

Antimicrobial Resistance and Characterization of *Salmonellae* Isolated From Chicken Meat and Its Products in Mansoura City, Egypt

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ABSTRACT

Purpose: This research aimed to evaluate the rate of resistance to antimicrobials and the recognition of *Salmonella* strains virulence-related genes in chicken meat and its products collected from various shops for poultry, and supermarkets with varying degrees of hygiene in Mansoura City, Egypt.

Methods: Three hundred chicken meat and its products samples were streaked on XLD agar plates followed by biochemical and serological identification of the isolates. 43 isolates from all examined samples were identified as *Salmonella*, assayed for susceptibility to 14 antimicrobials by the single diffusion method.

Results: The antimicrobial resistance percentages for the *Salmonella* isolates was the highest for streptomycin (100%) and lowest for gentamicin (2.3%). Out of the forty three isolates of *Salmonella*, thirty six (83.72%) displayed multiple antimicrobial resistance (MAR) for 3 or more antimicrobials. PCR identification of virulence genes for *Salmonella* strains showed that *S. enteritidis*, *S. typhimurium*, *S. papuana*, *S. infantis*, and *S. virchow* serovars were positive for *stn*, *hila* and *fimH* genes. *S. kentucky*, *S. wingrove*, and *S. bargnyser* serovars were positive for *hila* and *fimH* genes. *S. tamale* serovar was found to be positive for *fimH* and *stn* genes. *S. anatum* serovar was found to be positive for *hila* and *stn* genes. *S. larochelle* serovar was found to be positive for *fimH* gene. *S. typhimurium*, *S. kentucky*, *S. enteritidis*, *S. tamale*, *S. papuana*, *S. wingrove*, *S. anatum*, *S. virchow*, and *S. larochelle* serovars were positive for *sopA* gene.

Conclusion: The higher contamination of different chicken meat and its products with multidrug-resistant *Salmonella* indicates improper hygienic measures. Also, the higher MAR index and presence of virulence-related genes in *Salmonella* isolates has high risk potential for consumers.

Keywords

Antimicrobial resistance, *Salmonella*, Chicken meat, Virulence genes, MAR.

Introduction

Chicken and chicken products provide high biological value animal protein for consumers of all ages, where they provide all the necessary essential amino acids, a significant proportion of fatty acids that are unsaturated (Marangoni et al. 2015). Moreover, chicken meat continues to be incriminated in human salmonellosis outbreaks (Ravel et al. 2009). All chicken edible products are exposed to contamination from many sources inside and outside of the animal during the various stages of slaughter and processing. The detection of *Salmonellae* in the chicken production chain is therefore of great concern, particularly at retail level.

Owing to the appearance and spreading of antimicrobial-resistant and possibly more strains that are pathogenic, *Salmonella* is increasingly concerned (Furuya and Lowy 2006; Baker et al. 2018). Inappropriate use of antimicrobial agents as medicinal or preventative agents and its use for promotion of the growth in animal development can be the reason for the increase in resistant strains. Effective antimicrobial agents are necessary in severe cases of human salmonellosis. Antimicrobial-resistant *Salmonella* strains are highly risky because they can impair the successful treatment for human salmonellosis (Berrang et al. 2009).

Many of the *Salmonella* strains expressed multiple virulence factors that promote the pathogenicity and establish the method of transmission to the target hosts and the severity of the infection (Hensel 2004). The objective of this research was to recognize the rate of resistance to antimicrobials and the recognition of *Salmonella* strains virulence-related genes in chicken meat and its products.

Results and Discussion

The obtained results in Table (1) revealed that forty-three isolates from all examined samples were identified as *Salmonella*. Among the isolates of *Salmonella*, eleven various serotypes have been identified. *S. typhimurium* (23.26%) was the most prevalent, then *S. kentucky* (18.6%), *S. enteritidis* (16.28%), *S. tamale* (11.63%), *S. infantis* (6.98%), *S. papuana* (6.98%), *S. wingrove* (4.65%), *S. bargny* (4.65%), *S. Larochelle* (2.33%), *S. virchow* (2.33%), and *S. anatum* (2.33%). These results were comparable to the findings provided by (Abd-Elghany et al. 2015) and (Morshdy et al. 2015).

The high incidence of *Salmonellae* in chicken and chicken products reflected the public health hazards that could result from subsequent mishandling, improper cooking, and cross-contamination.

The isolated 43 *Salmonella* strains were assayed for susceptibility to 14 antimicrobials as displayed in Table (2). The antimicrobial resistance percentages for the *Salmonella* isolates was the highest for streptomycin (100%) followed by erythromycin (90.7%), norocillin (83.7%), cephalothin (72.1%), penicillin G (69.8%), nalidixic acid (62.8%), cephradine (51.1%), sulphamethoxazol (37.2%), clindamycin (32.5%), tetracycline (20.9%), ampicillin (11.6%), amikacin (9.3%), doxycycline (4.7%), and gentamicin (2.3%).

Antimicrobial resistance profile of the isolated 43 *Salmonella* strains revealed in Table (3). Out of *Salmonella* 43 isolates, 36 (83.72%) showed multiple antimicrobial resistance (MAR) for 3 or more antimicrobials. It was clear that the MAR index ranged from 1 to 0.071 with an average of 0.469. Multiple antimicrobial resistant *Salmonella* is recognized as an environmental hazard to the food supply and human health.

Nearly similar results were recorded by (Abd-Elghany et al. 2015). Higher results of 100% multi-resistant *Salmonella* strains were isolated by

(Carramiñana et al. 2004) from avian slaughterhouse in Spain, (KasimogluDogru, Ayaz, and Gencay 2010) from chicken carcasses in Turkey, (Shrestha et al. 2010) from poultry in Nepal, (Yildirim et al. 2011) from raw chicken carcasses in Turkey, and (Álvarez-Fernández et al. 2012) from poultry in Spain. Also, (Abd-Elghany et al. 2015) recorded 92.8% isolated multi-resistant strains of *Salmonella* from chickens and giblets in Egypt with a MAR index average of 0.582.

Lower results of multi-resistant *Salmonella* strains were isolated by (Nastasi, Mammina, and Cannova 2000) with a percentage of 2.3% in Southern Italy, (Antunes et al. 2003) with a percentage of 75% from poultry products in Portugal, (Abdellah et al. 2009) with a percentage of 75.43% in carcasses and giblets of chicken in Morocco, and 65.2% in Korea from poultry (Hur et al. 2011).

Nearly 90% of antimicrobials used in poultry, provided either prophylactically at subtherapeutic concentrations or to promote growth. Antimicrobial usage has long been documented to modify the genes of antimicrobial resistance. The microbial population encoded (resistome) and the influences of resistant bacteria persists for decades after antimicrobial usages has ended (Sommer and Dantas 2011).

The evidence that *S. typhimurium* has been among the serovars that have the highest mean antimicrobial resistance in this research is a disturbing report, because *S. typhimurium* has more significant effects on human health than other serotypes of *Salmonella*.

Results shown in Table (4) revealed PCR identification of enterotoxin (*stn*), hyper-invasive locus (*hila*), and fimbrial (*fimH*) virulence *Salmonella* genes. The results showed that *S. enteritidis*, *S. typhimurium*, *S. papuana*, *S. infantis*, and *S. virchow* serovars were positive for *stn*, *hila* and *fimH* genes. *S. kentucky*, *S.*

wingrove, and *S. bargnyserovars* were positive for *hilA* and *fimH* genes. *S. tamale* serovar had *stn* and *fimH* genes. *S. anatum* serovar had *stn* and *hilA* genes. *S. larochelle* serovar had the *fimH* gene.

Results shown in Table (4) revealed PCR identification of *sopA* virulence gene of *Salmonella* species. The results showed that *S. typhimurium*, *S. kentucky*, *S. enteritidis*, *S. tamale*, *S. papuana*, *S. wingrove*, *S. anatum*, *S. virchow*, and *S. larochelle* serovars were positive for *sopA* gene. From the other side, *S. infantis*, and *S. bargnyserovars* were negative for *sopA* gene.

Comparable results have been reported in other studies, including (EL-Hanafy 2019) who found that *S. enteritidis*, *S. kentucky* and *S. typhimurium* were positive for *stn*, *hilA* and *fimH* genes, *S. infantis* and *S. takoradi* were positive for *fimH* gene, and *S. papuana* was positive for *hilA* gene, (Abd-Elghany et al. 2015) who reported that *S. typhimurium*, *S. enteritidis*, *S. kentucky*, *S. anatum*, and *S. virchow* were positive for *stn* gene, and (Ahmed, El-Hofy, and Shafik 2016) who recorded that *stn* gene was identified in all isolates of *S. typhimurium* of human and chicken origin at Mansoura city, Egypt.

Detection of genes of virulence in isolated *Salmonella* strains clarified the high prevalence of related virulence genes among isolated strains and added extra evidence of the hazard of virulent salmonellosis posed by chicken and its products to humans.

Conclusion and Recommendations

These results have established that chicken meat is a major multi-resistant *Salmonella* reservoir, and concluded that effective antimicrobial treatment of salmonellosis caused by chicken-origin strains is difficult to accomplish. Chicken meat and its products therefore pose a major concern for the health of the public, and this directs for proper control of antimicrobials to minimize the

inappropriate usage of antimicrobial drugs in the food sector. To ensure food safety before consumption, an improper method of cooking of chicken meat and inadequate hygiene procedures before consumption should be avoided.

Materials and Methods

(1) Samples Collection

A sum of 300 samples of chicken meat and its products including raw thigh, frozen thigh, raw breast, frozen breast, gizzard, liver, heart, pane, luncheon, and burger (30 of each) were collected from various poultry shops and supermarkets with varying hygiene levels in Mansoura, Egypt. Samples collected were packed, described, transported to the ice box as quickly as possible and processed at the Research lab of Animal Health Research Institute, Mansoura.

(2) Isolation and Identification of *Salmonellae*

The applied technique was recommended by (Vassiliadis 1983). Twenty five grams of every hard sample were homogenised into 225 ml of buffered peptone water (BPW) under aseptic conditions for 2 min. by using sterile homogenizer. All of the samples were incubated at 35° C for 24 ± 2 hours. One ml from the pre-enrichment was added to 10 ml of the Rappaport Vassiliadis (RV) enrichment broth and was incubated at 41 ± 1° C for 24 hours. Loopfuls of RV broth enrichment were independently streaked onto xylose lysine deoxycholate (XLD) agar and were incubated at 37° C for 24 hours. Two or three of typical or suspected colonies (red colonies with or without a black centre on XLD) were chosen from every selective medium and were streaked onto nutrient agar slope which incubated at 37° C for 24 hours for more identification. Suspected isolates of *Salmonella* organisms were subjected to morphological identification (***International Standards Organization “ISO”, 2013***), biochemical identification (Holt 1984) and serological identification (Kauffman 1974).

(3) Antibiotic Resistance of isolated *Salmonellae* species (Antibiogramme)

Susceptibility to antimicrobials has been evaluated via the single diffusion method in accordance with (Srivani 2011) for *Salmonellae*. Discs of sensitivity with different concentrations have been used to evaluate the susceptibility of the isolated *Salmonella* strains (Oxoid Limited, Basingstoke, Hampshire, UK).

The Multiple Antibiotic Resistance Index (MAR) for every strain was determined on the basis of the formula specified by Singh et al. (2010) as follows:

MAR index= Resistance No. (Isolates categorized as intermediate were assumed to be sensitive to MAR index)/Total No. of antibiotics tested.

(4) Polymerase Chain Reaction (PCR) for isolated *Salmonellae* species

• Sequences of primers used for PCR identification:

Implementation of PCR for virulence factors identification for Enterotoxin (*stn*), hyper-invasive locus (*hilA*), fimbrial (*fimH*) and (*sopA*) genes was conducted essentially by the use of Primers (Pharmacia Biotech) as seen in the following Table. DNA Extraction using QIA amp kit by the method of (Shah et al. 2009):

Target gene	Oligonucleotide sequence (5' → 3')	Product size (bp)	References
<i>stn</i> (F)	5' CTTTGGTCGTAAAATAAGGCG '3	260	(Makino et al. 1999)
<i>stn</i> (R)	5' TGCCCAAAGCAGAGAGATTC '3		
<i>hilA</i> (F)	5' CTGCCGCAGTGTTAAGGATA '3	497	(Guo, Chen, and Beuchat 2000)
<i>hilA</i> (R)	5' CTGTGCCTTAATCGCATGT '3		
<i>fimH</i> (F)	5' GGA TCC ATG AAA ATA TAC TC '3	1008	(Menghistu 2009)
<i>fimH</i> (R)	5' AAG CTT TTA ATC ATA ATC GAC TC '3		
<i>sopA</i> (F)	5' TGGACTGAGAACGCTGTGGA '3	207	(Elabed et al. 2016)
<i>sopA</i> (R)	5' GTGGGCCAGTACGCTTACCA '3		

• DNA Amplification

Multiplex PCR for amplification of *stn*, *hila*, and *fimH* virulence genes (Singh et al. 2010) and *sopA* gene (Elabed et al. 2016).

Conflict of Interest

Neither of the authors have had any conflicts of interest to specify.

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List of Tables

Table 1. Distribution of *Salmonella* strains (n = 43) among chicken meat and its products samples (30 of each).

T= thigh, B=breast, G=gizzard, L=liver, H=heart, P=pane, L=luncheon, B=burger, r=raw, and f= frozen.

Serotypes	Tr	Tf	Br	Bf	G	L	H	P	L	B	Total	
											No.	%
<i>S. typhimurium</i>	3	1	1	-	2	1	-	1	1	-	10	23.26
<i>S. kentucky</i>	2	1	1	-	1	2	-	1	-	-	8	18.6
<i>S. enteritidis</i>	1	-	-	-	4	1	1	-	-	-	7	16.28
<i>S. tamale</i>	1	2	-	-	-	-	1	1	-	-	5	11.63
<i>S. infantis</i>	-	-	-	1	-	-	-	-	1	1	3	6.98
<i>S. papuana</i>	-	1	-	1	1	-	-	-	-	-	3	6.98
<i>S. wingrove</i>	-	-	-	-	-	1	1	-	-	-	2	4.65
<i>S. bargny</i>	-	-	-	-	-	-	1	1	-	-	2	4.65
<i>S. larochelle</i>	-	-	1	-	-	-	-	-	-	-	1	2.33
<i>S. virchow</i>	-	-	-	-	1	-	-	-	-	-	1	2.33
<i>S. anatum</i>	-	-	-	-	-	-	-	-	1	-	1	2.33

Table 2. Antimicrobial susceptibility of *Salmonella* strains isolated from the examined chicken meat and its products samples (n=43)

Antimicrobial agent	S ^a		I ^b		R ^c	
	NO	%	NO	%	NO	%
<i>Streptomycin (S)</i>	-	-	-	-	43	100
<i>Erythromycin (E)</i>	-	-	4	9.3	42	90.7
<i>Norocillin (NO)</i>	2	4.7	5	11.6	36	83.7
<i>Cephalothin (CN)</i>	7	16.3	5	11.6	31	72.1
<i>Penicillin G (P)</i>	10	23.2	3	7.0	30	69.8
<i>Nalidixic acid (NA)</i>	14	32.5	2	4.7	27	62.8
<i>Cephradine (CE)</i>	18	41.9	3	7.0	22	51.1
<i>Sulphamethoxazol (SXT)</i>	23	53.5	4	9.3	16	37.2

Clindamycin (CL)	29	67.4	-	-	14	32.5
Tetracycline (T)	33	76.7	1	2.3	9	20.9
Ampicillin (AM)	35	81.4	3	7.0	5	11.6
Amikacin (AK)	37	86.0	2	4.7	4	9.3
Doxycycline (DO)	38	88.3	3	7.0	2	4.7
Gentamicin (G)	41	95.4	1	2.3	1	2.3

S: Susceptible^a I: Intermediate susceptibility^b R: Resistant^c

Table 3. Antimicrobial resistance profile of *Salmonella* strains isolated from the examined chicken meat and its products samples (n=43).

NO	<i>Salmonella</i> strains	Antimicrobial resistance profile	MAR ^o index
1	<i>S. typhimurium</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ , T ^j , AM ^k , AK ^l , DO ^m , G ⁿ	1
2	<i>S. typhimurium</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ , T ^j , AM ^k	0.786
3	<i>S. typhimurium</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ	0.643
4	<i>S. typhimurium</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ	0.643
5	<i>S. typhimurium</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g	0.500
6	<i>S. typhimurium</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f	0.428
7	<i>S. typhimurium</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e	0.357
8	<i>S. typhimurium</i>	S ^a , E ^b , NO ^c	0.214
9	<i>S. typhimurium</i>	S ^a , E ^b	0.143
10	<i>S. typhimurium</i>	S ^a , E ^b	0.143
11	<i>S. kentucky</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ , T ^j , AM ^k , AK ^l , DO ^m	0.928
12	<i>S. kentucky</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ , T ^j	0.714
13	<i>S. kentucky</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h	0.571
14	<i>S. kentucky</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g	0.500
15	<i>S. kentucky</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e	0.357
16	<i>S. kentucky</i>	S ^a , E ^b , NO ^c , CN ^d	0.286
17	<i>S. kentucky</i>	S ^a , E ^b	0.143
18	<i>S. kentucky</i>	S ^a	0.071
19	<i>S. enteritidis</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ , T ^j , AM ^k	0.857

AK ^l			
20	<i>S. enteritidis</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ	0.643
21	<i>S. enteritidis</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g	0.500
22	<i>S. enteritidis</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f	0.428
23	<i>S. enteritidis</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e	0.357
24	<i>S. enteritidis</i>	S ^a , E ^b , NO ^c	0.214
25	<i>S. enteritidis</i>	S ^a , E ^b	0.143
26	<i>S. tamale</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ , T ^j , AM ^k , AK ^l	0.857
27	<i>S. tamale</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ , T ^j	0.714
28	<i>S. tamale</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h	0.571
29	<i>S. tamale</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f	0.428
30	<i>S. tamale</i>	S ^a , E ^b , NO ^c	0.214
31	<i>S. infantis</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ , T ^j	0.714
32	<i>S. infantis</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g	0.500
33	<i>S. infantis</i>	S ^a , E ^b	0.143
34	<i>S. papuana</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ , T ^j	0.714
35	<i>S. papuana</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f	0.428
36	<i>S. papuana</i>	S ^a , E ^b	0.143
37	<i>S. wingrove</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ	0.643
38	<i>S. wingrove</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g	0.500
39	<i>S. bargny</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g , SXT ^h , CL ⁱ	0.643
40	<i>S. bargny</i>	S ^a , E ^b , NO ^c	0.214
41	<i>S. larochelle</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f , CE ^g	0.500
42	<i>S. virchow</i>	S ^a , E ^b , NO ^c , CN ^d , P ^e , NA ^f	0.428
43	<i>S. anatum</i>	S ^a , E ^b , NO ^c	0.214
Average		0.469	

S:Streptomycin^a

E:Erythromycin^b

NO:Norocillin^c

CN:Cephalothin^d

P:Penicillin-G^e

NA:Nalidixic acid^f

CE:Cephadrine^g

SXT:Sulphamethoxazol^h

CL:Clindamycinⁱ

T:Tetracycline^j

AM:Ampicillin^k

AK:Amikacin^l

DO:Doxycycline^m

G:Gentamicinⁿ

MAR: Multiple Antibiotic Resistance^o

Table 4. Occurrence of virulence genes of *Salmonella* species isolated from the examined samples of chicken meat and its products.

Salmonella Serovars	stn	hilA	fimH	sopA
<i>S. typhimurium</i>	+	+	+	+
<i>S. kentucky</i>	-	+	+	+
<i>S. enteritidis</i>	+	+	+	+
<i>S. tamale</i>	+	-	+	+
<i>S. infantis</i>	+	+	+	-
<i>S. papuana</i>	+	+	+	+
<i>S. wingrove</i>	-	+	+	+
<i>S. bargny</i>	-	+	+	-
<i>S. anatum</i>	+	+	-	+
<i>S. virchow</i>	+	+	+	+
<i>S. larochelle</i>	-	-	+	+