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# IDENTIFICATION OF VIRULENCE GENES, β-LACTAMS AND QUINOLONES RESISTANCE-ASSOCIATED GENES AND INTEGRONS IN *SALMONELLA* ISOLATED FROM RETAIL CHICKEN MEAT AND GIBLETS IN EGYPT

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**ABSTRACT**

The study investigated the prevalence of virulent and antibiotic resistant *Salmonella* (S) in retail chicken meat (CM) and pooled giblets (PG) in Egypt. Seventy-two and 64 % of examined CM and PG samples harbored *Salmonellae*, respectively. When *S.* Infantis was the most predominant serovar, followed by S. Kentucky, and then S. Virchow. The antibiotic resistance experiment showed a high rate of multiple drug resistant (MDR) *Salmonellae*. Remarkably, 64.7% of the isolates were resistant to ciprofloxacin (the drug of choice for *Salmonella* infection). Molecularly, a noteworthy correlation between the MDR and the presence of integrons class 1 and 3. When, integrons 1 and 3 were detected in 100 and 92 % of identified MDR isolates, respectively, while class 2 integrons were not found. Surprisingly, 53.8 and 100% of the molecularly identified isolates were resistant to ciprofloxacin and nalidixic acid, despite the absence of plasmid-mediated quinolone resistance (PMQR)-associated genes (*qnrA, qnrB* and *qnrS, aac(6')-Ib-cr* and *qepA*) from all MDR isolates. This indicates that other genetic factors could be incriminated in this pattern. Beta-lactam resistance was explored by the existence of $bla_{TEM}$, $bla_{CTX}$, and $bla_{CMY-2}$ genes in most MDR isolates. Additionally, 4 virulence genes were detected in almost all serovars. Concerning virulence genes, *invA, hilA, sopB*, and *stn* were detected in 100, 100, 100 and 94.7 % of MDR serovars, respectively. While, *spvC* was detected in only 7.6 %, and *pef* was absent. The high prevalence of MDR virulent *Salmonella* strains in retail chicken meat and giblets in Egypt should be faced with a great worry, as consumers could be under a great public health risk.

**Keywords:** *Salmonella*, Foodborne pathogens, zoonoses, antibiotic resistance, integrons, virulence genes

## INTRODUCTION

The genus *Salmonella* (S), an *Enterobacteriaceae* member, is facultative aerobic intracellular bacteria that capable of causing varieties of illnesses in a wide range of hosts. *Salmonella* has been recognized as the leading cause of foodborne diseases in human, leading to 1.3 billion cases of gastroenteritis, 16 million cases of typhoid infection, and about 3 million deaths around the globe every year **(Bhunia, 2007)**. A variety of food items have been incriminated in human *Salmonellosis* outbreaks, especially those derived from animals such as beef, pork, poultry, and eggs. Nevertheless, poultry and poultry products are the most associated foods with *Salmonella* outbreaks in humans **(EFSA and ECDC, 2013; Antunes et al., 2016)**.

Most of *Salmonella* food poisoning outbreaks are caused by *Salmonella enterica* subspecies *enterica*, though, over 2,500 *Salmonella* serovars have been identified and new serovars are designated frequently **(Hassan et al., 2015)**. The pathogenicity of *Salmonella* is managed by several factors established by virulence genes that enable the pathogen to express its virulence in the host and eventually produce the characteristic symptoms of the disease, besides, antibiotic resistance-associated genes. The invasion gene *inv*A is unique for the genus *Salmonella*, so it represents a suitable DNA target in diagnostic approaches. *inv*A is situated in *Salmonella* pathogenicity islands coding to produce certain proteins, which are accountable for the invasion of the pathogen into the host cells **(Valdez et al., 2009)**. Plasmid-encoded fimbria (*pef*) locus assists the bacteria to adhere to the intestinal epithelial cells **(Friedrich et al., 1993)**, and *spv* is another plasmid-located virulence gene, which suppresses host innate immune system to bacterial infection (Yang et al., 2016). *Salmonella* outer proteins (*sop A-E*) encoded with *sop* gene are responsible for the pathogen penetration through cell membrane deformities and rearrangement of the host cell cytoskeletons **(Borges et al., 2013)**, besides *hilA*, which is considered a hyper invasive locus **(Lostroh et al., 2000)**. While, the virulence gene *stn* arbitrates the production of enterotoxins and was found to be linked to causing acute gastroenteritis in infected hosts **(Zou et al., 2012)**.

In addition to virulence, the emergence of antibiotic resistant strains of *Salmonella* has become a significant public health risk. It was found that the improper application of antibiotics in livestock production for preventive and therapeutic purposes, as well as growth promotion is a noteworthy factor in appearance of antibiotic resistant bacteria in animals and poultry. These resistant bacteria which is subsequently transferred to human through food chain is challenging the efforts of serving safe food for consumers **(Antunes et al., 2016)**. Factors related to the antibiotic resistance and virulence of *Salmonella* may be situated on chromosomes, plasmids, integrons and transposon. Integrons are genetic elements that play an important role in the dissemination of resistance genes between bacteria owing to the associated conjugative plasmids. There are two main groups of integrons: mobile integrons and chromosomal integrons **(Cambray et al., 2010)**. Based on the sequence of the encoded integrases, five different classes of mobile integrons have been identified. Even though, only classes 1, 2, and 3 have been reported in the spread of multidrug-resistance phenotypes, all 5 classes have been associated with antibiotic-resistance determinants **(Siriken et al., 2015)**. Furthermore, several non integron-related resistance genes have also been reported in *Salmonella*; such as quinolones resistance determinants (*qnrA, qnrB, qnrS, aac(6')-Ib-cr, qepA*) and β-lactams resistance-related genes ($bla_{TEM}$, $bla_{CTX}$, and $bla_{CMY-2}$) **(Robicsek et al., 2006; Wiesner et al., 2016)**. Accordingly, the identification of virulence genes, antibiotic resistance genes and integrons in *Salmonella* isolates from retail chicken became a very crucial approach for risk assessment of such pathogen in this food item.

Therefore, the present study was conducted to estimate the prevalence and antibiotic susceptibility/resistance of *Salmonella* species from retail chicken meat (CM) and pooled giblets (PG) in Beni-Suef governorate, Egypt, beside serological identification of the isolates. As well as, molecular identification of virulence genes, β-lactams and quinolones resistance-associated determinants and integrons (classes 1, 2, and 3) using Polymerase Chain Reaction (PCR).

## MATERIALS AND METHODS

### Sample collection

A total of 50 broiler chicken carcasses (around 40 days age at time of slaughtering) were randomly collected from retail poultry outlets in Beni-Suef governorate, Egypt during 2016 – 2017. All carcasses were purchased in fresh state directly after slaughtering in the retail market. Each carcass was represented by a specimen from muscle as chicken meat (CM) and another from gizzard, liver, and heart as pooled giblets (PG), with a total of 100 samples (50 CM and 50 PG). The collected samples were identified and wrapped separately in sterile polyethylene bags to be directly transferred without delay in an icebox to the laboratory for further preparation and examination.

### Isolation and identification of *Salmonella*

Isolation and morphological and biochemical identification of *Salmonella* spp. from CM and PG was done according to the standard protocol of **ISO 6579 (2002)**. Briefly, 25 g specimen was aseptically removed from each CM and PG of each carcass, and then homogenized with 225 mL of 0.1 % sterile buffered peptone water (Biolife; Italy). Afterwards, the homogenate was incubated at 36 ± 1 °C for 16-20 h. Then 0.1 and one mL of the pre-enrichment broth were inoculated into 10 mL of Rappaport-Vassiliadis (RV) broth (Biolife; Italy) and 10 mL of Müller-Kaufmann Tetrathionate (MKT) broth (Biolife; Italy), respectively. The enrichment broths were further incubated at 41.5 ± 0.5 °C (for RV) and 36 ± 1 °C (for MKT) during 18-24 h. A loopful from each broth after incubation was streaked onto each of *Salmonella-Shigella* (SS) and Xylose Lysine Desoxycholate (XLD) agar plates and incubated at 36 ± 1 °C for 18-24 h. Colorless colonies with black centers on SS and slightly transparent red colonies with black center on XLD agar plates were suspected as *Salmonella* and selected for further identification procedures. Suspected colonies of *Salmonella* were identified morphologically by Gram`s staining, and biochemically by oxidase, indole, methyl red, voges proskauer, citrate utilization, triple sugar iron (TSI), and urease tests. All morphologically and biochemically confirmed *Salmonella* isolates were consequently identified by serology based on somatic (O) and flagellar (H) antigens by slide agglutination using commercial antisera (SISIN, Berlin) following the Kauffman-White scheme **(Popoff et al., 2004)**. See the schematic protocol in Figure 1.

### Antibiotic sensitivity/resistance testing

All serologically identified *Salmonella enterica* subspecies *enterica* serovars were tested for their antibiotic susceptibility pattern by disc diffusion technique according to the Clinical and Laboratory Standards Institute, CLSI (2018). Commercial discs of antibiotic (Oxoid, UK) soaked with cefotaxime (30 µg), ampicillin (10 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), amikacin (30 µg), piperacillin-tazobactam (100/10 µg), amoxicillin-clavulanic acid (20/10 µg), nalidixic acid (30 µg), aztreonam (30 µg), and tetracycline (30 µg) (Oxoid, UK) were used. Multidrug resistant isolate (MDR) is defined as that isolate resist three or more antibiotics belonging to different antibiotic categories.

### Molecular detection of *Salmonella* virulence genes and integrons using PCR

MDR *Salmonella* serovars were molecularly identified for the presence of 6 virulence genes (*hilA, stn, pef, invA, sopB and spvC*), 8 antibiotic resistance determinants, out of them, 5 are linked to quinolones resistance (*qnrA, qnrB, qnrS, aac(6`)-Ib-cr and qepA*) and the rest three antibiotic resistance genes are related to beta-lactams resistance ($bla_{TEM}$, $bla_{CMY-2}$ and $bla_{CTX}$), in addition to identification of integrons classes 1,2 and 3. Genomic DNA was extracted from overnight bacterial cultures using Qiagen DNA extraction kit (Qiagen, Germany) according to the manufacturer's instructions. Specific primers obtained from Metabion (Germany) for each target gene were used for DNA amplification using uniplex PCR. The sequences of primers and sizes of amplified segments are listed in Table 1. Primers were utilized in a 25 µL reaction tube containing 12.5 µL of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µL of each primer of 20 pmol concentrations, 6 µL of DNA template, and 4.5 µL of nuclease free water. The reactions were performed in an Applied Biosystem 2720 thermal cycler. Briefly, initial denaturation step was done at 94 °C for 5 min, then followed by 35 cycles of 94 °C for 45 sec, afterwards, 40 sec of annealing was applied according to the temperatures showed in Table 1. Subsequently, an extension step at 72 °C for 45 sec and a final extension step at 72 °C for 10 min were conducted. The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. 20 µL of the PCR products were loaded in each gel slot. The fragment sizes were determined using Gelpilot 100 bp and 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) and Gene ruler 100 bp ladder (Thermo Scientific, Germany). Afterwards, the gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

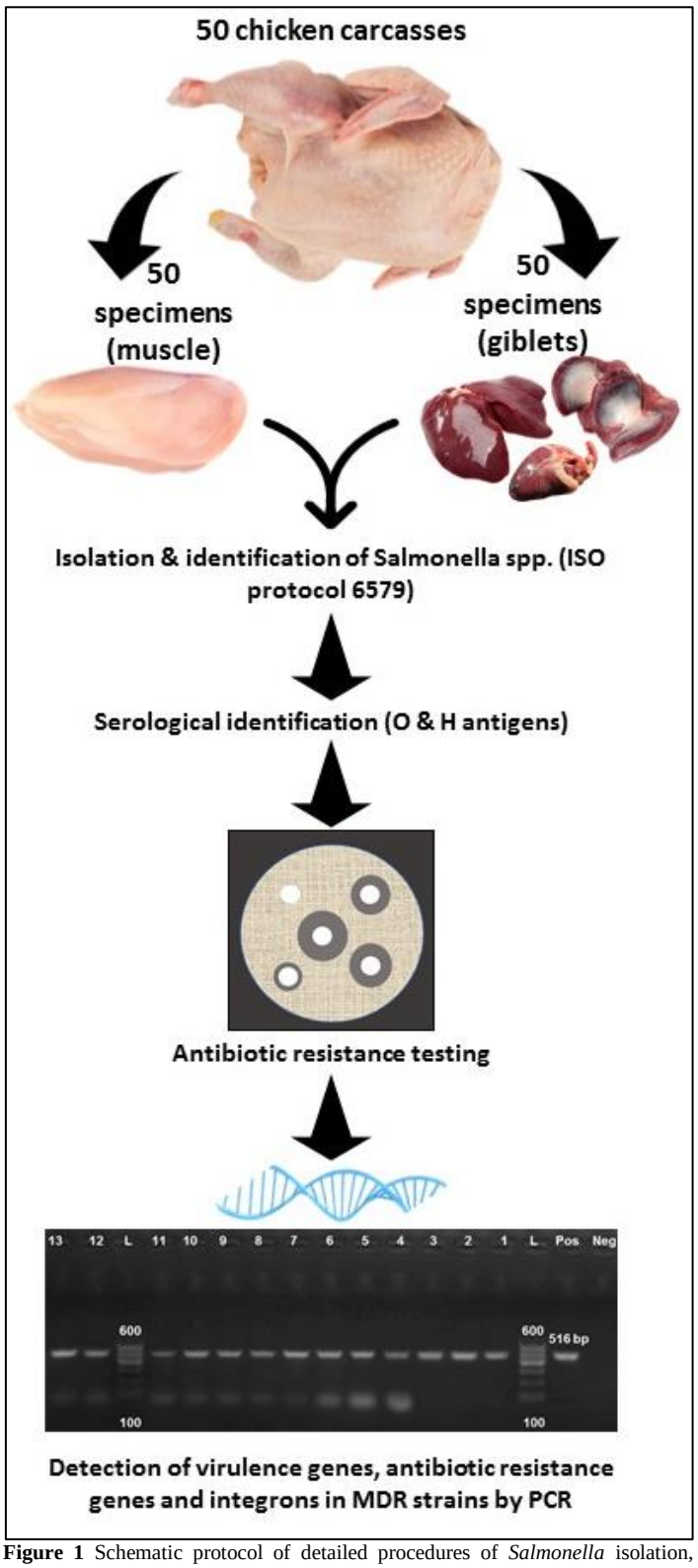


**Figure 1** Schematic protocol of detailed procedures of *Salmonella* isolation, identification and antibiotic resistance testing during the study. MDR (multidrug resistant).

**Table 1** Primers sequences of target genes, amplicon sizes and annealing temperatures

| Target gene | Primers sequences | Amplified segment (bp) | Resistance /virulence * | Annealing temperature (°C) | Reference |
|---|---|---|---|---|---|
| *qnrA* | ATTTCTCACGCCAGGATTTG | 516 | (R) Quinolones | 53 | Robicsek et al. (2006) |
| | GATCGGCAAAGGTTAGGTCA | | | | |
| *qnrB* | GATCGTGAAAGCCAGAAAGG | 469 | (R) Quinolones | | |
| | ACGATGCCTGGTAGTTGTCC | | | | |
| *qnrS* | ACGACATTCGTCAACTGCAA | 417 | (R) Quinolones | | |
| | TAAATTGGCACCCTGTAGGC | | | | |
| *aac(6')-Ib-cr* | CCCGCTTTCTCGTAGCA | 113 | (R) Quinolones | 53 | Lunn et al. (2010) |
| | TTAGGCATCACTGCGTCTTC | | | | |
| *qepA* | CGTGTTGCTGGAGTTCTTC | 403 | (R) Quinolones | 50 | Cattoir et al. (2008) |
| | CTGCAGGTACTGCGTCATG | | | | |
| $bla_{TEM}$ | ATCAGCAATAAACCAGC | 516 | (R) Beta-Lactams | 54 | Colom et al. (2003) |
| | CCCCGAAGAACGTTTTC | | | | |
| $bla_{CMY-2}$ | TGGCCAGAACTGACAGGCAAA | 462 | (R) Beta-Lactams | 54 | Pérez-Pérez and Hanson (2002) |
| | TTTCTCCTGAACGTGGCTGGC | | | | |
| $bla_{CTX}$ | ATGTGCAGYACCAGTAARGTKATGGC | 593 | (R) Beta-Lactams | 60 | Archambault et al. (2006) |
| | TGGGTRAARTARGTSACCAGAAYCAGCGG | | | | |
| *Int1* | CCTCCCGCACGATGATC | 280 | (R) Disseminate the resistance | 50 | Kashif et al. (2013) |
| | TCCACGCATCGTCAGGC | | | | |
| *Int2* | TTATTGCTGGGATTAGGC | 250 | (R) Disseminate the resistance | | |
| | ACGGCTACCCTCTGTTATC | | | | |
| *Int3* | AGTGGGTGGCGAATGAGTG | 484 | (R) Disseminate the resistance | | |
| | TGTTCTTGTATCGGCAGGTG | | | | |
| *hilA* | CATGGCTGGTCAGTTGGAG | 150 | (V) | 59 | Yang et al. (2014) |
| | CGTAATTCATCGCCTAAACG | | | | |
| *Stn* | TTGTGTCGCTATCACTGGCAACC | 617 | (V) | 59 | Murugkar et al. (2003) |
| | ATTCGTAACCCGCTCTCGTCC | | | | |
| *Pef* | TGTTTCCGGGCTTGTGCT | 700 | (V) | 54 | |
| | CAGGGCATTTGCTGATTCTTCC | | | | |
| *invA* | GTGAAATTATCGCCACGTTCGGGCAA | 284 | (V) | 54 | Oliveira et al. (2003) |
| | TCATCGCACCGTCAAAGGAACC | | | | |
| *sopB* | TCAGAAGRCGTCTAACCACTC | 517 | (V) | 58 | Huehn et al. (2010) |
| | TACCGTCCTCATGCACACTC | | | | |
| *spvC* | ACCAGAGACATTGCCTTCC | 467 | (V) | 60 | |
| | TTCTGATCGCCGCTATTCG | | | | |

* The role of the target gene; either antibiotic resistance or virulence activity. (R) means that the target gene is responsible for antibiotic resistance, while (V) means that the target gene is responsible for specific virulence activity of the strain.

## RESULTS

### Prevalence and serotyping of *Salmonella* spp. in CM and PG

According to the results of morphological and biochemical identification of *Salmonella* isolates, it was found that out of 50 CM samples, 36 samples harbored *Salmonella* (72%), while out of 50 PG samples, 32 contained *Salmonella* (64%) (data not shown). The serotyping of 36 and 32 *Salmonella* isolates from CM and PG revealed that *S.* Infantis represented 52.8 and 50% in CM and PG, respectively. While *S.* Kentucky was identified with incidences of 36.1 and 25% of *Salmonella* isolates from CM and PG, respectively. Each of *S.* Ferruch and *S.* Kottbus represented 6.25% of PG isolates, conversely, it was failed to find them in CM. Although, *S.* Colindale represented 5.55% of CM *Salmonella* isolates, it was none in PG. On the other hand, *S.* Virchow represented 5.55 and 12.5% in CM and PG, respectively (Table 2).

**Table 2** Distribution of *Salmonella* serovars in examined chicken meat and pooled giblets

| **Serovars (antigenic formula)** | **Chicken meat (n=50)** | **Pooled giblets (n=50)** | **Total (n=100)** |
|---|---|---|---|
| | **No. (%*) (%**)** | **No. (%*) (%**)** | **No. (%*) (%**)** |
| *S.* Infantis (O: 6,7,14; $H_1$: r; $H_2$: 1,5) | 19 (38) (52.8) | 16 (32) (50) | 35 (35) (51.47) |
| *S.* Ferruch (O: 8; $H_1$: e, h; $H_2$: 1,5) | 0 (0) (0) | 2 (4) (6.25) | 2 (2) (2.94) |
| *S.* Kentucky (O: 8, 20; $H_1$: i; $H_2$: $Z_6$) | 13 (26) (36.1) | 8 (16) (25) | 21 (21) (30.88) |
| *S.* Kottbus (O: 6, 8; $H_1$: e, h; $H_2$: 1,5) | 0 (0) (0) | 2 (4) (6.25) | 2 (2) (2.94) |
| *S.* Virchow (O: 6,7,14; $H_1$: r; $H_2$: 1,2) | 2 (4) (5.55) | 4 (8) (12.5) | 6 (6) (8.82) |
| *S.* Colindale (O: 6,7; $H_1$: r; $H_2$: 1,7) | 2 (4) (5.55) | 0 (0) (0) | 2 (2) (2.94) |
| **Total *Salmonella* isolates, no. (%)** | **36 (72)** | **32 (64)** | **68 (68)** |

Where %* represents the percentage in relation to the number of examined samples, while %** represents the percentage in relation to the number of *Salmonella* isolates.

**Antibiotic resistance/susceptibility of *Salmonella* serovars**

The results illustrated in Table 3 show the antibiotic resistance/susceptibility of *Salmonella* serovars (n= 68) isolated from CM and PG. High rates of resistance were explored by the serotyped *Salmonella* isolates, it was evident that the highest rate of resistance was against nalidixic acid, when all isolates showed resistance against it (100%), followed by tetracycline (89.7%), cefotaxime (67.5%), and then 64.7% of the isolates were resistant to both ciprofloxacin and ampicillin, followed by ceftazidime (45.6%), and amikacin (35.3%) comes after, then amoxicillin-clavulanic acid (29.4%), while the lowest resistance was against each of piperacillin-tazobactam and aztreonam (23.5%). All isolates of *S.* Virchow (n=6) were resistant to all antibiotics investigated during the study expect piperacillin-tazobactam and aztreonam. Likewise, the two isolates of *S.* Kottbus (100%) isolated from PG were resistant to all tested antibiotics. Additionally, high levels of resistance were found in *S.* Kentucky, followed by *S.* Infantis and then *S.* Ferruch and *S.* Colindale.

Interestingly, 64.7% (44 out of 68) of the isolates were resistant to ciprofloxacin, which is considered the drug of choice against *Salmonella* spp. in animals and humans, whereas, the other 24 isolates (35.3%) showed intermediate resistance, thus, none of the isolates exhibited any sensitivity to ciprofloxacin. Therefore, we report in the present study the emergence of ciprofloxacin-resistant isolates of *S.* Kentucky (100%), *S.* Virchow (100%), *S.* Kottbus (100%), and *S.* Infantis (42.8%) from CM and PG in Egypt (Table 3).

**Table 3** Antibiotic resistance of isolated *Salmonella* serovars from chicken meat and pooled giblets.

| Origin/serovars (n) | Antibiotics<br>Number of resistant serovars (resistant %) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **AK** | **CIP** | **AMP** | **AMC** | **TZP** | **CTX** | **CAZ** | **ATM** | **NA** | **TE** |
| **Chicken meat (36)** | **11 (30.6)** | **24 (66.7)** | **23 (63.9)** | **10 (27.8)** | **9 (25)** | **29 (80.6)** | **17 (47.2)** | **8 (22.2)** | **36 (100)** | **33 (91.7)** |
| *S.* Infantis (19) | 5 (26.3) | 9 (47.4) | 6 (31.6) | 0 (0) | 3 (15.8) | 15 (78.9) | 3 (15.8) | 2 (10.5) | 19 (100) | 18 (94.7) |
| *S.* Kentucky (13) | 4 (30.8) | 13 (100) | 13 (100) | 8 (61.5) | 6 (46.15) | 12 (92.3) | 12 (92.3) | 6 (46.2) | 13 (100) | 13 (100) |
| *S.* Virchow (2) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 0 (0) | 2 (100) | 2 (100) | 0 (0) | 2 (100) | 2 (100) |
| *S.* Colindale (2) | 0 (0) | 0 (0) | 2 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 2 (100) | 0 (0) |
| **Pooled giblets (32)** | **13 (46.6)** | **20 (62.5)** | **21 (65.6)** | **10 (31.3)** | **7 (21.9)** | **23 (71.9)** | **14 (43.8)** | **8 (25)** | **32 (100)** | **28 (87.5)** |
| *S.* Infantis (16) | 4 (25) | 6 (37.5) | 5 (31.3) | 0 (0) | 2 (12.5) | 11 (68.8) | 2 (12.5) | 2 (12.5) | 16 (100) | 14 (87.5) |
| *S.* Ferruch (2) | 0 (0) | 0 (0) | 2 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 2 (100) | 0 (0) |
| *S.* Kentucky (8) | 3 (37.5) | 8 (100) | 8 (100) | 4 (50) | 3 (37.5) | 6 (75) | 6 (75) | 4 (50) | 8 (100) | 8 (100) |
| *S.* Kottbus (2) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) |
| *S.* Virchow (4) | 4 (100) | 4 (100) | 4 (100) | 4 (100) | 0 (0) | 4 (100) | 4 (100) | 0 (0) | 4 (100) | 4 (100) |
| Total sensitive (%) | 36 (52.9) | 0 (0) | 13 (19.1) | 32 (47.1) | 24 (35.3) | 14 (20.6) | 27 (39.7) | 39 (57.3) | 0 (0) | 7 (10.3) |
| Total intermediate (%) | 8 (11.8) | 24 (35.3) | 11 (16.2) | 16 (23.5) | 28 (41.2) | 2 (2.9) | 10 (14.7) | 13 (19.2) | 0 (0) | 0 (0) |
| Total resistant (%) | 24 (35.3) | 44 (64.7) | 44 (64.7) | 20 (29.4) | 16 (23.5) | 52 (67.5) | 31 (45.6) | 16 (23.5) | 68 (100) | 61 (89.7) |

AK (amikacin 30 µg), CIP (ciprofloxacin 5 µg), AMP (ampicillin 10 µg), AMC (amoxicillin-clavulanic acid 20/10 µg), TZP (piperacillin-tazobactam 100/10 µg), CTX (cefotaxime 30 µg), CAZ (ceftazidime 30 µg), ATM (aztreonam 30 µg), NA (nalidixic acid 30 µg), and TE (tetracycline 30 µg).

**Integron profile and antibiotic resistance genes**

The data illustrated in Table 4 clarify the antibiotic resistance pattern, integron profile (classes 1, 2 & 3) and antibiotic resistance-associated genes in 13 isolates which were selected from MDR *Salmonella* serovars isolated from CM and PG. The selected 13 isolates showed variable degrees of MDR which ranged from 0.2 (resistant to 2/10 antibiotics) to one (resistant to 10/10 antibiotics). Integron class 1 was detected in 100% of the isolates, while integron class 3 existed in 92.3% of the isolates. Interestingly, the only isolate that had not integron 3 showed the lowest MDR pattern (0.2). On the contrary, integron class 2 was absent in all isolates (0%).

Regarding plasmid-mediated quinolone resistance (PMQR) genes, it was surprising that the three most significant *qnr* genes (*qnrA, qnrB and qnrS*) were absent in all molecularly identified isolates, and *aac(6')-Ib-cr* and *qepA* either. However, 53.8% and 100% of the isolates were found resistant to ciprofloxacin and nalidixic acid, respectively (Table 4).

Concerning β-lactams resistance-related genes, $bla_{TEM}$, $bla_{CMY-2}$ and $bla_{CTX}$ were found in 100%, 30.7% and 53.8% of the isolates, respectively (Table 4).

**Table 4** Resistance pattern, integron classes (1, 2 and 3) and antibiotic resistance genes among MDR *Salmonella* serovars isolated from chicken meat (CM) and pooled giblets (PG).

| No. | Serovar (Origin) | Resistance pattern | MDR ratio | Integron profile | | | Antibiotic resistance genes | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | **Int1** | **Int2** | **Int3** | **PMQR genes** | **$bla_{TEM}$** | **$bla_{CMY-2}$** | **$bla_{CTX}$** |
| 1 | Kentucky (CM) | AK, CIP, AMP, AMC, TZP, CTX, CAZ, ATM, NA, TE | 1 | + | - | + | - | + | + | - |
| 2 | Kentucky (PG) | CIP, AMP, CTX, CAZ, NA, TE | 0.6 | + | - | + | - | + | + | - |
| 3 | Kentucky (CM) | CIP, AMP, AMC, TZP, CTX, CAZ, NA, TE | 0.8 | + | - | + | - | + | + | - |
| 4 | Infantis (CM) | CTX, NA, TE | 0.3 | + | - | + | - | + | + | - |
| 5 | Virchow (CM) | AK, CIP, AMP, AMC, CTX, CAZ, NA, TE | 0.8 | + | - | + | - | + | - | + |
| 6 | Kentucky (CM) | CIP, AMP, NA, TE | 0.4 | + | - | + | - | + | - | + |
| 7 | Infantis (PG) | AK, NA, TE, CTX | 0.4 | + | - | + | - | + | - | + |
| 8 | Infantis (CM) | NA, TE, CTX | 0.3 | + | - | + | - | + | - | + |
| 9 | Infantis (CM) | CIP, AMP, TZP, CTX, CAZ, ATM, NA, TE | 0.8 | + | - | + | - | + | - | - |
| 10 | Infantis (CM) | CIP, CTX, NA, TE | 0.4 | + | - | + | - | + | - | + |
| 11 | Infantis (PG) | AK, AMP, NA, TE | 0.4 | + | - | + | - | + | - | + |
| 12 | Infantis (CM) | AK, AMP, CTX, NA, TE | 0.5 | + | - | + | - | + | - | + |
| 13 | Infantis (PG) | NA, TE | 0.2 | + | - | - | - | + | - | - |

AK (amikacin 30 µg), CIP (ciprofloxacin 5 µg), AMP (ampicillin 10 µg), AMC (amoxicillin-clavulanic acid 20/10 µg), TZP (piperacillin-tazobactam 100/10 µg), CTX (cefotaxime 30 µg), CAZ (ceftazidime 30 µg), ATM (aztreonam 30 µg), NA (nalidixic acid 30 µg), and TE (tetracycline 30 µg). CM: chicken meat, PG: pooled giblets. MDR ratio (multiple drug resistance ratio), for instance, MDR 0.6 means that this strain was resistance to 6 out of 10 antibiotics tested (6/10=0.6). PMQR genes**,** plasmid-mediated quinolone resistance genes (*qnrA, qnrB, qnrS, aac(6')-Ib-cr* and *qepA).*

**Molecular identification of virulence-associated genes**

The results of molecular identification of virulence genes in 13 MDR isolates of *Salmonella* were illustrated in Table 5. The data showed that each of the invasion gene *inv*A, the hyper invasive locus *hilA* and *sopB* gene were detected in all isolates (100%). While, *stn* gene was found 94.7%. Conversely, the plasmid-encoded fimbria (*pef*) locus was not detected in any of the identified isolates, as well as the other plasmid-located virulence gene *spvC* was distinguished in only one out of 13 isolates.

**Table 5** Virulence genes among MDR *Salmonella* serovars isolated from chicken meat (CM) and pooled giblets (PG).

| No. | Serovar (Origin) | Virulence genes | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***invA*** | ***hilA*** | ***Stn*** | ***Pef*** | ***sopB*** | ***spvC*** |
| 1 | Kentucky (CM) | + | + | + | - | + | - |
| 2 | Kentucky (PG) | + | + | + | - | + | - |
| 3 | Kentucky (CM) | + | + | + | - | + | - |
| 4 | Infantis (CM) | + | + | + | - | + | - |
| 5 | Virchow (CM) | + | + | + | - | + | - |
| 6 | Kentucky (CM) | + | + | + | - | + | - |
| 7 | Infantis (PG) | + | + | + | - | + | + |
| 8 | Infantis (CM) | + | + | + | - | + | - |
| 9 | Infantis (CM) | + | + | + | - | + | - |
| 10 | Infantis (CM) | + | + | + | - | + | - |
| 11 | Infantis (PG) | + | + | + | - | + | - |
| 12 | Infantis (CM) | + | + | + | - | + | - |
| 13 | Infantis (PG) | + | + | - | - | + | - |

## DISCUSSION

The surprisingly higher rates of *Salmonella* spp. in the present study than previous reports **(Ammar et al., 2016; Gharieb et al., 2015)** could be attributed to the slaughter of live birds inside the poultry retail markets with absence of veterinary supervision and without even a minimum hygienic measure during different stages of carcass preparation, processing, and handling. Additionally, cross contamination from workers, equipment and utensils used during carcass preparation could be very important source of contamination **(Antunes et al., 2016)**.

Although, *S.* Typhimurium has not been detected in the present study, it was determined as a predominant serovar in poultry meat in some previous studies in other areas in Egypt such as **Gharieb et al. (2015)**. It could be attributed to the difference in the location of sample collection in the current study, as to the best of our knowledge, this is the first study to emphasize the prevalence of *Salmonella* serovars in retail poultry meat in Beni-Suef, Egypt. Alternatively, *S.* Infantis and *S.* Kentucky were reported as predominant *Salmonella* serovars in poultry meat in the present study. Incidentally, *S.* Infantis is capable of triggering septicemia and death in both children and adults **(Fonseca, 2006)**. As well as, the European Food Safety Authority and European Centre for Disease Prevention and Control **(EFSA and ECDC, 2015)** denoted that *S.* Infantis is the second most predominant serovar in broiler meat and the fourth most dominant one in human non-typhoidal salmonellosis in Europe. Regarding *S.* Kentucky, **Weill et al. (2006)** detected 197 *S.* Kentucky isolates in French travelers during 2000 through 2005, among them 17 ciprofloxacin-resistant strains were detected in 16 patients got the infection during or instantly after travel to Egypt (10 patients), Kenya, Tanzania, and Sudan. Consistent with the current result, **Weill et al. (2006)** reported that poultry is the main animal reservoir of *S.* Kentucky. Concerning to *S.* Virchow, the third highly reported serovar in this study, however it causes mild infection in humans, it produces severe illness in immunocompromised persons; therefore, the European Union has given it a priority for control of entry the food chain **(Arnold, 2010)**.

Resistance to ciprofloxacin has been reported exceptionally in non-typhoidal *Salmonella* isolates and only in *S.* Typhimurium, *S.* Choleraesuis, and *S.* Schwarzengrund **(Weill et al., 2006)**. Fascinatingly, we report in the present study the emergence of ciprofloxacin-resistant isolates of *S.* Kentucky (100%), *S.* Virchow (100%), *S.* Kottbus (100%), and *S.* Infantis (42.8%) from CM and PG in Egypt, which is considered the drug of choice against *Salmonella* infection in animals and humans **(CLSI, 2018)**. Conferring the last report of EFSA and **ECDC (2016)** on antibiotic resistance in zoonotic and indicator bacteria from humans, animals and food, *S.* Infantis significantly contributed to the overall numbers of MDR *Salmonellae* in Europe, when isolates from broilers showed resistance to third generation cephalosporins and great resistance to ciprofloxacin. Therefore, a high worry still leftovers for the public health significance of *S.* Infantis. It was proposed that the routine practice of using antimicrobials in food animal and poultry production is engaged in the emergence of antibiotic resistant bacterial strains and are subsequently transferred to human beings through the food chain **(Stürenburg and Mack, 2003; Threlfall, 2002)**.

As regard to integrons, in accordance with the present study, the previous report of **Siriken (2015)** concluded that class 1 is the most widely spread and clinically reported integron in MDR *Salmonellae*. Worthy mentioning that integron containing isolates are more antibiotic resistant than those lacking integrons **(Fluit AC, Schmitz, 2004)**, alike this study. Quinolones are very significant antibiotic substances for overcoming bacterial infections in both animals and humans, thus quinolone resistance is considered a noteworthy public health risk. The high quinolones-resistance rate (ciprofloxacin and nalidixic acid) of *Salmonella* serovars reported in the present study, despite absence of quinolones resistance-related genes indicates that other determinants could be encountered. A similar scenario was reported by **Myšková and Íšková (2017)**. This could be explained considering the concepts of **Jacoby (2005)** who suggested that quinolone resistance is mostly attributed to mutation in chromosomes that modify the antibiotic target enzymes, DNA gyrase (*gyrA* and *gyrB*) and DNA topoisomerase IV (*parC* and *parE*) or trigger the efflux systems. Moreover, according to **Piddock (1999)**, a single point mutation in *gyrA* can arbitrate resistance to the nonfluorinated quinolone (100% of the identified isolates in the present study showed resistance to nalidixic acid) and reduce susceptibility to fluoroquinolones (53.8% of the identified isolates in the present study showed resistance to ciprofloxacin), while mutation in the *gyrB*, *parC* and *parE* is rare in *Salmonella* **(Eaves et al., 2004)**. In addition to quinolones resistance, the antibiotic resistance to expanded spectrum cephalosporines, which are strongly recommended for the treatment of salmonellosis, is determined mainly by the existence of extended spectrum β-lactamases (ESBL) and plasmid-mediated AmpC β-lactamases (PABL) genes of which $bla_{\text{TEM}}$, $bla_{\text{CTX}}$ and $bla_{\text{CMY-2}}$ are the most common **(Kang et al., 2013)**. This explains the high level of antibiotic resistance to that group in the present study, as 61.5, 23.0, 23.0, 76.9, 38.4, and 15.3% of the isolates were resistant to ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, ceftazidime and aztreonam from β-lactams group, respectively.

In addition to the antibiotic resistance, the existence of *invA*, *hilA*, *sopB* and *stn* virulence genes in almost all MDR *Salmonella* isolates indicates the high pathogenicity of these isolates to animal, poultry and humans. Since *invA* and *hilA* genes are responsible for penetration of *Salmonella* bacterium into the host cells **(Valdez et al., 2009; Lostroh et al., 2000)**. While *sopB*, in addition to its role in host cell membrane invasion, is also responsible for rearrangement of the host cell cytoskeletons **(Borges et al., 2013)**. As well as, *stn* gene is in charge of production of enterotoxins and is linked to triggering acute gastroenteritis in infected hosts **(Zou et al., 2012)**. Consequently, the retail chicken meat marketed in Egypt constitutes high public health risks to consumers. Thus, it should be faced with a high level of care and consideration by the legal authorities.

## CONCLUSION

In conclusion, the high rate of *Salmonellae* in the present study is attributed to the slaughter of live birds without veterinary supervision inside low hygienic poultry retail markets. *S.* Infantis and *S.* Kentucky are the top among the mostly isolated *Salmonella* enterica serovars from poultry meat in Egypt. The emergence of ciprofloxacin-resistant isolates of *S.* Kentucky, *S.* Virchow, *S.* Kottbus, and *S.* Infantis from CM and PG was reported for the first time in Egypt. Isolates with class 1 integron showed a high level of MDR. Class 2 was absent in all isolates. The determined high rate of quinolones-resistance of *Salmonella* serovars, despite absence of quinolones resistance-related genes, indicates that other genetic factors could be incriminated. The high level of resistance to β-lactams is attributed to the high incidence of β-lactams resistance-related genes ($bla_{\text{TEM}}$, $bla_{\text{CMY-2}}$ and $bla_{\text{CTX}}$). Eventually, the existence of high incidence of virulence genes within MDR *Salmonella* serovars gives us an alarm and should be faced with a great worry because consumers could be under a great public health risk.

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