Received: 01 October 2022, Accepted: 21 October 2022

Original Research

Journal of Advanced Veterinary Research
(2022) Volume 12, Issue 6, 743-752

Incidence, Phenotypic and Genotypic Antimicrobial Resistance of Zoonotic *Salmonella* spp. Isolated from Broiler Chicken and Human in Egypt

Eman Y. Tohamy[1], Nahil Y. Dorgham[1], Ahmed A. Askora[1], Abdallah M.A. Merwad[2*]

[1]Department of Botany and Microbiology, Faculty of Science, Zagazig University, Zagazig, 44511, Egypt.
[2]Department of Zoonoses, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt.

***Correspondence**
Abdallah M.A. Merwad
Department of Zoonoses, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt.
E-mail address: merwad.abdallah@yahoo.com

Abstract

This study investigated incidence, phenotypic-genotypic antimicrobial resistance in zoonotic *Salmonella*e from broiler chicken and human in Egypt. Two hundred and forty samples were randomly collected from broilers including (liver, gizzard, intestine, n.= 60 of each) and from workers (hand swabs, n.= 60) at poultry outlets. Isolation, biochemical and serological identifications of *Salmonella* spp. were performed. Antimicrobial susceptibility testing of *Salmonella* serotypes was done using disc diffusion method. The multiple antibiotic resistance (MAR) index of *Salmonella* serotypes was calculated. Genotypic detection of antimicrobial resistance genes [bla_{TEM}, *flo*R and *tet*A(A)] was identified in phenotypically resistant *Salmonella*e using PCR. The incidence of *Salmonella* spp. was 5% in each of liver and intestine of broilers, and 1.66% in gizzard of broilers; and 3.33% in hand swabs of workers. The serotypes of S. Typhimurium were distributed into liver of broilers (3 out of 4, 75%) and into intestine of broilers (1 out of 4, 25%). The distribution of S. Enteritidis was 33.3% (1 out of 3) in gizzard and 66.7% (2 out of 3) was distributed in intestine of broiler. Two isolates of S. Kentucky (100%) were distributed in hand swabs from workers. The peak resistance (100%) of 9 *Salmonella* isolates was found to each of chloramphenicol and ampicillin followed by a highest resistance (88.8%) to doxycycline The profile of each S. Typhimurium and S. Enteritidis isolates from broiler chicken reached the peak resistance (100%) for ampicillin, chloramphenicol and doxycycline The multiple antibiotic resistance (MAR) index of *Salmonella* isolates was ranged from 0.23 to 0.54 with an average of 0.34. The bla_{TEM}, *tet*A(A) and *flo*R genes were identified with similar distribution percentage of 66.7% in S. Typhimurium isolates from liver. all isolates of S. Enteritidis from gizzard, S. Typhimurium from intestine, S. Enteritidis from intestine and S. Kentucky from hand swabs harbored similar distribution percentage (100%) for each bla_{TEM}, *tet*A(A) and *flo*R gene. Further studies are required to predict biological tools such as bacteriophages during poultry production to minimize entry of multidrug resistant (MDR) *Salmonella*e from broiler chicken to human food chain.

KEYWORDS
Salmonellosis, Broilers, Humans, Phenotypic resistance, Antimicrobial resistance genes

INTRODUCTION

Salmonellosis is one of the most serious issues affecting the chicken industry, as well as a serious food safety risk (Hassan *et al*., 2021). *Salmonella* is a leading cause of foodborne illness in humans, with 16 million cases of typhoid fever, 1.3 billion cases of gastroenteritis, and 3 million deaths worldwide each year (Bhunia, 2018). *Salmonella* outbreaks have been linked to a variety of foods, particularly those derived from animals, such as meat, poultry, and eggs (Bouchrif *et al*., 2009). Animal-originated foods, particularly chickens are represented as major reservoirs for dissemination of *Salmonella*e (Vo *et al*., 2006; Jackson *et al*., 2013). However, poultry meat & their products are considered as the most common sources of *Salmonella* food poisoning in people and has been linked to numerous human salmonellosis outbreaks. *Salmonella* is transmitted to poultry meat by cross-contamination with excrement, water, equipment, and workers' hands throughout the slaughtering, scalding, defeathering, and preparation procedures, particularly at low-hygienic poultry retail shops (Saeed *et al*., 2013).

There are around 2500 *Salmonella* serovars in the world. *Salmonella* enterica Typhi (S. Typhi) and *Salmonella* enterica Paratyphi (S. paratyphi) cause typhoid fever and paratyphoid fever, respectively, in humans (Chaudhry *et al*., 2003), whereas salmonellosis is an umbrella term that encompasses invasive infection with all serovars of *Salmonella*, as well as the normally gut-confined infections of food poisoning (Fàbrega and Vila, 2013). S. Enteritidis and S. Typhimurium, which can be transferred to humans, are widely found in poultry (Abd EL-Ghany *et al*., 2012). It has been discovered that the chicken industry can account for up to 50% of salmonellosis outbreaks (Antunes *et al*., 2016). El-Shaboury and Basha (2009) identified five *Salmonella* strains as S. Typhimurium in Egyptian broiler chicken farms in Alexandria, while Mohamed *et al.* (2009) serotyped isolates as S. Enteritidis and S. Typhimurium in Assiut governorates. In the Dakhlia governorate, S. Enteritidis was found in chicken meat and a patient with food poisoning symptoms (Ammar *et al*., 2009). *Salmonella*e infections were later isolated from broiler *flo*cks in both Eastern and Northern Egypt (Ammar *et al*., 2016; El-Sharkawy *et al*., 2017). *Salmonella* serovars (S. Typhimurium, S. Enteritidis, S. Anatum,

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
ISSN: 2090-6277/2090-6269/ © 2011-2022 Journal of Advanced Veterinary Research. All rights reserved.

S. Heidelberg, S. Muenster, and S. Kentucky) have recently been discovered in chicken meat products sold in local supermarkets in 2019 (Shsltout *et al*., 2019).

The increased spread of multidrug-resistant *Salmonella* spp. is owing to haphazard antibiotic use, which has resulted in increased illness severity. Antimicrobial resistant bacteria such as *Salmonella* have evolved as a serious public health concern as a result of extensive abuse of antimicrobial drugs in food animal production as a means of growth (Antunes *et al*., 2016). One of the main causes of the rise of multidrug resistance bacteria is the improper use of antibiotics in poultry farms in underdeveloped countries, particularly Egypt (Okeke *et al*., 2005). The misuse of the antimicrobial agents in veterinary medicine could result in the emerging of multidrug-resistant bacteria (MDR) including *Salmonella* (Sallam *et al*., 2014). The antimicrobial-resistant microorganism and the antimicrobial resistance genes could be transmitted to humans through food derived from animals particularly poultry meat and their products (Zhao *et al*., 2020). *Salmonella* recovered from broiler environment contain class 1 integrons, that are genetic elements that could integrate antimicrobial resistance gene within the *Salmonella* host genome (Goldstein *et al*., 2001). There exists a diversity of integron-associated resistance genes in poultry litter such as resistances to β-lactams, chloramphenicol, and aminoglycosides (Lu *et al*., 2003; Nandi *et al*., 2004; Smith *et al*., 2007).

The multiple antibiotic resistance (MAR) index is a cost effective, rapid, easy, and valid method used for tracing the bacterial source (Adzitey, 2015; Khan *et al*., 2015; Davis and Brown, 2016). The high-risk sources of faecal contaminations of meat, poultry meat could be distinguished through the MAR indexing of bacterial isolates including Escherichia coli and *Salmonella* spp. (Parveen *et al*., 1997; Khan *et al*., 2015). In Egypt, molecular detection of antimicrobial resistance genes including bla_{TEM} & *tet*A(A), *flo*R genes in *Salmonella* isolated from poultry in Egypt (Abd El-Tawab *et al*., 2015; Lebdah *et al*., 2017). This study was carried out to investigate the incidence, serotyping and phenotypic resistance of *Salmonella* serotypes as well as genotypic detection of antimicrobial resistance genes in recovered *Salmonella* isolates from broiler chicken and workers at poultry outlets at Sharkia Province, Egypt.

MATERIALS AND METHODS

Collection and preparation of broiler and human samples

Two hundred and forty samples were randomly collected from broilers including (liver, gizzard, intestine, n.= 60 of each) and from workers (hand swabs, n.= 60) at poultry outlets from two localities (Zagazig and El-Salheia cities) at Sharkia province, Egypt during the period extending from December 2018 till July 2019. Oral consent was obtained from workers prior to sample collection. The collected broiler samples were wrapped in sterile polyethylene bags then directly transferred in an insulated ice box under complete aseptic conditions without delay to the Laboratory of Zoonoses Department, Faculty of Veterinary Medicine, Zagazig University for further preparation and examination. Regarding the collected samples from workers, each hand swap was placed 9 ml of buffered peptone water (BPW) 0.1% under aseptic conditions then placed in an ice box followed by direct transfer to the Laboratory.

Pre-enrichment of broiler and human samples

Twenty five grams each of liver, gizzard and intestine of broilers were aseptically transferred into a sterile homogenizer flask that contains 225 ml of sterile buffered peptone water (0.1%). The contents were subjected to homogenization at 2000 rpm for 2.5 minutes using a homogenizer. All broiler samples and hand swabs from workers were subjected for an incubation at 37ºC for 24 h as a pre-enrichment step.

Isolation of Salmonella species from both broiler and worker samples

The isolation of *Salmonella* species was performed according to the protocol of ISO 6579 (ISO, 2002) and Pavic *et al*. (2010) with minor modifications. After the pre-enrichment step, 1 ml of the pre-enriched samples were exposed to an inoculation into a tube harboring 10 ml of sterile Rappaport-Vassiliadis soy peptone broth (Biolife; Italy) for the selective enrichment. All inoculated broths were subjected to an incubation at 41.5±0.5 °C for 24 h. Afterwards, 10 µl loopful from each incubated broth was streaked onto Xylose Lysine Desoxycholate agar (XLD). All the inoculated plates were incubated at 37 °C for 24 h. The slightly transparent red colonies with black center on XLD agar were suspected as *Salmonella*. The characteristic colonies of *Salmonella*e were further streaked on nutrient agar plates and then incubated at 37 °C for 24 h for purification, and then on nutrient agar slopes for further identification and biochemical characterizations.

Morphological and biochemical identifications

The initial identification step was performed by using Gram's stain smears and oxidase test. The isolates revealing Gram's stain positive and/or oxidase positive were not included. The other isolates were biochemically investigated using indole, methyl red, Voges– Proskauer, citrate utilization, triple sugar iron (TSI), and urease tests (Ewing, 1986). The bacterial colonies revealing *Salmonella* specific IMViC pattern (– + – +) were further inoculated on TSI slants, and the bacterial colonies that revealed alkaline slant (pink) and acidic butt (yellow) with or without H2S production (blackening) were further tested for the urea hydrolysis on urea agar slant. The urease negative bacterial isolates were biochemically identified as *Salmonella* isolates (Chen *et al*., 2013).

Serological identification of Salmonella isolates

Somatic (O) and flagellar (H) antigens were used to identify all biochemically verified *Salmonella* isolates by slide agglutination with commercial antisera (SISIN, Berlin) following the Kauffman–White system (Popoff *et al*., 2004). The serological identifications were performed at the Serology Unit, Animal Health Research Institute, Dokki, Egypt, and the Bacteriology Laboratory, Central Laboratories of Ministry of Health, Egypt.

Antimicrobial susceptibility testing

Antimicrobial susceptibilities of *Salmonella* isolates were determined, in accordance with the guidelines of the Clinical and Laboratory Standards Institute. The antimicrobial discs and their concentrations as well as the diameters of the zones of inhibition for the tested strains were demonstrated. *Salmonella* isolates were tested by modified Kirby-Bauer disk diffusion method on Müller-Hinton Agar as per CSLI recommendations (CSLI, 2018). The antibiotics tested in this study include Chloramphenicol (30 µg), Azetronam (30 µg), Ciprofloxacin (5 µg), Tobramycin (10 µg), Amikacin (30 µg), Cefoxitin (30 µg), Sublactam (20 µg), Gentamycin (10 µg), Impenem (10 µg), Cefepime (30 µg), Ampicillin (10

µg), Doxycycline (30 µg) and Cefoperazone (75 µg). Interpretation the results of antibiotic susceptibility tests were carried out according to standard interpretative zone diameters suggested in CLSI guidelines (Vinueza-Burgos *et al*., 2019). The bacterial response to antibiotic was interpreted as: R: Resistant, I: Intermediate and S: Sensitive.

The multiple antibiotic resistance (MAR) index of *Salmonella* serotypes was calculated according to the formula stipulated by Singh *et al*. (2010) as the following equation:
MAR index = Number of antibiotics with resistance profile / the number of used antibiotics.

Genotypic detection of antimicrobial resistance genes

The extraction of DNA was carried out for the eight serotyped *Salmonella* using QIAamp DNA Mini Kit (Qiagen, Gm 6H, Hilden, Germany) according to the manufacturer kits. The oligonucleotide primer sequences and the PCR conditions for detection of bla_{TEM}, *flo*R and *tet*A(A) antimicrobial resistance genes were performed according to Colom *et al*. (2003); Doublet *et al*. (2003) and Randall *et al*. (2004), respectively (Table 1).

Statistical analysis

The Chi-2 test was done on contingency tables to investigate if there were significant differences between isolate sources in terms of isolate's incidence. The significance was recorded when P- value was <0.05. This analysis was done using GraphPad prism software version 8 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

RESULTS

Incidence and serotypes of Salmonella spp. in broiler chicken and human

Our study revealed that the overall incidence of *Salmonella* spp. was 3.75% (9 out of 240) as mentioned in Table 2 and Fig. 1. Regarding broilers, the incidence of *Salmonella* spp. was 5% (3 out of 60, each) in each of liver and intestine, while was 1.66% (1 out of 60) in gizzard of broilers (Table 2). There was no significant difference between organ source and the incidence of *Salmonella* infection (DF=3; P=0.7) as listed in Table 2. The incidence of *Salmonella* spp. was 3.33% (2 out of 60) in hand swabs of workers at poultry outlets. Regarding broilers, the serological identifications of *Salmonella*e were distinguished into S. Typhimurium (4 out of 7 isolates, 57.1%) and into S. Enteritidis (3 out of 7 isolates, 42.9%) as illustrated in Table 3. Only two isolates of S. Kentucky (100%) were detected in hand swabs of human workers (Table 3). From the 4 serotyped S. Typhimurium isolates, three isolates

Table 1. oligonucleotide sequences and PCR conditions for detection of antimicrobial resistance genes in *Salmonella* isolates recovered from broilers and workers.

Gene	Primer Sequence (5′–3′)	Size (bp)	PCR cycling conditions	Reference
bla_{TEM}	TEM-C ATCAGCAATAAACCAGC TEM-H CCCCGAAGAACGTTTTC	516	94˚C-5 min. 35 cycles (45˚C -30s,54˚C -40s,72˚C - 45s); 72˚C -10 min	Colom *et al*. (2003)
floR	cml01 TTTGGWCCGCTMTCRGAC cml15 SGAGAARAAGACGAAGAAG	494	94˚C- 5 min. 35 cycles (94˚C -30s, 50˚C -40s,72˚C - 45s) ;72˚C -10 min	Doublet *et al*. (2003)
*tet*A(A)	F GGTTCACTCGAACGACGTCA R CTGTCCGACAAGTTGCATGA	576	94˚C-5min. 35cycles (94˚C -30s,50˚C -40s,72˚C -45s); 72˚C -10min	Randall *et al.* (2004)

Table 2. Incidence of *Salmonella* species in broilers and workers in poultry outlets at Sharkia Province.

Sample source		No. of examined samples	No. of positive samples	%. of infection	X2 test	DF, P-value
Broilers	Liver	60	3	5	X2 = 1.2	3, 0.7
	Gizzard	60	1	1.66		
	Intestine	60	3	5		
workers	Hand swabs	60	2	3.33		
Total		240	9	3.75		

X2 test was done to test the association between organ source and incidence of infection. P- value were tested at a significance level of 0.05.

Table 3. Distribution of *Salmonella* serotypes in examined broiler liver, gizzard and intestine (n= 7 isolates) and in hand swabs of workers (n=2 isolates).

Serotypes from broilers	Antigenic formula	Broilers						Total	
		Liver		Gizzard		Intestine		No.	(%)
		No.	(%)	No.	(%)	No.	%		
S. Typhimurium	O: 1,4,5,12 H: i: 1,2	3	(75)	0	0	1	(25)	4	(57.1)
S. Enteritidis	O:1,9,12 H: g, m: -	0	(0)	1	(33.3)	2	(66.7)	3	(42.9)
Serotypes from workers	Antigenic formula	Hand swabs from workers						Total	
		No.		(%)				No.	(%)
S. Kentucky	O:8,20 H: i : Z6	2		(100)				2	(100)

were distributed in liver of broilers with a percentage of 75%, and one strain (25%) was detected in intestine of broilers (Table 3). The gizzard of broilers was free from S. Typhimurium. Out of the recovered three isolates of S. Enteritidis, one isolate (33.3%) was detected in gizzard, and 2 strains (66.7%) was distributed in intestine of broilers (Table 3). Notably, the liver of broilers was free from S. Enteritidis.

Phenotypic resistance and multiple antibiotic resistance of Salmonella serotypes

In this study, the phenotypic resistance of 9 *Salmonella* isolates from broilers and workers were investigated against 13 antimicrobials using the disk diffusion method as illustrated in Table 4. The peak resistance (100%) of 9 *Salmonella* isolates was detected to each of chloramphenicol and ampicillin followed by a highest resistance (88.8%, 8/9) to doxycycline and then moderate resistance of 44.4% (4/9) to cefepime and 33.3% (3/9) to cefoperazone (Table 4). Also, a lower resistance (11.1%, 1/9) was detected to each of azetronam ciprofloxacin sulbactam and gentamycin. In Table (4), all recovered *Salmonella* isolates from broilers and humans were 100 % sensitive to each of amikacin and impenem. Regarding the phenotypic resistance of *Salmonella* serotypes, all isolates of S. Typhimurium (4); S. Enteritidis (3) and S. Kentucky (2) showed resistance to chloramphenicol and ampicillin (Table 5). All isolates of S Typhimurium serotypes (4) and S. Enteritidis

Table 4. Antimicrobial susceptibility of 9 *Salmonella* isolates originated from examined broiler samples and hand swabs of workers at poultry outlets using disc diffusion method.

Antimicrobial code	R	I	S
(Disc concentration µg)	NO. (%)	NO. (%)	NO. (%)
C (30)	9(100)	0(0.00)	0(0.00)
ATM (30)	1(11.1)	1(11.1)	7(77.7)
CIP (5)	1(11.1)	5(55.5)	3(33.3)
TOB (10)	0(0.00)	4(44.4)	5(55.5)
AK (30)	0(0.00)	0(0.00)	9(100)
FOX (30)	3(33.3)	4(44.4)	2(22.2)
SAM (20)	1(11.1)	6(66.6)	2(22.2)
CN (10)	1(11.1)	1(11.1)	7(77.7)
IPM (10)	0(0.00)	0(0.00)	9(100)
FEP (30)	4(44.4)	1(11.1)	4(44.4)
AM (30)	9(100)	0(0.00)	0(0.00)
DO (30)	8(88.8)	1(11.1)	0(0.00)
CEP (75)	3(33.3)	0(0.00)	6(66.6)

No: Number , %: percentage R: Resistance; I: Intermediate; S: Sensitive; C: Chloramphenicol ATM: Azetronam; CIP: Ciprofloxacin; TOP: Tobramycin; AK: Amikacin; FOX:Cefoxitin; SAM: Sublactam; CN: Gentamycin; IPM: Impenem; FEP:Cefepime; ATM:Azetronam;CIP:Ciprofloxacin;TOP:Tobramycin; AK:Amikacin; FOX:Cefoxitin SAM:Sublactam;CN:Gentamycin; IPM:Impenem FEP: Cefepime; AM: Ampicillin; DO:Doxycycline ; CEP:Cefoperazone.

Table 5. The antimicrobial resistance/susceptibility of isolated *Salmonella* serotypes from broilers and human hand swabs; the data are represented by number of isolates.

Antimicrobials (Disc concentration µg)	S. Typhimurium (n.= 4)			S. Enteritidis (n.= 3)			S. Kentucky (n.= 2)		
	R	I	S	R	I	S	R	I	S
C (30)	4	0	0	3	0	0	2	0	0
ATM (30)	1	0	3	0	1	2	0	0	2
CIP (5)	1	1	2	0	2	1	0	2	0
TOB (10)	0	2	2	0	2	1	0	0	2
AK (30)	0	0	4	0	0	3	0	0	2
FOX (30)	2	2	0	1	1	1	0	1	1
SAM (20)	1	3	0	0	3	0	0	0	2
CN (10)	1	0	3	0	0	3	0	1	1
IPM (10)	0	0	4	0	0	3	0	0	2
FEP (30)	3	0	1	1	0	2	0	1	1
AM (30)	4	0	0	3	0	0	2	0	0
DO (30)	4	0	0	3	0	0	1	1	0
CEP (75)	3	0	1	0	0	3	0	0	2

R: Resistance; I: Intermediate; S: Sensitive; C: Chloramphenicol ATM: Azetronam; CIP: Ciprofloxacin; TOP: Tobramycin; AK: Amikacin; FOX:Cefoxitin; SAM: Sublactam; CN: Gentamycin; IPM: Impenem; FEP:Cefepime; ATM:Azetronam;CIP:Ciprofloxacin;TOP:Tobramycin; AK:Amikacin; FOX:Cefoxitin SAM:Sublactam;CN:Gentamycin; IPM:Impenem FEP: Cefepime; AM: Ampicillin; DO:Doxycycline ; CEP:Cefoperazone.

serotypes (3) showed maximum resistance to doxycycline, while only one isolate of S. Kentucky revealed resistance to doxycycline (Table 5). The resistance to each of cefoperazone and cefepime was detected in 3 out of 4 recovered S. Typhimurium serotypes, while the resistance to cefoxitin was detected in 2 out of 4 S. Typhimurium serotypes. Only one isolate out of 3 S. Enteritidis serotypes showed resistance to cefoxitin and cefepime, while only one strain of 2 S. Kentucky serotypes exhibited resistance to doxycycline (Table 5).

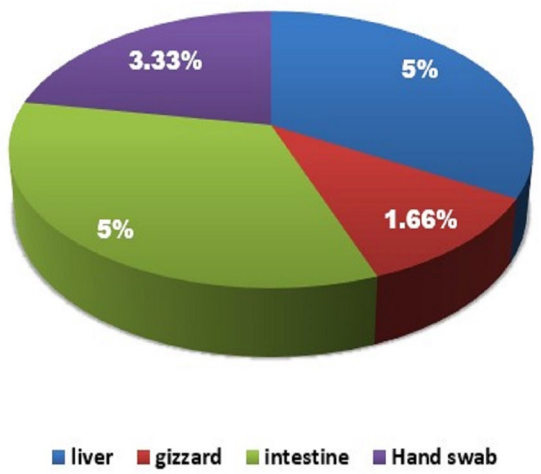

Fig. 1. Pie chart showing incidence of *Salmonella* species in liver, gizzard and intestinal samples from broilers and in hand swabs from workers at poultry outlets.

The multiple antibiotic resistances (MAR) index was determined for 9 *Salmonella* isolates based on the results of disc diffusion method. The MAR index of the isolates ranged from (0.23 to 0.54) with an average is 0.34 (Table 6). Also, from Table (6), the predominant MAR index (0.31) was found in 4 isolates of *Salmonella* (2 isolates of S. Typhimurium from liver, 1 isolate of S. Enteritidis from gizzard and 1 isolate of S. Typhimurium from liver), which were resistant to 4 antibiotics. One isolate of S. Enteritidis of intestine origin was found to have the highest MAR index of 0.54 which was resistant to 7 antimicrobials out of 13 tested antibiotics. Moreover, slightly higher MAR index (0.46) was detected in one isolate of S. Typhimurium from liver as such isolate was resistant to 6 out of 13 tested antibiotics (Table 6).

Genotypic detection of antimicrobial resistance genes

The genotypic identification of antimicrobial resistance genes [bla_{TEM}, *flo*R and *tet*A(A)] was molecularly identified in 9 *Salmonella* serotypes from broilers and human workers using conventional PCR with PCR products of 516, 494 and 576 bp, respectively (Fig. 2). The total distributions of antimicrobial resistance genes including bla_{TEM}, *tet*A(A) and *flo*R gene among *Salmonella* isolates were 88.9%, 66.7%& 88.9%, respectively (Table 7). Among 9 tested isolates, bla_{TEM}, *tet*A(A) and *flo*R genes were identified with similar distribution percentage of 66.7% (2 out of 3) in S. Typhimurium isolates of liver origin (Figs. 2A, 2B and 2C). Notably, all isolates of S. Enteritidis from gizzard, S. Typhimurium from intestine, S. Enteritidis from intestine and S. Kentucky from hand swabs harbored similar distribution percentage of 100% for each bla_{TEM}, *tet*A(A) and *flo*R gene (Fig. 2 and Table 7).

DISCUSSION

Salmonellosis is a serious bacterial infection that mostly affects poultry flocks. It poses a serious threat to food safety (Vinueza-Burgos *et al*., 2019). *Salmonella* is also one of the most common zoonotic infections found in food (WHO, 2016). *Salmonella*e infections in chicken can be divided into two categories: non-motile serotypes such as S. Pullorum and S. Galli-

Table 6. Antimicrobial resistance profile and MAR Index of *Salmonella* isolates from broilers and hand swabs from workers (n=9).

Samples source	*Salmonella* Strain code	Resistance pattern	No. of antibiotics showing resistance	No. of Isolates (%)	Resistance profile	MAR index
Liver (3 isolates)	S1	R1	4	1(33.3)	C, DO, CIP, AM	0.31
	S2	R2	6	1(33.3)	C, CN, DO, SAM, AM, FEB	0.46
	S3	R3	4	1(33.3)	C, FOX, DO, AM	0.31
Gizzard (1 isolate)	S4	R4	4	1(100)	C, AM, FEB, CEP	0.31
Intestine (3 isolates)	S5	R5	4	1(33.3)	C, DO, AM, CEP	0.31
	S6	R6	7	1(33.3)	ATM, C, FOX, DO, AM, FEB, CEP	0.54
	S7	R7	3	1(33.3)	C, DO, AM	0.23
Hand swabs (2 isolates)	S8	R8	5	1(50)	C, FOX, DO, AM, FEB	0.39
	S9	R9	3	1(50)	C, DO, AM	0.23

No.: Number; %: percentage; MAR index: multiple antibiotic resistance, S: *Salmonella* strain code; R: resistance profile. S1, S2&, S3: 3 isolates of S. Typhimurium from liver. S4: S. Enteritidis from gizzard. S5: S.Typhimurium from intestine. S6&S7: 2isolates of S. Enteritidis from intestine. S8&S9:2 isolates of. S. Kentucky from hand swabs.

Table 7. Distribution of antimicrobial resistance genes in *Salmonella* isolated from broilers and workers (n=9 isolates)

Source of isolates	*Salmonella* serotype (No.)	bla_{TEM} gene		*tet*A(A) gene		*flo*R gene	
		No.	(%)	No.	(%)	No.	(%)
Liver	S. Typhimurium (3)	2	-66.7	2	-66.7	2	-66.7
Gizzard	S. Enteritidis (1)	1	-100	0	0	1	-100
Intestine	S. Typhimurium (1)	1	-100	0	0	1	-100
	S. Enteritidis (2).	2	-100	2	-100	2	-100
Hand swabs	S. Kentucky (2)	2	-100	2	-100	2	-100
Total distribution	*Salmonella* isolates (9)	8	-88.9	6	-66.7	8	-88.90%

narum, which cause pullorum illness and fowl typhoid, respectively (Barrow and Neto, 2011). The second type of infection is caused by a group of motile *Salmonella* serotypes known as paratyphoid *Salmonella*e. Human salmonellosis outbreaks have been linked to the ingestion of poultry products infected with *Salmonella* Enteritidis and *Salmonella* Typhimurium (Vose *et al*., 2011). The current study showed that there was no significant difference between organ source and incidence of *Salmonella* infection (P=0.7). Our study revealed that that the overall incidence of *Salmonella* spp. was 3.75%. Regarding the incidence in broiler chickens, *Salmonella* spp. was distributed in each of liver and intestine with a prevalence rate of 5%; while it was 1.66% in gizzard of broilers. Nearly similar incidence of *Salmonella* (2.55%) was detected in healthy broiler flocks in Kalyobia, Egypt (Abd El-Ghany *et al*., 2012). Also, our study was nearly close to the finding of Suresh *et al*. (2011), where they reported the prevalence of *Salmonella* in various body parts of marketed broiler chickens in Southern India as the following: 1.40% in cloaca; 6.90% in crop, 5.05% in ceca and 4.04% in intestine. Also, our study was consistent with previous studies that recorded lower prevalence rates of *Salmonella* in broiler chickens:.7.14% in Egypt (Hassan *et al*., 2021); 10% in Sharkia, Egypt (Gharieb *et al*., 2015) and 9.2% in broiler chickens (Al-Abadi and AL-Mayah, 2012). In El-Gharbia, ElBehera, Kafr-Elshikh, Alexandria and MarsaMatrouh Provinces in Egypt *Salmonella* was isolated with a total distribution of 7.5%; and 9% from liver and 9% from intestine of broiler chicks (Sedeik *et al*., 2019). Also, nearly similar finding of 7.8% was recorded by Shang *et al*. (2018). In total 615 samples collected from intestine, liver and gall bladder, 67 (10.9%) *Salmonella* strains were isolated from 41 broiler chicken flocks in Kafr El-Sheikh Province in Delta Egypt (El-Sharkawy *et al*., 2017). Our study reported an incidence rate of *Salmonella* (3.33%) in hand swabs of workers at poultry outlets. This finding was nearly similar to findings of Hassan *et al*. (2016) and Gharieb *et al*. (2015), where they cited a prevalence of 4% in human stool in Egypt. The lower incidence of *Salmonella* in broiler chickens and workers at poultry outlets in the present study indicated moderate hygienic measures observed in poultry markets at Sharkia Province during slaughtering and evisceration. Also, the cross contamination from workers' hands, equipment and utensils used during carcass preparation, subsequent handling of the raw poultry carcasses and ready-to-eat products together with the ingestion of improperly cooked poultry meat could act as the most frequent sources of infection by *Salmonella* reported in humans (Saeed *et al*., 2013; Yildirim *et al*., 2010).

On the contrary, higher prevalence rates of *Salmonella* was detected in broiler meat, skin, and pooled giblets (liver, gizzard, and heart) was 76, 80, and 64%, respectively, in Benisuef Province, Egypt (Hassan *et al*., 2016). Also, Roshdy *et al*. (2020) cited the highest isolation rates of *Salmonella* spp. were recovered from broilers of 1-7 days – old in summer (40%) followed by broilers of more than 7 days old in summer (33.3%). In addition, previous studies recorded higher incidence of *Salmonella* in broiler chickens: 30% (Temelli *et al*., 2012); 49.9% (Islam *et al*., 2014); 32.6% in Taiwan (Lin *et al*., 2021); 31.5% in Brazil (Perin *et al*., 2020); 15.6% in Beheira Governorate, Egypt (Ammar *et al*., 2019). The high prevalence of *Salmonella* spp. in the previous studies comparable to the present study could attributed to the low hygienic measures noticed in the poultry retail markets during slaughtering, scalding, defeathering, evisceration, carcass cutting and handling. These procedures permit cross contaminations from diseased birds or contaminated carcass to healthy and clean ones. In addition, the shortage of veterinary supervisions inside these poultry markets might lead to slaughtering of diseased chickens (Hassan *et al*., 2016).

Regarding recovered serotypes of *Salmonella*e from broilers in our study, four isolates out of seven were identified into S. Typhimurium (57.1%) and 3 isolates out of seven were distinguished into S. Enteritidis (42.9%). Only two isolates of S. Kentucky (100%) were detected in hand swabs of workers at poultry outlets. The distribution percentage of S. Typhimurium was 75% (3 out of 4) in liver of broilers and was 25% (1 out of 4) in intestine

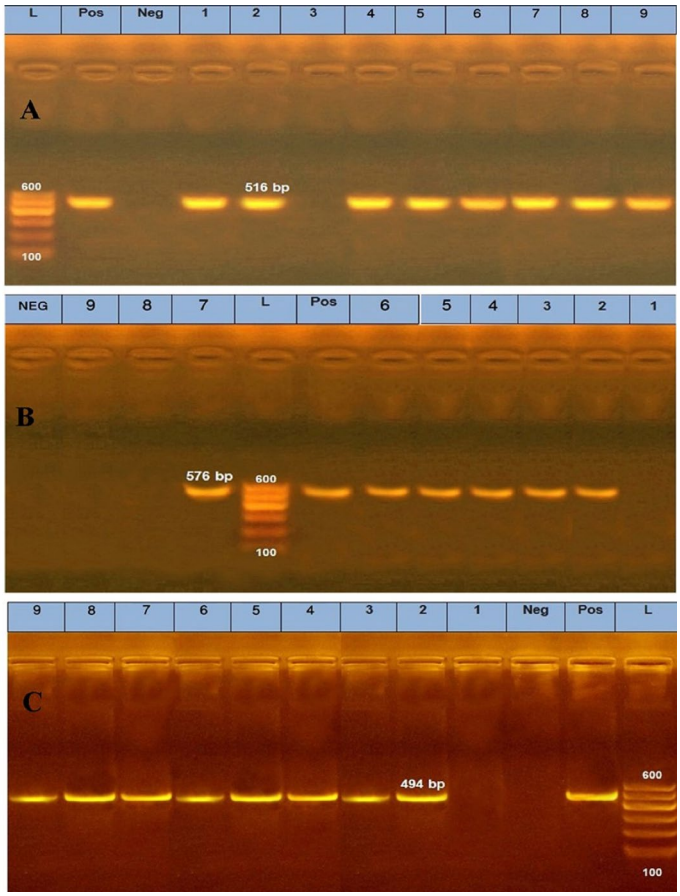

Fig. 2. Agarose gel electrophoresis of antimicrobial resistance genes in *Salmonella* isolates recovered from broilers and workers.
2A) Molecular detection of bla_{TEM} gene in *Salmonella* isolates using PCR. L: ladder 100bp; Pos: positive control of *Salmonella* Typhimurium carrying bla_{TEM} gene; Neg: negative control; lanes 1&2 :positive S. Typhimurium isolate of liver origin for bla_{TEM} gene (516 bp); lane 3 : negative S.Typhimurium isolate of liver origin; Lane 4: Positive S. Enteritidis isolates from gizzard; lanes 5&6:positive S. Enteritidis isolates from intestine, lane 7: positive S.Typhimurium from intestine; lanes 8&9: positive S. Kentucky from hand swabs of workers.
2B) Molecular detection of *tet*A(A) gene in *Salmonella* isolates using PCR. L: ladder 100bp; Pos: positive control of *Salmonella* Typhimurium carrying *tet*A(A) gene; Neg: negative control; lane 1: negative S. Typhimurium of liver origin; lanes 2&3: positive S. Typhimurium isolates from liver bearing *tet*A(A) gene (576 bp); lanes 4&5: positive S. Enteritidis isolates from intestine; lanes 6&7: positive S. Kentucky from hand swabs; lanes 8&9: negative S. Enteritidis and S. Typhimurium from gizzard and intestine, respectively.
2C) Molecular detection of *flo*R gene in *Salmonella* isolates using PCR. L: ladder 100bp; Pos: positive control of *Salmonella* Typhimurium carrying *flo*R gene; Neg: negative control; lane 1: negative S. Typhimurium of liver origin, lanes 2&3: lanes 2&3: positive S. Typhimurium isolates from liver bearing *flo*R gene (494 bp); lanes 4&5: positive S. Enteritidis and S. Typhimurium from gizzard and intestine, respectively; lanes 6&7: positive S. Enteritidis from intestine; lanes 8&9: positive S. Kentucky from hand swabs

of broilers. While S. Enteritidis serotypes was distributed as 33.3% (1 out of 3) in gizzard and into 66.7% (2 out of 3) in intestine of broilers. In Egypt, Ammar *et al*. (2019) detected S. Enteritidis, S. Infantis, S. Kentucky, S. Maloma , S. Bardo and S. Typhimurium from broiler chickens with the percentages of 43.3, 16.6, 16.6, 6.7, 6.7 and 3.3% respectively. Moreover, the serotype S. Enteritidis was isolated from two samples (2%), while S. Typhimurium was isolated from three samples (3%) of chicken meat in Egypt (Tarabees *et al*., 2017). In Egypt, out of 10 *Salmonella* enterica isolates, 3 serotypes were identified into 4 isolates of *Salmonella* Kentucky (40%) followed by *Salmonella* Blegdam and *Salmonella* Virchow; 3 isolates ;30% for each (1). Serotyping of *Salmonella* isolates from broiler carcasses revealed S. Enteritidis (5 isolates), S. Typhimurium (3 isolates), S. Infantis, S. Bargny, S. Newport, S. Magherafelt (2 isolates) and lastly S. Apeyeme in (1 isolate) as was formerly reported by Roshdy *et al*. (2020). Besides, Recovered *Salmonella* strains were serotyped as 58 (86.6%) S. Typhimurium, 6 (9%) S. Enteritidis (El-Sharkawy *et al*., 2017). In Beni Suef, Egypt; the predominant serotype in broiler carcasses was *Salmonella* Infantis (56.36%) followed by *Salmonella* Kentucky (25.45%), and then *Salmonella* Enteritidis with a percentage of 5.45% (Hassan *et al*., 2016). The recovered serotypes S. Typhimurium and S. En-

teritidis from Broiler chickens in the present study indicated the great zoonotic and public health importance of such *Salmonella*e and the possibility for transmission to human workers at poultry outlets during the defeathering, slaughtering and evisceration process of broiler chickens. So, collaboration between human and veterinary practitioners is very crucial to increase the awareness and education toward salmonellosis in broilers and humans. Therefore, it is an urgent necessity for strengthening environmental and behavioral intervention plans to minimize the burden of *Salmonella* infections in broiler chickens and their products at poultry outlets (Abd EL-Ghany, 2020).

One of the main causes of the rise of multidrug resistance bacteria is the improper use of antibiotics in poultry farms in Egypt; and these multidrug-resistant bacteria, which include both S. Typhimurium and S. Enteritidis, have the ability to infect humans, resulting in systemic infections (Ma *et al*., 2007). In the current study, the peak resistance (100%) of 9 *Salmonella* isolates was found to chloramphenicol and ampicillin followed by a highest resistance (88.8%) to doxycycline and then moderate resistance of 44.4% to cefepime and 33.3% to cefoperazone and finally a lower resistance (11.1%) was detected to azetronam, ciprofloxacin sulbactam and gentamycin. It was clear that nine *Salmonella* isolates from broilers and humans were 100 % sensitive to amikacin and impenem. In Egypt, *Salmonella* isolates showed high resistance to cefuroxime (100%), nalidixic acid (93%) and amoxicillin/clavulanic acid (83%). while the resistance to cefepime was (53%), streptomycin (40%), sulfamethaxezole/trimethoprim (40%), ampicillin (37%), doxycycline (37%) and 30% to gentamicin. Besides, all strains were sensitive to amikacin and norfloxacin (100%); followed by kanamycin (97%) and cefotaxime (83%) (Ammar *et al*., 2019). In Benisuef, Egypt, *Salmonella* Kentucky strains showed high rates of resistance against the majority of the used antimicrobials, where 100% were resistant to ciprofloxacin, ampicillin, nalidixic acid and tetracycline; moreover, 85.7% displayed resistance against both of cefotaxime and ceftazidime. while few of them were found sensitive to some antimicrobials such as amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime and aztreonam, while 71.4% isolates of *Salmonella* Kentucky were susceptible to amikacin; and *Salmonella* Enteritidis, it was sensitive to all tested antimicrobials except nalidixic acid (Hassan *et al*., 2016). In a study carried out in South African by Zishiri *et al*. (2016), *Salmonella* isolates from chickens exhibited resistance to tetracycline (93%), trimethoprim–sulfamethoxazole (84%), gentamicin (48%), ampicillin (47%), chloramphenicol (31%), and streptomycin (12%). Concerning the resistance of *Salmonella* serotypes, 89.7% of S. Typhimurium isolates were susceptible to streptomycin, and 94.8% of S. Typhimurium strains were sensitive to trimethoprim/sulphamethoxazole, and 5.2% of isolates were resistant to ampicillin, chloramphenicol, and tetracycline; while all S. Enteritidis isolates were sensitive to all tested antimicrobial agents (El–Sharkawy *et al*., 2017).

Our study was contradicted to the finding of Cardoso *et al*. (2006), who reported 100% of *Salmonella* isolates sensitive to doxycycline. Our results agree with Snow *et al*. (2007) who reported that all *Salmonella* isolates from commercial layer flocks in UK were sensitive to amikacin. Our results could be attributed to the fact that these antimicrobials of low efficiency are cheap, easily affordable, and frequently used for humans and poultry without medical prescription, so it could be used with incorrect doses. In poultry, these antibiotics are used either for therapeutic purposes or as growth promoting feed additives, that result in the development of resistance in the enteric microflora of poultry. Therefore, the pathogenic microorganisms such as *Salmonella* acquire resistance from this microflora and transfer it to the human strains through food chain, which helps to the appearance of multidrug resistant *Salmonella*e that represent a public health risk and potentially affect the efficacy of medications in humans (Gharieb *et al*., 2015; Hassan *et al*., 2016). The high prevalence of resistant or multi-resistant *Salmonella* isolates in the present study might be attributed to the widespread and excessive use of antimicrobials in veterinary medicine fields, including food and animal production. In Egypt, some of these antimicrobials have been used on poultry farms as growth-promoters, thus cross-resistance or co-resistance mechanisms could be the etiology of the resistance noticed to the drugs (Capita *et al*., 2013).

In our study, the resistance profile of S. Typhimurium of poultry origin was 100% for each of ampicillin, chloramphenicol and doxycycline then followed by 75% to cefoperazone and 50% to cefoxitin and lastly a lower resistance (25%) to gentamicin, ciprofloxacin and azetronam. This result agreed with the finding of El-Sharkawy *et al*. (2017), where S. Typhimurium isolated from poultry in Egypt exhibited maximum resistance (100%) to ampicillin, chloramphenicol and tetracycline. However, S. Typhimurium in the current study showed the maximum sensitivity (100%) to imipenem. On the contrary, the resistance profile of S. Typhimurium isolated from poultry droppings and humans in Nigeria was 93.4% to ampicillin, 69.8% to ceftriaxone and 1.0% to imipenem (Ibrahim *et al*., 2022). It was evident from our study that S. Enteritidis isolates showed a peak resistance (100%) to ampicillin, chloramphenicol and doxycycline followed by a lower resistance (33.3%) to cefoxitin and cefepime. Similarly, the antimicrobial resistance profile of S. Enteritidis isolated from chicken in Egypt displayed the peak resistance (100%) to ampicillin and tetracyclines (Abdelaziz *et al*., 2020). Otherwise, S. Enteritidis isolates of poultry origin were sensitive to all antimicrobial agents in Egypt (El-Sharkawy *et al*., 2017). Also in this study, the resistance profile of S. Kentucky of human origin was 100% to chloramphenicol and ampicillin followed by a moderate resistance of 50% to doxycycline. In Morocco, S. Kentucky isolates were multi-resistant to amoxicillin, tetracycline and chloramphenicol (Karraouan *et al*., 2017). This study pointed out that existence of multidrug resistant isolates of *Salmonella* requires the wisdom through the application of such antimicrobials to poultry to decrease the emergence of MDR human pathogens (Abdelaziz *et al*., 2020).

MAR indexing is used as a necessary tool for risk assessment in identifying the contamination from high-risk sources (CLSI, 2017). In the current study, the MAR index of *Salmonella* isolates was ranged from 0.23 to 0.54 with an average of 0.34. Moreover, the predominant MAR index (0.31) was found in 4 isolates of *Salmonella* which were resistant to 4 antibiotics. One isolate of S. Enteritidis of intestine origin was found to have the highest MAR index of 0.54 which was resistant to 7 antimicrobials. Moreover, slightly higher MAR index (0.46) was detected in one isolate of S. Typhimurium from liver as such isolate was resistant to 6 antibiotics. Our finding was in agreement with similar studies that reported MAR index greater than 0.2. In India, MAR index of *Salmonella* enterica isolates from poultry was ranged from 0.06 to 0.56 with 0.37 being the predominant in 8 strains resistant to 6 different antimicrobials (Khan *et al*., 2015). In Nigeria, MAR index for S. Typhimurium strains was higher than 0.2 (Ibrahim *et al*., 2022). In Egypt, MAR indexes of S. Enteritidis and S. Typhimurium isolated from broiler chickens were 0.5 and 0.83, respectively (Tarabees *et al*., 2019). In Iran, MAR index was ranged from 0.45-0.81 with an average of 0.63 in 4 *Salmonella* isolates (Mir *et al*., 2022). In South Africa, the value of MAR index was 0 to 0.87 with the predominant index being 0.31 in fecal and environmental samples recovered from chicken, duck, pig, sheep and cattle (Mthembu *et al*., 2019). On the contrary, the lowest value of MAR index was detected in 3 isolates of S. Enteritidis with 0.1 in Egypt (Hassan *et al*., 2016). Therefore, higher MAR index than 0.2 for S. Typhimurium and S. Enteritidis of poultry origin in the present study indicated that those *Salmonella* isolates were gained from a high risk and contaminated sources where antimicrobials are frequently used for therapy or as growth promotors in the feed additives (Khan *et al*., 2015).

The present study revealed the overall distribution of antimicrobial resistance genes [bla_{TEM}, *flo*R and *tet*A(A)] in *Salmonella* enterica serovars recovered from poultry and workers were 88.9% (8/9), 66.7% (6/8) & 88.9% (8/9), respectively. This finding was nearly in accordance with previous studies: Aziz *et al*. (2018) detected frequency distribution of bla_{TEM} and *tet*A(A) gene were 83.3% and 91.7%, respectively in Egypt; and Das *et al*. (2022) cited

distribution percentage of 95.4% for *bla*$_{TEM}$ and 81.4% for *tet*A gene in Bangladesh. However, higher distribution frequency of *bla*$_{TEM}$ and *tet*A(A) gene (100%, for each) in *Salmonella* isolates in Egypt was reported (Ezzat *et al*., 2019). In India, *tet*A and *bla*$_{TEM}$ gene in *Salmonella* isolates had lower distribution percentages of 56.7% and 30%, respectively (Inbaraj *et al*., 2022). In Pakistan, Khan *et al*. (2019) found that the frequency *bla*$_{TEM}$ (76%) and *tet*A (64%) was lower compared to the present study.

In the present study, *bla*$_{TEM}$ and *flo*R genes were distributed in 3 out of 4 S. Typhimurium isolates (75%, each) of from poultry sources; however, *tet*A (A) gene was only detected in 2 out of 3 S. Typhimurium isolates from liver with a frequency of 66.7%. *bla*$_{TEM}$ and *flo*R genes were the most predominant resistance genes detected in all 3 isolates of S. Enteritidis (100%) from gizzard and intestine sources of poultry, while *tet*A(A) gene was only distributed (100%) in 2 isolates of S. Enteritidis of intestinal origin. Also, S. Kentucky from hand swabs harbored similar distribution percentage of 100% for each *bla*$_{TEM}$, *tet*A(A) and *flo*R gene. In Egypt, *bla*$_{TEM}$ gene was detected in 11/15 S. Typhimurium isolates of duck source with frequency distribution of 73.3% followed by lower distribution (46.7%,7/15) for *flo*R gene (Khalifa *et al*., 2021). Also, 84.5% of S. Typhimurium and 50% of S. Enteritidis isolates of poultry origin in Egypt were harboured *tet*A (A) gene (El Sharkawy *et al*., 2017). However, S. Typhimurium was detected to harbour *bla*$_{TEM}$ and *tet*A gene with distribution percentages of 94.9% and 84.1%, respectively; while S. Enteritidis harbored lower distribution percentage of 48.8% for *bla*$_{TEM}$ and of 73.8% for *tet*A gene in Pakistan (Khan *et al*., 2019). Our study showed that the higher distribution of *bla*$_{TEM}$ and *tet*A(A) resistance genes in S. Typhimurium and S. Enteritidis isolates, that were phenotypically resistant to ampicillin and tetracyclines, reflects the common use of ampicillin and tetracyclines during poultry production for controlling bacterial infection and for promotion poultry growth (Aslam *et al*., 2012); and therefore the existence of these resistance genes on genetic mobile elements could facilitate their transfer (Schwarz *et al*., 2005).

CONCLUSION

The incidence of S. Typhimurium and S. Enteritidis in broiler chickens reflects the possibility of cross contamination from workers' hands, equipment and utensils used during carcass preparation and evisceration, subsequent handling of the raw poultry carcasses and constitutes a zoonotic hazard. The high prevalence of MDR *Salmonella*e in the present study could be attributed to the widespread and excessive use of antimicrobials on poultry farms as growth-promoters. Also, higher MAR index than 0.2 for S. Typhimurium and S. Enteritidis of poultry origin reflected higher contamination sources where antibiotics are excessively used for therapy and growth promoters. Besides, higher distribution of *bla*$_{TEM}$ and *tet*A(A) resistance genes in S. Typhimurium and S. Enteritidis isolates indicated the common use of ampicillin and tetracycline in broiler poultry farms. Further intervention studies are recommended to minimize the circulation of MDR strains of zoonotic *Salmonella*e from broiler chickens and their products at poultry outlets, and to predict biological tools such as bacteriophages during poultry production to mitigate entry MDR *Salmonella*e from broiler chicken to human food chain.

ACKNOWLEDGMENTS

The authors wish to thank staff members of Zoonoses Department, Faculty of Veterinary Medicine Zagazig University, Egypt for their support to achieve the practical work of the study at their Laboratory of Zoonoses Department.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abdelaziz, I.S., El-Tawab, A., Awad, A., Maarouf, A.A., Elhofy, F.I., 2020. Bacteriological and molecular studies on *Salmonella* isolated from duckling farms at Kaliobia, Egypt. Benha Vet. Med. J. 39,169-174.

Abd El-Ghany, W.A., El-Shafii, S.S., Hatem, M.E., 2012. A survey on *Salmonella* species isolated from chicken flocks in Egypt. Asian J. Anim. Vet. Adv. 7, 489-501.

Abd El-Ghany, W.A., 2020. Salmonellosis: A food borne zoonotic and public health disease in Egypt. J. Infect. Dev. Ctries. 14, 674-78.

Abd El-Tawab, A., El-Hofy, F.I., Ammar, A.M., Nasef, S.A., Nabil, N.M., 2015. Molecular studies on antimicrobial resistance genes in *Salmonella* isolated from poultry flocks in Egypt. Benha Vet. Med. J. 28, 176-87.

Adzitey, F., 2015. Antibiotic resistance of Escherichia coli isolated from beef and its related samples in Techiman Municipality of Ghana. Asian J. Anim. Sci. 9, 233-40.

Al-Abadi, I.K.M., AL-Mayah, A.A.S., 2012. Isolation and identification of *Salmonella* spp. from chicken and chicken environment in Basrah Province. Afr. J. Biol. Sci. 7, 33-43.

Ammar, A.M., Abdeen, E.E., Abo-Shama, U.H., Fekry, E., Kotb E., 2019. Molecular characterization of virulence and antibiotic resistance genes among *Salmonella* serovars isolated from broilers in Egypt. Lett. Appl. Microbiol. 68, 188-195.

Ammar, A.M., Mohamed, A.A., Abd El-Hamid, M.I., El-Azzouny, M.M., 2016. Virulence genotypes of clinical *Salmonella* serovars from broilers in Egypt. J. Infect. Dev. Ctries. 10, 337-46.

Ammar, A.M., Ahmed, Y.A.E., Asawy, A.M.I., Ibrahim, A.A., 2009. Bacteriological studies on *Salmonella* Enteritidis isolated from different sources in Dakhlia Governorate. Assiut Vet. Med. J. 56, 125-135.

Antunes, P., Mourão, J., Campos, J., Peixe, L., 2016. Salmonellosis: the role of poultry meat. Clin. Microbiol. Infect. 22, 110-21.

Aslam, M., Checkley, S., Avery, B., Chalmers, G., Bohaychuk, V., Gensler, G., Reid-Smith, R., Boerlin, P., 2012. Phenotypic and genetic characterization of antimicrobial resistance in *Salmonella* serovars isolated from retail meats in Alberta, Canada. Food Microbiol. 32,110-17.

Aziz, S.A.A., Abdel-Latef, G.K., Shany, S.A., Rouby, S.R., 2018. Molecular detection of integron and antimicrobial resistance genes in multidrug resistant *Salmonella* isolated from poultry, calves and human in Beni-Suef governorate, Egypt. Beni-Suef Univ. J. Basic Appl. Sci. 7, 535-42.

Barrow, P.A., Neto, O.F., 2011. Pullorum disease and fowl typhoid—new thoughts on old diseases: a review. Avian Pathol. 40,1-13.

Bhunia, A.K., 2018. *Salmonella* enterica. In Foodborne Microbial Pathogens. Springer, New York, NY. pp. 271-287.

Bouchrif, B., Pagliett,i B., Murgia, M., Piana, A., Cohen, N., Ennaji, M.M., Timinouni, M., 2009. Prevalence and antibiotic-resistance of *Salmonella* isolated from food in Morocco. J. Infect. Dev. Ctries. 3, 35-40.

Capita, R., Alonso-Calleja, C., 2013. Antibiotic-resistant bacteria: a challenge for the food industry. Crit. Rev. Food Sci. Nutr. 53, 11-48.

Cardoso, M.O., Ribeiro, A.R., Santos, L.R.D., Pilotto, F. de Moraes, H.L., Salle, C.T.P, Nascimento, V.P.D., 2006. Antibiotic resistance in *Salmonella* Enteritidis isolated from broiler carcasses. Braz J Microbiol. 37, 368-371.

Chaudhry, R., Mahajan, R.K., Diwan, A., Khan, S., Singhal, R., Chandel, D.S., Hans, C., 2003. Unusual presentation of enteric fever: three cases of splenic and liver abscesses due to *Salmonella* Typhi and *Salmonella* Paratyphi A. Trop Gastroenterol. 24,198-199.

Chen, L., Zhang, J., Yang, X., Wu, Q., Xu, M., 2013. Prevalence and characterization of *Salmonella* spp. from foods in South China. Wei Sheng Wu Xue Bao. 53, 1326-33.

CLSI (Clinical and Laboratory Standards Institute), 2017. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100.

Colom, K., Pérez, J., Alonso, R., Fernández-Aranguiz, A., Lariño, E., Cisterna, R., 2003. Simple and reliable multiplex PCR assay for detection of bla TEM, bla SHV and bla OXA–1 genes in Enterobacteriaceae. FEMS Microbiol lett, 223, 147-51.

CSLI (Clinical and Standards Laboratory Institutes), 2018. Performance standards for antimicrobial susceptibility testing.2018; M100S, (28 edition)., Wayne, PA, USA. 38.

Das, T., Rana, E.A., Dutta, A., Bostami, M.B., Rahman, M., Deb, P., Nath, C., Barua, H., Biswas, P.K., 2022. Antimicrobial resistance profiling and burden of resistance genes in zoonotic *Salmonella* isolated from broiler chicken. Vet Med Sci. 8, 237-44.

Davis, R., Brown, P.D., 2016. Multiple antibiotic resistance index, fitness and virulence potential in respiratory Pseudomonas aeruginosa from Jamaica. J Med Microbiol, 65, 261–271.

Doublet, B., Lailler, R., Meunier, D., Brisabois, A., Boyd, D., Mulvey, M.R., Chaslus-Dancla, E., Cloeckaert, A., 2003. Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster in *Salmonella* enterica serovar Albany. Emerg Infect Dis. 9, 585.

El-Shaboury, F.A., Basha, O.A.A., 2009. Epidemiological studies on salmonellosis in broiler chicken farms in Alexandria governorate. Assuit Vet Med J. 55, 1-10.

El-Sharkawy, H., Tahoun, A., El-Gohary, A.E.G.A, El-Abasy, M., El-Khayat, F., Gillespie, T., El-Adawy, H., 2017. Epidemiological, molecular characterization and antibiotic resistance of *Salmonella* enterica serovars isolated from chicken farms in Egypt. Gut Pathog. 9,1-12.

Ewing, W.H., 1986. Edwards and Ewing's Identification of Enterobacteriaceae. Elsevier; 4th edition.

Ezzat, M., Elsotohy, M., Esawy, A.E., Wahdan, A., 2019. Detection of some antibiotic resistant genes within *Salmonella* serovars isolated from broiler chickens. SCVMJ 24, 147-58.

Fàbrega, A., Vila, J., 2013. *Salmonella* enterica serovar Typhimurium skills to succeed in the host: virulence and regulation. Clin Microbiol Rev., 26, 308-341.

Gharieb, R.M., Tartor, Y.H., Khedr, M.H., 2015. Non-Typhoidal *Salmonella* in poultry meat and diarrhoeic patients: prevalence, antibiogram, virulotyping, molecular detection and sequencing of class I integrons in multidrug resistant strains. Gut Pathog. 7, 34.

Goldstein, C., Lee, M.D., Sanchez, S., Hudson, C., Phillips, B., Register, B., 2001. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrob. Agents Chemother. 45, 723–726.

Hassan, W.H., Hassan, H.S., Hassan, W.M., Shany, S.A., Osman, G.S., 2021. Identification and Characterization of *Salmonella* species isolated from broiler chickens. J. Vet. Med. Res. 28, 21-29.

Hassan, A.R.H., Salam, H.S., Abdel-Latef, G.K., 2016. Serological identification and antimicrobial resistance of *Salmonella* isolates from broiler carcasses and human stools in Beni-Suef, Egypt. Beni-Suef Univ J Basic Appl Sci. 5, 202-207.

Ibrahim, T., Ngwai, Y.B., Ishaleku, D., Tsaku, P.A., Nkene, I.H., Abimiku, R.H., 2022. Detection of extended spectrum beta-lactamase (ESBL)–production in *Salmonella* Typhimurium isolated from poultry birds in Nasarawa State, Nigeria. Sci. Afri. 16, e01243.

Inbaraj, S., Agrawal, R.K., Thomas, P., Mohan, C., RK, S.A., Verma, M.R., Chaudhuri, P., 2022. Antimicrobial resistance in Indian isolates of non typhoidal *Salmonella* of livestock, poultry and environmental origin from 1990 to 2017. Compar. Immunol. Microbiol. Infect. Dis. 80, 101719.

ISO (International Organization for Standardization), 2002. ISO-6579.Microbiology--General guidance on methods for the detection of *Salmonella*. https://www.iso.org/standard/12985.html

Islam, M.M., Islam, M.N., Sharifuzzaman F.M., Rahman, M.A., Sharifuzzaman, J. U, Sarker, E.H., Shahiduzzaman, M., Mostofa, M., Sharifuzzaman, M.M., 2014. Isolation and identification of Escherichia coli and *Salmonella* from poultry litter and feed. Int. J. Nat. Soc. Sci. 1, 1-7.

Jackson, B.R., Griffin, P.M., Cole, D., Walsh, K.A., Chai, S.J., 2013. Outbreak-associated *Salmonella* enterica serotypes and food commodities, United States, 1998-2008. Emerg Infect Dis. 19, 1239-1244.

Karraouan, B., Ziyate, N., Ed-Dra, A., Amajoud, N., Boutaib, R., Akil, A., El Allaoui, A., El Ossmani, H., Zerouali, K., Elmdaghri, N., Bouchrif, B., 2017. *Salmonella* Kentucky: Antimicrobial resistance and molecular analysis of clinical, animal and environment isolates, Morocco. The Journal of Infection in Developing Countries 11, 368-370.

Khalifa, Z.K.M., Ibrahim, A.A.E.H., Abd El-motelib, T.Y., Abd El-Aziz, A.M., 2021. Molecular characterization of antibacterial resistance genes of *Salmonella* in ducks. Assuit Vet. Med. J. 6752-66.

Khan, J.A., Irfan, A.M., Soni, S.S., Maherchandani, S., Soni, S. S., Maherchandani, S., 2015. Antibiogram and multiple antibiotic resistance index of *Salmonella* enterica isolates from poultry. Journal of Pure and Applied Microbiology 9, 2495–2500.

Khan, S.B., Khan, M.A., Ahmad, I., ur Rehman, T., Ullah, S., Dad, R., Sultan, A., Memon, A.M., 2019. Phentotypic, gentotypic antimicrobial resistance and pathogenicity of *Salmonella* enterica serovars Typimurium and Enteriditis in poultry and poultry products. Microbial Pathogen. 129, 118-124.

Krumperman, P.H., 1983. Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. Appl Environm Microbiol. 46, 165-170.

Lebdah, M.A., Mohammed, W.M., Eid, S., Hamed, R.I., 2017. Molecular detection of some antimicrobial resistance genes in *Salmonella* species isolated from commercial layers in Egypt. Zag. Vet. J. 45, 29-38.

Lin, C.H., Adams, P.J., Huang, J.F., Sun, Y.F., Lin, J.H., Robertson, I.D., 2021. Prevalence and risk factors for *Salmonella* spp. contamination of slaughtered chickens in Taiwan. Prev. Vet. Med. 196,105476.

Lu, J., Sanchez, S., Hofacre, C., Maurer, J.J., Harmon, B.G., Lee, M.D., 2003. Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. Appl. Environ. Microbiol. 69, 901-908.

Ma, M., Wang, H., Yu, Y., Zhang, D., Liu, S., 2007. Detection of antimicrobial resistance genes of pathogenic *Salmonella* from swine with DNA microarray. J. Vet. Diagn. Invest. 19,161-7.

Mir, R., Salari, S., Najimi, M., Rashki, A., 2022. Determination of frequency, multiple antibiotic resistance index and resistotype of *Salmonella* spp. in chicken meat collected from southeast of Iran. Vet. Med. Sci. 8, 229-36.

Mohamed, F., Mohamed, M., Shata, N., Manaa, A., 2009. Detection and identification of *Salmonella* isolated from chickens by PCR. Assiut Vet. Med J. 55, 211-225.

Mthembu, T.P., Zishiri, O.T., El Zowalaty, M.E., 2019. Molecular detection of multidrug-resistant *Salmonella* isolated from livestock production systems in South Africa. Infect Drug Resist. 12, 3537-48.

Nandi, S., Maurer, J.J., Hofacre, C., Summers, A.O., 2004. Gram positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. Proc. Natl. Acad. Sci. U S A. 101, 7118-22.

Okeke, I.N., Laxminarayan, R., Bhutta, Z.A., Duse, A.G., Jenkins, P., O'Brien, T.F, Klugman, K.P., 2005. Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lancet Infect. Dis. 5, 481-493.

Parveen, S., Murphree, R.L., Edmiston, L., Kaspar, C.W., Portier, K.M., Tamplin, M.L., 1997. Association of multiple-antibiotic-resistance profiles with point and nonpoint sources of Escherichia coli in Apalachicola Bay. Appl. Environ. Microbiol. 63, 2607–12.

Pavic, A., Groves, P.J., Bailey, G., Cox, J.M., 2010. A validated miniaturized MPN method, based on ISO 6579: 2002, for the enumeration of *Salmonella* from poultry matrices. J. Appl. Microbiol. 109, 25-34.

Perin, A.P., Martins, B.T.F., Barreiros, M.A.B., Yamatogi, R.S., Nero, L.A., dos Santos Bersot, L., 2020. Occurrence, quantification, pulse types, and antimicrobial susceptibility of *Salmonella* sp. isolated from chicken meat in the state of Paraná, Brazil. Braz. J. Microbiol. 51, 335-345.

Popoff, M.Y., Bockemühl, J., Gheesling, L.L., 2004. Supplement 2002 (no. 46) to the Kauffmann–White scheme. Res. Microbiol. 155, 568-570.

Randall, L.P., Cooles, S.W., Osborn, M.K., Piddock, L.J.V., Woodward, M.J., 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella* enterica isolated from humans and animals in the UK. J. Antimicrob Chemother, 53, 08-216.

Roshdy, H, Mohamed, S., Elsebaey, H., El-Demerdash, G., 2020. Effect of age and season on *Salmonella* infection in broiler chickens with special reference to virulence and antibiotic resistance genes. Zag. Vet. J. 48, 328-39.

Saeed, A.A., Hasoon, M.F., Mohammed, M.H., 2013. Isolation and molecular identification of *Salmonella* Typhimurium from chicken meat in Iraq. J. World's Poult Res. 3, 63-67.

Sallam, K. I., Mohammed, M. A., Hassan, M. A., Tamura, T., 2014. Prevalence, molecular identification and antimicrobial resistance profile of *Salmonella* serovars isolated from retail beef products in Mansoura, Egypt. Food Control 38, 209-14.

Schwarz, S., Cloeckaert, A. and Roberts, M.C., 2005. Mechanisms and spread of bacterial resistance to antimicrobial agents. In: Antimicrobial resistance in bacteria of animal origin. (Frank M. Aarestrup, Editor), ASM Press. pp.73-98.

Sedeik, M.E., Nahed, A., Awad, A.M., Elfeky, S.M., Abd El-Hack, M.E., Hussein, E.O.S., Alowaimer, A.N., Swelum, A.A., 2019. Isolation, conventional and molecular characterization of *Salmonella* spp. from newly hatched broiler chicks. AMB Express 9, 136.

Shang, K., Wei, B., Kang, M., 2018. Distribution and dissemination of antimicrobial-resistant *Salmonella* in broiler farms with or without enrofloxacin use. BMC Vet. Res. 14, 257.

Shsltout, F., Nada, S.M., Fawzy, W.S., 2019. Prevalence of *Salmonella* in some chicken meat products. Benha Vet. Med. J. 36, 33-39.

Singh, S., Yadav, A.S., Singh, S.M., Bharti, P., 2010. Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. Food Res. Intern. 43, 2027-30.

Smith, J.L., Drum, D.J., Dai, Y., Kim, J.M., Sanchez, S., Maurer, J.J., 2007. Impact of antimicrobial usage on antimicrobial resistance in commensal Escherichia coli strains colonizing broiler chickens. Appl. Environ. Microbiol. 73, 1404-1144.

Snow, L.C., Davies, R.H., Christiansen, K.H, Carrique-Mas, J.J, Wales, A.D, O'connor, J.L, Evans, S.J., 2007. Survey of the prevalence of *Salmo-*

nella species on commercial laying farms in the United Kingdom. Vet. Rec. 161, 471-476.

Suresh, T., Hatha, A.A M, Harsha, H.T, Lakshmanaperumalsamy, P., 2011. Prevalence and distribution of *Salmonella* serotypes in marketed broiler chickens and processing environment in Coimbatore City of Southern India. Food Res Int. 44, 823-825.

Tarabees, R., Elsayed, M.S., Shawish, R., Basiouni, S., Shehata, A.A., 2017. Isolation and characterization of *Salmonella* Enteritidis and *Salmonella* Typhimurium from chicken meat in Egypt. J. Infect. Dev. Ctries. 11, 314-319.

Tarabees, R., Helal, G., Younis, G., 2019. Molecular characterization of virulence genes associated with *Salmonella* spp. Isolated from poultry. J. Curr. Vet. Res. 1, 36-46.

Temelli, S., Eyigor, A., Carli, K.T., 2012. *Salmonella* detection in poultry meat and meat products by the Vitek immunodiagnostic assay system easy *Salmonella* method, a LightCycler polymerase chain reaction system, and the International Organization for Standardization method 6579. Poult. Sci. 91,724-31.

Vinueza-Burgos, ., Baquero, M., Medina, J., De Zutter, L., 2019. Occurrence, genotypes and antimicrobial susceptibility of *Salmonella* collected from the broiler production chain within an integrated poultry company. Int. J. Food Microbiol. 299, 1-7.

Vo, A.T.T., van Duijkeren, E., Fluit, A.C., Heck, M.E.O.C., Verbruggen, A., Maas, H.M.E., 2006. Distribution of *Salmonella* enterica serovars from humans, livestock and meat in Vietnam and the dominance of *Salmonella* Typhimurium phage type 90. Vet. Microbiol., 113, 153–58.

Vose, D., Koupeev, T., Mintiens, K.A., 2011. quantitative microbiological risk assessment of *Salmonella* spp. in broiler (Gallus gallus) meat production. EFSA Supporting Publications 8, 183E.

WHO (World Health Organization), 2016. Interventions for the control of non-typhoidal *Salmonella* spp. Thorgeir L. (Ed.), Beef and Pork: Meeting Report and Systematic Review, Microbiological Risk Assessment Series., No. 30, Rome, Italy pp. 1-10.

Yildirim, Y., Gonulalan, Z., Pamuk, S., Ertas, N., 2011. Incidence and antibiotic resistance of *Salmonella* spp. on raw chicken carcasses. Food Res Int. 44,725-28.

Zhao, X., Hu, M., Zhang, Q., Zhao, C., Zhang, Y., Li, L., 2020. Characterization of integrons and antimicrobial resistance in *Salmonella* from broilers in Shandong, China. Poultry Science 99, 7046–54.

Zishiri, O.T., Mkhize, N., Mukaratirwa, S., 2016. Prevalence of virulence and antimicrobial resistance genes in *Salmonella* spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. Onderstepoort J. Vet. Res. 83, a1067.