (31%), and streptomycin (12%) [56].

Most of the phenotypically antibiotic resistance isolates were positive for some of the antibiotic resistance marker genes for each of the screened antibiotics.

The blaTEM gene was detected only in 65.5% of ampicillin resistant S. enterica serovar Typhimurium isolates. All of the isolated strains of S. enterica serovar Enteritidis were susceptible to ampicillin and were negative for blatEM. The three non-typable Salmonella strains showed phenotypical resistance to ampicillin without harbouring the blatEM gene, indicating that these strains possess another ampicillin resistance mechanism.

In this study, tetracycline resistance in the S. enterica serovar Typhimurium isolates correlated with the presence of tetC (96.6%), and tetA (84.5%). All tested strains were negative for tetB codon. tetA codon was also found in all of the non-typable Salmonella strains. All S. enterica serovar Enteritidis were sensitive to tetracycline. However, two of the strains were harbour both tetC and tetA determinants and one strain was harbouring tetA determinant. These cassettes were silent in this serotype strain in vitro, however, they may turn on in vivo.

All of the S. enterica serovar Enteritidis and non-typable Salmonella strains were sensitive to trimethoprim–sulphamethoxazole and all these strains were negative for the dfrA1 codon and did not possess integron that contains dfrA12 trimethoprim resistance cassette. Although one strain of S. enterica serovar Enteritidis carried sul1 gene but not possessed any trimethoprim genes. All S. enterica serovar Typhimurium isolates were sensitive to trimethoprim–sulphamethoxazole despite 98% of isolates being positive for sul3 and 57% being positive for sul1, both of which confer sulphamethoxazole resistance. Interestingly, the three S. Typhimurium strains that were resistant to trimethoprim–sulphamethoxazole were found to harbour the 2 kpb integron that contains the dfrA12 trimethoprim resistant marker.

All of the Salmonella isolates were sensitive to the streptomycin despite the presence of streptomycin modifying enzyme gene cassettes (aadA1, aadA2 and strA). This suggests that some of the antimicrobial resistance genes are silent in bacteria in vitro; however, these silent genes can spread to other bacteria or turn on in vivo, especially under antimicrobial pressure which in agreement with previous reports [31, 57].

The catI gene, encoding chloramphenicol acetyltransferase, was identified in all resistant strains. In S. enterica serovar Typhimurium, the cat2 gene was not found in any of the tested strains. The floR gene which also confers chloramphenicol resistance was detected in 80% of S. enterica serovar Typhimurium strains. One of the non-typable Salmonella strains carried the cat1 gene but the other two isolates did not possess cat1, cat2 or floR gene indicating that these two strains
harbour another chloramphenicol resistance mechanism. Of six *S. enterica* serovar Enteritidis strains, one strain possessed both, *cat*1 and, *flo*R, and one strain harboured only the *cat*1 gene, however, phenotypically they were all sensitive to chloramphenicol indicating that this resistant cassette is silent in vitro in this *Salmonella* serovar.

Multiple drug resistance genes have been found to be clustered on individual mobile elements, which mean that multi-resistance can be readily transferred and increase the multi-drug resistant bacterial population as reported previously [58].

Gene cassettes are a major source of the resistance genes found in clinical, commensal, and environmental isolates of bacteria that are resistant to antibiotics [59, 60]. Most commonly, they are found in association with class 1 or class 2 integrons [61].

In this study, a class one integron in three *S. enterica* serovar Typhimurium strains with size of 2 kb was identified. The sequencing data indicated that these integrons contained *dfr*A12-*orfV*-aad*A2*. The presence of the *dfr*A12-*orfV*-aad*A2* open reading frames revealed the basis for the streptomycin and trimethoprim/sulphamethoxazole resistance seen in these strains. It also provides an indication of the mapping distribution of antibiotic resistance alleles in this region of the *Salmonella* genome/chromosome.

In this study the higher infection rate in the investigated flocks may regarding to low biosecurity and hygienic measures inside these farms and easily to spread the infection through different reservoirs and the workers in the farms.

The screening of antimicrobial resistance in the *Salmonella* strains isolated in this study provides evidence for confirming the mechanisms employed by *S. enterica serovars* to resist cluster antibiotics used for treatment of broiler chicken in Egypt. Future work, in this regard, should address if allele distribution in chicken and human *Salmonella* isolates from the same region share the same resistance mechanisms in order to highlight potential horizontal gene transfer by this zoonotic organism and the origin of antimicrobial resistance in human isolates. Finally, we believe that this is the first report of the presence of a class one integron in the *S. enterica* serovar Typhimurium serotype together with the verification of the location of some resistance genes that are within or associated with the class one integron.

**Authors’ contributions**

HES, AT, ME, TG, and AAE participated in the conception and design of the study. HES, and AT were performed farm and laboratory work. HES, AT, ME, TG, YK, AAE, HN, HE and HVH analysed the data and wrote the manuscript. FE participated in manuscript revision. All authors contributed to the analysis and supported the manuscript discussion. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

The data supporting the findings of this study are contained within the manuscript.

**Ethics approval and consent to participate**

This study was carried out in strict accordance with the recommendations of the Egyptian Network of Research Ethics Committees (ENREC) which complies with the international laws and regulation regarding ethical considerations in research.

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