Phenotypes, antibacterial-resistant profile, and virulence-associated genes of *Salmonella* serovars isolated from retail chicken meat in Egypt

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ABSTRACT

Aim: The present study was designed to investigate the occurrence and distribution of *Salmonella* serotypes in chicken meat samples, and to explore the susceptibility of the strains to antimicrobials, as well as their virulence-associated genes.

Materials and Methods: Two-hundred retail chicken meat samples from different shops, as well as 25 stool specimens from retail shop workers, were included in the study. The collected samples were examined bacteriologically for the presence of salmonella. *Salmonella* isolates were serotyped using a slide agglutination test for O and H antigens and were screened for the presence of five virulence genes (*stn, pef, invA, sop*B, and *avrA*) using a uniplex polymerase chain reaction assay and for their susceptibility to 18 antimicrobial agents using the disk diffusion method.

Results: Thirty-one *Salmonella* isolates belonging to 12 different serovars were identified. *Salmonella* Enteritidis and *Salmonella* Kentucky were the dominant serovars (22.6% each). *Salmonella* isolates displayed a high antibiotic resistance against erythromycin, sulfamethoxazole/trimethoprim, doxycycline, cephalexin, cefaclor, tetracycline, polymyxin B, cefuroxime, vancomycin, and streptomycin. All *Salmonella* isolates exhibited multidrug resistance (MDR) and demonstrated different virulence genes. The majority of *Salmonella* serovars (87.1%) harbored *sop*B gene, 54.8% carried *avr*A and *pef* genes, while all isolates carried *inv*A and *stn* genes.

Conclusion: The presence of virulent MDR Salmonellae in raw chicken meat could allow the possibility of transmission of these resistant serovars to humans. Therefore, strict hygienic measures should be followed on the whole poultry production chain to decrease the potential transmission of *Salmonella* infection from poultry meat to humans.

Keywords: antibacterial susceptibility, broilers meat, multidrug resistance, Salmonella, virulence.

Introduction

Foodborne pathogens such as Salmonella (S) enterica species represent an important worldwide public health problem. Salmonella remains one of the major zoonotic bacterial foodborne pathogens, resulting in 93.8 million annual cases among patients having gastroenteritis, with an estimated 155,000 deaths each year [1]. Poultry is considered the main reservoir for a significant number of infections with S. enterica species, involving cross-contamination events at both the farm and retail levels [2]. A diverse incidence of salmonellosis has been reported recently [3]. The clinical manifestations of the disease among human patients ranged from self-limiting gastroenteritis to severe invasive meningitis, septicemia, and osteomyelitis [4]. The majority of cases are caused by the fecal contamination of foods of animal origin or the consumption of poultry or its products [5].

Copyright: Awad, *et al.* Open Access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/ publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated. Monitoring *Salmonella* infection on poultry farms is a challenging problem and relies mainly on the application of biosecurity measures in the farm setting and the use of antibacterial agents [6]. However, the excessive use of antibacterial medications in the poultry industry, either as growth promoters or to treat disease, could result in the emergence of antibacterial resistance among *Salmonella* isolates with potential health hazards [7]. Multidrug-resistant (MDR) bacteria from poultry origins have the potential to transfer to humans through the food chain, a danger that has triggered serious public concern [8].

The degree of the pathogenicity of *Salmonella* infections in the host cell has been attributed to the presence of virulence genes which have a significant role in the occurrence of systemic infections [9]. The presence of the *inv*A gene in *Salmonella* pathogenicity islands is associated with epithelial invasion and the production of proteins of the type III secretion system (T3SS) [10], while the *stn* gene mediates enterotoxin production and is often associated with acute gastroenteritis [11]. The plasmid-encoded fimbriae (*pef*) mediates intestinal adhesion in certain *Salmonella* serotypes [12], while the multiple function effector proteins encoded by the *avr*A and *sop*B genes

in the T3SS could facilitate endothelial uptake and invasion [13]. Therefore, recognizing the existence and characterization of *Salmonella* in retail chicken meat is important for emerging effective treatment approaches to control salmonellosis.

Currently, it is difficult to assess the impact of retail chicken meat MDR on public health due to the limited availability of data in the study area. MDR in retail chicken meat is not systematically monitored in most Egyptian governorates, and the existing data are scarce.

The present study was designed to investigate the occurrence and distribution of *Salmonella* serotypes in chicken meat samples, and to explore the susceptibility of the strains to antimicrobials, as well as their virulence-associated genes

Materials and Methods

Ethical approval

This study did not need ethical approval. However, the procedures performed in the current study were in accordance with the ethical standards of the institutional and/or national research committee and those of Mansoura University.

Samples collection

A total of 200 samples were collected from 100 chicken carcasses (two samples from each carcass) from local retail chicken meat shops at Mansoura city, Dakahlia Province, Egypt, during the period between September 2017 and December 2017. In addition, 25 stool specimens were collected from retail shops workers in sterile cups. For chicken meat, the samples were collected separately in sterile polyethylene bags to prevent cross-contamination and kept in clean sterile containers to be transferred within an hour through insulated coolers containing cold packs bag to the laboratory for *Salmonella* isolation and identification.

Bacteriological examination

The procedures of *Salmonella* isolation and identification were performed, according to standard methods (ISO6579:2002; International Organization for Standardization 2002). Briefly, 25 g from each chicken meat sample were cut into small pieces by sterile scissors. Then add to a stomach bag containing 225 mL sterile Buffered Peptone Water (BPW) (Oxoid, Hampshire, England). For the stool samples,

1 g was added to 9 ml BPW, then all the samples were incubated at 37°C for 18 h. 0.1 mL of the pre-enriched broth was transferred to 10 mL of Rappaport-Vassiliadis broth (Oxoid, Hampshire, England) and incubated at 42°C for 24 h. A loopful of the enriched broth was then streaked on xylose lysine desoxycholate agar and incubated for 24 h at 37°C. Biochemical examination of the suspected *Salmonella* colonies was performed, according to the (ISO6579:2002; International Organization for Standardization 2002) guidelines [14].

Serotyping

Serotyping of the biochemically identified *Salmonella* isolates was accomplished according to the Kauffmann–White–Le Minor technique on the basis of surface antigen identification using polyclonal antisera (Difco Laboratories, Detroit, USA) to determine the O (somatic) and H (flagellar) antigenic epitopes at the Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University.

Molecular identification of *Salmonella* virulence-associated genes

Genomic DNA was extracted from the identified Salmonella serovars, according to Ramadan et al. [15]. The polymerase chain reaction (PCR) assays were conducted in individual reactions using an applied Biosystems 96-well Thermal Cycler to detect five Salmonella associated virulence genes (stn, pef, invA, sopB, and avrA). The sets of primer sequences and corresponding amplicon sizes are illustrated in Table-1. All PCR reactions were performed in a total volume of 25 µL consisting of 12.5 µL of 2×PCR master mixes (Promega, Madison, USA), 6 µL DNA templates, and 1 µL of each primer (Metabion, Germany) brought to 25 µL using DNA/RNA free water. PCR reactions and thermal conditions used were performed, according to the referenced authors (Table-1) [9,16,17]. An aliquot of each amplified product was subjected to electrophoresis in a 1.5% agarose gel. The separated bands were visualized and photographed under an ultraviolet transilluminator.

Antibacterial susceptibility testing

Salmonella serovars were tested for their antibiotic susceptibility against 18 different antimicrobial

 Table-1: Primers sequences used in PCR for detection of virulence genes in Salmonella serovars.

Primer	Sequence	Virulence factor	Amplified product	References
stn	TTG TGT CGC TAT CAC TGG CAA CC ATT CGT AAC CCG CTC TCG TCC	Enterotoxin/Chromosome	617 bp	[9]
Pef	TGT TTC CGG GCT TGT GCT CAG GGC ATT TGC TGA TTC C	Plasmid encoded fimbriae/Plasmid	700 bp	
invA	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	Salmonella species/SPI-1	284 bp	[16]
<i>sop</i> B	TCA GAA GRC GTC TAA CCA CTC TAC CGT CCT CAT GCA CAC TC	Translocated effector protein/SPI-5	517 bp	[17]
avrA	CCT GTA TTG AGC GTC TGG AGA GCT TCG TTG AAT GTC C	Effector protein/SPI-1	422 bp	

PCR=Polymerase chain reaction

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Identified serotypes	Number of serotypes (31)	Prevalence %	Group	Antigenic structure	
				0	н
Salmonella Kentucky	7	22.6	C3	8,20	i:Z6
Salmonella Enteritidis	7	22.6	D1	1,9,12	g,m:-
Salmonella Typhimurium	6	19.4	В	1,4,5,12	i:1,2
Salmonella Labadi	2	6.5	C3	8,20	d:Z6
Salmonella Molade	2	6.5	C2	8,20	Z10:Z6
Salmonella Larochelle	1	3.2	C1	6,7	e,h:1,2
<i>Salmonella</i> Takoradi	1	3.2	C2	8,20	i:1,5
<i>Salmonella</i> Tamale	1	3.2	C3	8,20	Z29:e,n,Z15
<i>Salmonella</i> Papuana	1	3.2	C1	6,7	r,e,n,z15
Salmonella Infantis	1	3.2	C1	6,7	r:1,5
<i>Salmonella</i> Inganda	1	3.2	C1	6,7	Z10:1,5
Salmonella Bargny	1	3.2	C3	8,20	i:1,5

agents belonging to different antimicrobial classes through the disk diffusion method following Clinical and Laboratory Standards Institute guidelines [18]. The following antibiotic disks (Oxoid, Basingstoke, Hampshire, UK) were used: Polymyxin B (PB; 300 µg), azithromycin (AZM; 15 µg), cephalexin (CL; 30 µg), cefuroxime (CXM; 30 µg), cefaclor (CEC; 30 µg), erythromycin (E; 15 µg), sulfamethoxazole/trimethoprim (SXT; 23.75/1.25 µg), streptomycin (S; 10 µg), neomycin (N; 30 µg), vancomycin (VA; 30 µg), norfloxacin (NOR; 10 µg), doxycycline (DO; 30 µg), penicillin (P; 10 µg), gentamycin (CN; 10 µg), tetracycline (TE; 30 µg), amoxicillin (AX; 25 µg), AX/clavulanic acid (AMC; 30 µg), and rifampin (RA; 5 µg). The isolates were defined as MDR if they exhibited resistance to three or more antimicrobial classes

Results

Out of the total examined chicken samples (n=200), 31 (15.5%) Salmonella isolates were recovered. None of the examined workers stool samples' yielded positive results. The identified serogroups among Salmonella isolates were B, C1, C2, C3, and D. Group C3 was the predominant group in the obtained isolates (Table-2). Twelve serotypes were identified including, Salmonella Enteritidis (n=7), Salmonella Kentucky (n=7), Salmonella Typhimurium (n=6), Salmonella Molade (n=2), Salmonella Labadi (n=2), Salmonella Takoradi (n=1), Salmonella Papuana (n=1), Salmonella Tamale (n=1), Salmonella Larochelle (n=1), Salmonella Infantis (n=1), Salmonella Inganda (n=1), and Salmonella Bargny (n=1). The Salmonella isolates demonstrated a high resistance against E (96.78%), followed by SXT (93.55%), DO and CL (93.55%), CXM (83.33%), CEC (87.19%), S (80.65%), PB (83.33%), TE (83.88%), P (70.97%), AX (67.8%), AMC (83.88%), VA (83.88%), RA (70.97%), and AZM (58.07%) (Table-3). In contrast, all recovered Salmonella isolates displayed a high sensitivity to NOR (100%), CN (96.77%), and N (83.87%). MDR (resistance to three or more antimicrobials) was observed in all tested isolates (Table-4).

Table-3: Percentages of antimicrobial resistance among

 Salmonella serovars.

Antibiotic used (µg/disc)	Disk code	Sensitivity (%)	Resistant (%)
Erythromycin (15 µg)	Е	3.22	96.78
Azithromycin (15 µg)	AZM	41.93	58.07
Cephalexin (30 µg)	CL	6.45	93.55
Cefuroxime (30 µg)	CXM	16.12	83.33
Cefaclor (30 µg)	CEC	12.90	87.19
sulfamethoxazole	SXT	6.45	93.55
trimethoprim,			
(23.75/1.25 µg)			
Doxycycline (30 µg)	DO	6.45	93.55
Tetracycline (30 µg)	TE	16.12	83.88
Polymyxin B (300 µg)	PB	16.12	83.33
Streptomycin (10 µg)	S	19.35	80.65
Gentamycin (10 µg)	CN	96.77	3.23
Neomycin (30 µg)	Ν	83.87	16.13
Pencillin G (10 µg)	Р	29.03	70.97
Amoxacillin (25 µg)	AX	32.26	67.8
Amoxacillin/clavulanic	AMC	16.12	83.88
acid (30 µg)			
Norfloxacin (10 µg)	NOR	100	0.00
Rifampin (5 µg)	RA	29.03	70.97
Vancomycin (30 µg)	VA	16.12	83.88

E=Erythromycin, AZM=Azithromycin, CL=Cephalexin, CXM=Cefuroxime, CEC=Cefaclor, SXT=Sulfamethoxazole/ trimethoprim, DO=Doxycycline, TE=Tetracycline,

PB=Polymyxin B, S=Streptomycin, CN=Gentamycin,

N=Neomycin, P=Penicillin, AX=Amoxicillin,

AMC=AX/clavulanic acid, NOR=Norfloxacin, RA=Rifampin, VA=Vancomycin

All *Salmonella* isolates were screened by uniplex PCR for the identification of virulence-associated genes (*invA*, *stn*, *sopB*, *avrA*, and *pef*) and all were positive for at least two of the screened genes. Both *invA* and *stn* genes were detected in 100% of the tested isolates, while *sopB* was harbored by 27 isolates (87.1%) and both *avrA* and *pef* were found in 17 isolates (54.8%). Interestingly, *S*. Enteritidis and *S*. Kentucky harbored high percentages of the virulence genes (Table-4).

Discussion

S. enterica infections are considered an economically relevant disease in the poultry industry in Egypt and a serious public health potential worldwide.

Currently, there are over 2500 recognized *Salmonella* serovars [19]. In the present study, 15.5% (31/200) of the examined chicken meat samples were *Salmonella* positive. Similar results were previously reported from Central Ethiopia [20].

The frequency of S. Typhimurium in poultry meat has been reported previously in Egypt from Assiut city markets [21] and from Mansoura city [22]. In the former study, the authors have isolated Salmonella from 44% of the examined raw chicken meat: while in the latter study, the authors have isolated different Salmonella serotypes (including S. Typhimurium, S. Kentucky, S. Molade, and S. Bargny) from processed chicken in a percent of 25.6% (30/120). On the other side, Tarabees et al. [23] isolated S. Enteritidis from 2% (2/100) and S. Typhimurium from 3% (3/100) of chicken meats. In contrast, a higher prevalence of Salmonella contamination was recorded in South Thailand (67.5%) [24] and China (54.0%) [25]. According to a meta-analysis of European Published surveys, the incidence of Salmonella spp. in poultry meat commercialized in Europe is estimated to be 7.10% (95% confidence

interval: 4.60-10.8%) [26]. The diversity in the prevalence rates of Salmonella from retail chicken meat could reflect the differences in the level of hygiene followed during chicken handling and processing, the sampling time of the year, the sampling design, as well as diagnostic methods followed. The predominant Salmonella serovars identified in the present study were S. Enteritidis (n=7), S. Kentucky (n=7), and S. Typhimurium (n=6). Similar findings were previously reported in Egypt [22,27]. In the latter study, 77.4% (24/31) of the Salmonella isolates belonged to serogroups C and B, aligning with results previously reported by Abdel-Maksoud et al. [28]. In the current study, seven isolates were serotyped as S. Enteritidis. According to the European Food Safety Authority report, approximately 74% of human zoonotic salmonellosis is caused by S. Enteritidis and S. Typhimurium [29]. It is imperative to discover the most common serotypes in the examined raw chicken meat to determine the point (s) of contamination at different stages during slaughtering, scalding, defeathering, evisceration, processing and handling, particularly in low hygienic poultry retail outlets.

Table-4: Virulence genes and antibiotic resistance profiles of Salmonella serovars.

Pattern	Serovars	Virulence genes	Antimicrobial resistance profile
1	S. Kentucky	stn, pef, invA	TE, CXM, VA, E, PB, CEC, S, STX, P, CL
2	S. Kentucky	sopB, stn, invA	RA, CXM, VA, E, PB, CEC, DO, S, AMC, STX, AX, P, CL
3	S. Kentucky	sopB, stn, invA	CN, TE, CXM, E, AZM, CEC, DO, S, AMC, ATX, P, CL
4	S. Kentucky	sopB, stn, invA	RA, CXM, VA, E, AZM, PB, CEC, DO, S, AMC, STX, AX, P, CL
5	S. Kentucky	sopB, stn, avr, pef, invA	CN, RA, TE, CXM, VA, E, AZM, PB, CEC, DO, S, AMC, STX, AX, P, CL
6	S. Kentucky	sopB, stn, avr, pef, invA	N, TE, CXM, PB, DO, STX, CL
7	S. Kentucky	sopB, stn, pef, avr, invA	CN, N, RA, TE, CXM, VA, E, AZM, PB, CEC, DO, AMC, STX, AX, P, CL
8	S. Enteritidis	sopB, stn, avr, pef, invA	RA, TE, CXM, VA, E, AZM, PB, CEC, DO, S, AMC, STX, AX, P, CL
9	S. Enteritidis	sopB, stn, avr, invA	RA, TE, VA, E, PB, CEC, DO, S, AMC, AX, P, CL
10	S. Enteritidis	sopB, stn, pef, invA	CN, RA, TE, CXM, VA, E, PB, CEC, DO, S, AMC, STX, AX, CL
11	S. Enteritidis	sopB, stn, pef, invA	TE, CXM, VA, E, AZM, PB, CEC, DO, S, AMC, STX, AX, CL
12	S. Enteritidis	sopB, stn, avr, invA	TE, CXM, E, AZM, PB, CEC, DO, S, AMC, STX, AX, CL
13	S. Enteritidis	sopB, stn, avr, pef, invA	N, RA, TE, CXM, VA, E, PB, CEC, DO, S, AMC, STX, AX, P, CL
14	S. Enteritidis	sopB, stn, avr, pef, invA	RA, TE, CXM, VA, E, AZM, PB, CEC, DO, S, AMC, STX, AX, P, CL
15	S. Typhimurium	sopB, stn, pef, invA	TE, VA, E, DO, AMC, STX, P, CL
16	S. Typhimurium	sopB, stn, pef, avr, invA	TE, CXM, VA, E, S, STX, P
17	S. Typhimurium	sopB, stn, invA	RA, TE, CXM, E, AZM, PB, CEC, DO, S, AMC, STX, CL
18	S. Typhimurium	sopB, stn, avr, invA	RA, TE, VA, E, PB, CEC, DO, S, AMC, AX, P, CL
19	S. Typhimurium	sopB, stn, avr, invA	RA, CXM, VA, E, AZM, PB, CEC, DO, S, AMC, STX, AX, P, CL
20	S. Typhimurium	stn, pef, invA	CN, RA, TE, CXM, VA, E, AZM, PB, CEC, DO, S, AMC, STX, AX, P, CL
21	S. Labadi	sopB, stn, invA	RA, TE, CXM, VA, E, AZM, PB, CEC, DO, S, AMC, STX, AX, P, CL
22	S. Labadi	sopB, stn, avr, pef, invA	CN, N, RA, TE, CXM, VA, E, PB, CEC, DO, S, STX, AX, CL
23	S. Molade	sopB, stn, invA	RA, TE, CXM, VA, E, AZM, CEC, DO, S, AMC, STX, CL
24	S. Molade	sopB, stn, avr, pef, invA	RA, TE, CXM, VA, E, AZM, PB, CEC, DO, S, AMC, STX, AX, P, CL
25	S. Inganda	sopB, stn, avr, invA	RA, TE, CXM, VA, E, AZM, PB, CEC, DO, S, STX, CL
26	S. Bargny	sopB, stn, avr, pef, invA	RA, TE, CXM, VA, E, PB, CEC, DO, AMC, STX, AX, CL
27	S. Larochelle	stn, invA	RA, TE, CXM, VA, E, PB, DO, S, AMC, STX, P, CL
28	S. Takoradi	sopB, stn, pef, invA	VA, E, PB, CEC, DO, S, AMC, STX, P, CL
29	S. Tamale	sopB, stn, avr, invA	E, PB, CEC, DO, AMC, AX, P
30	S. Papuana	sopB, stn, avr, pef, invA	RA, TE, CXM, VA, E, AZM, CEC, DO, S, AMC, STX, AX, P, CL
31	S. Infantis	stn, invA	CN, N, RA, TE, CXM, VA, E, AZM, PB, CEC, DO, AMC, STX, AX, P, CL

S. Kentucky=*Salmonella* Kentucky, *S.* Enteritidis=*Salmonella* Enteritidis, *S.* Typhimurium=*Salmonella* Typhimurium, *S.* Labadi=*Salmonella* Labadi, *S.* Molade= *Salmonella* Molade, *S.* Inganda=*Salmonella* Inganda, *S.* Bargny=*Salmonella* Bargny, *S.* Larochelle=*Salmonella* Larochelle, *S.* Takoradi=*Salmonella* Takoradi, *S.* Tamale=*Salmonella* Tamale, *S.* Papuana=*Salmonella* Papuana, *S.* Infantis=*Salmonella* Infantis, TE=Tetracycline, CXM=Cefuroxime, VA=Vancomycin, E=Erythromycin, PB=Polymyxin B, CEC=Cefaclor, S=Streptomycin, STX=Trimethoprim/sulfamethoxazole, P=Penicillin, CL=Cephalexin, RA=Rifampin, DO=Doxycycline, AMC=AX/clavulanic acid, CN=Gentamycin, AZM=Azithromycin, N=Neomycin

The antibacterial susceptibility testing revealed that the identified Salmonella serovars were sensitive to NOR (100%), CN (96.77%), and N (83.87%). Nearly, similar data were previously reported [30], while 83.88% of the obtained Salmonella serovars were resistant to AMC and 96.78% were resistant to E. Several studies have demonstrated that various patterns of antibacterial resistance exist in Salmonella isolates [27,30]. A high rate of MDR was found against most of the commonly used antibiotics. These findings should alert the farm owners to use alternative medications to combat the bacteria instead of using the traditional antibiotics against which bacterial resistance has become common. The current findings are similar to those previously reported in Spain and Turkey [31,32], and MDR Salmonella isolates from poultry origin have been reported worldwide [4,33,34]. The pattern of MDR observed in the present study highlights the drawbacks of the uncontrolled use of antimicrobials, which contributes to increasing the incidence of resistant pathogens among human infections. Hence, a continuous surveillance of antimicrobial resistance of zoonotic bacterial strains such as Salmonella spp. isolated from food and production environments will contribute to a successful control program.

The virulence of the obtained Salmonella strains is associated with a series of genes which is responsible for invasion, colonization, and spread within the infected host. The present study demonstrated various virulence genes among Salmonella serovars. For example, the invA and stn genes were harbored by 100% of Salmonella serovars, which are in agreement with Osman et al. [35] and Ahmed et al. [36]. It has been suggested that the *inv*A gene is a specific marker for the molecular detection of Salmonella serotypes, while the stn gene is responsible for enterotoxin production [36]. In the current study, the prevalence of the sopB gene was 87.1%. This gene plays a significant role in the occurrence of diarrhea through activating secretory pathways or facilitating inflammation and altering ion balances within cells [36]. The avrA gene was detected in 54.8% of the obtained isolates. Other reports have indicated that this gene was only observed in serovars that cause severe salmonellosis in humans [37]. The *pef* gene was also identified in 54.8% of the isolates, highlighting the significance of fimbriae in the infection process. A similar detection rate (68%) was previously reported by Hudson et al. [37]. In contrast, a low frequency (6.7%) of the *pef* gene in Salmonella isolates was previously detected [36]. Interestingly, none of the examined stool samples vielded positive results, notwithstanding the fact that the risk of contracting the infection from contaminated products cannot be excluded.

Conclusion

Our findings provide evidence for the presence of MDR *Salmonella* serovars with a wide distribution of

virulence genes in chicken meat. These findings raise the suspicion that retail chicken meat often harbors zoonotic *Salmonella* serovars, representing a potential public health hazard. The possibility of transmission of these MDR serovars to humans requires regular monitoring of the production chain and amended farming practices, with particularly strict regulation of antibiotic usage.

Authors' Contributions

AA designed the study, conducted the experiments, and revised the manuscript, MG wrote the manuscript and participated in carrying out experiments, and EK and AS shared in performing the experiments and reviewing the manuscript. All authors read and approved the final manuscript for publication.

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Competing Interests

The authors declare that they have no competing interests.

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