Research Article

Influence of Hygienic Measures on Enterobacteriaceae Prevalence and Antimicrobial Resistance in Poultry Farms

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Abstract | Enterobacteriaceae threaten the success of the poultry industry, as they cause great economic losses due to the high mortalities and the high treatment cost. They cause serious public health hazards, as they are the major cause of food-borne infections. The study aimed to investigate the prevalence of Enterobacteriaceae in twelve poultry farms located in Qalubia Governorate, Egypt, and their antimicrobial resistance. A total number of 2160 samples included litter, pen litter, stored feed, feed from the feeders, water, drinkers, droppings, dust, swabs from walls, birds' cloaca, worker's hands, and wheels of vehicles. The results showed that there was a negative relationship between the prevalence of Enterobacteriaceae and the hygienic measures enforced in the poultry farms under study. The highest prevalence of *Salmonella* and *E. coli* was recorded in duck farms (36.2% and 54.3% respectively) and the lowest prevalence was recorded in breeder chicken farms (10.2% and 29.3% respectively). The isolated *Salmonella* species showed high resistance (100%) against doxycycline, ampicillin, and amoxicillin, while *E. coli* species showed resistance (90%) against oxytetracycline, doxycycline, ampicillin, and amoxicillin. Both *Salmonella* and *E. coli* species were highly susceptible to gentamicin. Gene *tet*A and *bla*SHV were detected in Gene *tet*A and *bla*SHV were detected in 28.5% & 57.1% of *Salmonella,* respectively, and 70% & 60% of *E. coli* serotypes, respectively. The application of good biosecurity programs including strict measures in poultry farms is the preferable method to reduce the risk of pathogenic bacteria and reduce the use of antibiotics.

Keywords | Poultry farms, Hygiene, Enterobacteriaceae, Antimicrobial Resistance, Resistance gens.

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INTRODUCTION

Poultry is an important source of protein due to its cheaper prices and high nutritional value. Therefore, poultry farming has become one of the most important industries worldwide and has significantly developed and increased in recent years (Hussain et al., 2015). Poultry hygiene is a critical point, especially in intensive farms with closed housing that facilitates the spreading of bacterial contamination such as Enterobacteriaceae (Nechyporenko et al., 2018). Enterobacteriaceae are widely distributed in nature and can be found in soil, feed, and water. They normally inhabit the gastrointestinal tract of birds, humans, and animals and might cause serious infections in poultry under certain circumstances.

Salmonella, *Escherichia coli* (*E. coli*), and *Shigella* are the major cause of food-borne infection all around the world.

Poultry and its products are considered the main vehicle for these pathogens causing serious economic losses and huge public health hazards, in addition to huge numbers of annual mortalities (Kamboh et al., 2018). *Salmonella* causes serious clinical symptoms and high mortality at young ages less than 6 weeks, older chicks may show stunting and uneven growth (Kim et al., 2007). *E. coli* causes economic loss in broilers due to high mortality and weight loss and a decrease in egg production and profitability in layer and breeder farms (Vandekerchove et al., 2004). *Shigella* can infect chickens (shigellosis) causing gastroenteritis, the development of serious complications, and death and it is known for its zoonotic capabilities (Pashazadeh et al., 2017). Antimicrobials are widely used in poultry farms as one of the methods to control the bacterial diseases that result from improper hygiene; they aren't only used to treat and prevent infectious diseases but also as prophylaxis, and growth promotors. On the other side, bacteria had built up great resistance to antibacterial substances. This complex situation increases the development of multi-drug resistant bacteria (MAR) in poultry (Saliu et al., 2017).

The present study aimed to investigate the prevalence of some Enterobacteriaceae species such as *Salmonella*, *E. coli*, and *Shigella* in different poultry farms and monitor the antimicrobial resistance of the isolated Enterobacteriaceae species with the detection of some resistance genes such as *tet*A and *bla*SHV.

MATERIAL AND METHODS

Sampling

A total number of 2160 samples and swabs were collected from twelve poultry farms, 180 samples, and swabs from each farm in three visits per farm, and five samples were collected per visit from each type of samples and swabs. The samples included litter, pen litter, stored feed, feed from the feeders, water, drinkers, droppings, dust, swabs from walls, birds' cloaca, worker's hands, and wheels of vehicles, 180 samples per each. The collection of samples was approved with Institutional Approval Number (BUFVTM 04-07-22).

Poultry farms

The present study was carried out on twelve poultry farms. The selection of the farms was based on their geographical location, hygienic level, housing system, and type of production. All farms are located in Qalyubia Governorate, Egypt, as well as all farms use public chlorinated tap water as a water source. The most used antibiotics in studied poultry farms were oxytetracycline, doxycycline, amoxicillin, and flumequine. The basic information on poultry farms under study and the applied hygienic measures were listed in (Table 1).

Preparation of samples

One gram of each sample was put in nine ml of buffer peptone water (BPW) to be ready for bacteriological examination (Soliman and Hassan, 2017).

Isolation and identification of Enterobacteriaceae

Isolation of *Salmonella*: The previously prepared samples were incubated aerobically in BPW at 37°C for 24 h. From the pre-enrichment tubes, one ml was inoculated into nine ml Rappaport Vassiliadis (RV) broth and incubated aerobically at 42°C for 24 h. A loop full of selectively enriched broth was streaked separately onto Xylose Lysine Desoxycholate (XLD) agar and Hektoen enteric (HE) agar and incubated at 37°C for 24 h. The suspected colonies were pink with or without black centers colonies on XLD and clear colonies with or without black centers on HE agar. One colony from the presumptive *Salmonella* colonies was subcultured onto XLD agar until the pure homogenous colonies were obtained. The pure suspected colonies were subcultured onto nutrient agar plates for further identification. These procedures were carried out after (Hassan and Osama, 2021).

Isolation of *E. coli*: The previously prepared samples were incubated aerobically in BPW at 37°C for 24 h. A loop full of the non-selective enriched broth was streaked onto Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 h. The suspected colonies were metallic green in reflected light with a blue-black center in transmitted light. The typical colonies were subcultured onto EMB agar until the pure cultures with homogenous colonies were obtained. The pure colonies were subcultured onto nutrient agar plates for further identification. These procedures were carried out after (Jozić et al., 2018).

Isolation of *Shigella*: The samples were pre-enriched in peptone water and incubated anaerobically at 41.5°C for 20 h. A loop full of the non-selective enriched samples was inoculated onto McConkey agar (low selectivity), XLD agar (moderate selectivity), and HE agar (high selectivity) and incubated at 37°C for 24 h, the suspected colonies were smooth colorless colonies on McConkey agar, pale red on XLD agar, and ranged from clear to pale green colonies on HE agar. The pure suspected colonies were subcultured onto McConkey agar plates for further identification. These procedures were carried out after (ISO, 2004).

Biochemical identification of Enterobacteriaceae: The purified suspected colonies of *Salmonella*, *E. coli,* and *Shigella* were identified based on biochemical tests panel following the standard test protocol described in FDA's Bacteriological Analytical Manual (FDA, 2012), (Gupta et al., 2017), and (Omara et al., 2017), respectively.

Table 1: The basic information and hygienic measures of poultry farms under study.

Farms	Type of the flock	Housing system	Ventilation	Feed and watering system	Fence	Foot bath	Worker hygiene	visitors control	hygienic disposal of wastes	Cleaning and disinfection program
Farm 1	Broiler chicken	Open deep litter	Mechanical and natural	Manual	-	-	Bad	Bad	Not applied	Weak
Farm 2	Broiler chicken	Open deep litter	Mechanical and natural	Manual	-	-	Fair	Fair	Not applied	Fair
Farm 3	Broiler chicken	Open deep litter	Mechanical and natural	Automated	+	+	Fair	Fair	applied	Fair
Farm 4	Layer chicken	Open deep litter	Natural	Manual	-	+	Fair	Fair	applied	Good
Farm 5	Layer chicken	Open deep litter	Natural	Manual	-	_	Bad	Bad	Not applied	Weak
Farm 6	Layer chicken	Battery, open system	Mechanical and natural	Automated	+	+	Fair	Fair	applied	Good
Farm 7	Broiler breeder chicken	Closed deep litter	Mechanical	Automated	+	+	Good	Good	applied	Good
Farm 8	Broiler breeder chicken	Closed deep litter	Mechanical	Automated	_	+	Good	Good	applied	Good
Farm 9	Broiler breeder chicken	Closed deep litter	Mechanical	Automated	+	+	Good	Good	applied	Good
Farm 10	Breeder ducks	Open deep litter	Natural	Manual	_	_	Bad	Bad	Not applied	Weak
Farm 11	Breeder ducks	Open deep litter	Natural	Manual	_	_	Bad	Bad	Not applied	Weak
Farm 12	Breeder ducks	Open deep litter	Natural	Manual	_	_	Bad	Bad	Not applied	Weak

(+) Positive, (-) Negative

Table 2: Antimicrobial discs, concentration, and interpretation of their action on the isolated Enterobacteriaceae.

Antimicrobial agent	Disc content (ug)	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Oxytetracycline (O_{30})	30	≤ 11	12-14	≥15
Doxycycline (DO_{30})	30	≤ 10	11-13	≥14
Enrofloxacin (EX_5)	5	≤ 12	13-16	≥17
Norfloxacin (NX_{10})	10	≤ 12	13-16	≥17
Flumequine (UB_{30})	30	≤ 10	11-13	≥14
Ciprofloxacin(CIP_5)	5	≤ 15	16-20	≥21
Ampicillin (AMP_{10})	10	≤ 13	14-16	≥17
Amoxicillin (AMX_{10})	10	≤ 13	14-16	≥17
Cefotaxime (CTX_{30})	30	≤ 13	14-20	≥21
Ceftriaxone (CTR_{30})	30	≤ 13	14-20	≥21
Gentamicin (GEN_{10})	10	≤ 12	13-14	≥15

(≤) equal or less, (≥) equal or more

Table 3: The prevalence of *Salmonella* and *E. coli* in collected samples and swabs from different poultry farms (mean ± SE).

Parameters	*Salmonella*					*E. coli*				
	Broiler chicken Farms	Breeder chicken farms	Layer chicken farms	Duck farms	Total	Broiler chicken Farms	Breeder chicken farms	Layer chicken farms	Duck farms	Total
Wall	6.7± 3.33^{eB}	0± 0^{eC}	6.7± 6.67^{dB}	17.8± 4.01^{fA}	7.8	17.8± 7.78^{fgB}	17.8± 5.21^{dB}	33.3± 10^{efA}	17.7± 7.03^{hB}	21.7
Stored feed	0± 0^{fB}	0± 0^{eB}	0± 0^{eB}	6.7± 4.71^{gA}	1.7	2.2± 2.22^{iD}	4.4± 2.94^{eC}	13.6± 6.61^{hB}	20± 4.71^{gA}	10
Feeder	26.7± 8.16^{cB}	6.7± 3.33^{deC}	0± 0^{eD}	62.2± 7.78^{bcA}	23.9	48.9± 7.54^{cC}	35.6± 5.56^{cD}	53.3± 6.67^{cB}	93.3± 4.71^{abA}	54.5
Water source	0± 0^{fA}	0± 0^{eA}	0± 0^{eA}	0± 0^{hA}	0	4.4± 2.94^{hiB}	0± 0^{eC}	6.7± 4.71^{hB}	33.3± 7.45^{deA}	14.5
Drinker	35.6± 6.48^{bB}	6.7± 4.71^{deD}	15.6± 9.3^{cC}	75.6± 7.29^{aA}	33.3	20± 8.82^{efD}	33.3± 7.45^{eC}	40± 8.82^{deB}	86.7± 5.77^{bcA}	41.7
Stored litter	6.7± 4.71^{eB}	0±0^{eD}	4.4± 4.44^{deC}	15.6± 5.56^{fA}	6.7	33.3± 8.16^{dB}	0± 0^{eD}	46.7± 6.67^{dA}	24.4± 6.48^{fgC}	26.1
Pen litter	51.1± 9.49^{aB}	35.6± 6.48^{aD}	44.4± 9.88^{aC}	66.7± 6.67^{bA}	49.5	68.9± 8.89^{bC}	68.9± 4.84^{abC}	84.4± 5.56^{aB}	97.8± 2.22^{aA}	80
Dust	13.3± 5.77^{dB}	13.3± 4.71^{cB}	8.9± 4.84^{dC}	33.3± 5.77^{dA}	17.2	28.9± 5.88^{dB}	17.8± 7.03^{dC}	33.3± 5.77^{efA}	31.1± 4.84^{efA}	27.8
Cloaca	53.3± 6.67^{aB}	22.2± 6.19^{bD}	28.89± 6.76^{bC}	64.4± 8.01^{bcA}	42.2	75.6± 7.29a^{bB}	64.4± 4.44^{bC}	75.6± 8.68^{bB}	84.4± 5.6^{cA}	75
Droppings	48.9± 6.76^{aB}	31.1± 8.24^{aD}	44.4± 9.30^{aC}	60± 8.82^{cA}	46.1	80± 6.67^{aC}	75.6± 5.56^{aD}	88.9± 7.54^{aB}	95.6± 2.94^{aA}	85
Hand	0±0^{fB}	2.2± 2.22^{deAB}	0±0^{eB}	4.4± 2.94^{ghA}	1.7	11.1± 5.88^{ghD}	17.8± 5.21^{dC}	24.4± 8.68^{gB}	40± 9.43^{dA}	23.3
Wheel	11.1± 5.88^{deB}	6.7 ±4.71^{deC}	0±0^{eD}	26.7± 11.06^{eA}	11.1	26.7± 10.00^{deB}	17.8± 5.21^{dC}	31.1± 9.49f^{gA}	26.7± 8.16e^{fgB}	25.6
Total	21.1	10.2	12.8	36.2	20	33.7	29.3	44.4	54.3	40.4

a, b & c: There is no significant difference (P>0.05) between any two means for each farm separately, within the same column have the same superscript letter.

Table 4: Different *Salmonella* and *E. coli* serotypes were isolated from different poultry farms.

Salmonella strains	Broiler chicken farms	Breeder chicken farms	Layer chicken farms	Duck farms	Percentage of total serotypes (%)
S. Agona	+	+	+	+	39.76
S. Kentucky	+	+	-	+	24.1
*S.*Derby	+	+	-	+	16.87
S. Typhimurium	+	+	+	+	8.43
S. Enteritidis	+	-	+	+	7.22
S. Molade	-	-	-	+	2.4
*S.*Virchow	-	-	-	+	1.2
O26	-	+	+	+	22.9
O44	+	+	+	+	20.83
O119	-	+	-	+	14.58
O86	-	+	-	+	11.11
O124	-	+	+	+	6.94
O114	+	+	-	+	6.94

O55	+	-	-	+	5.56
O112	+	-	-	-	4.17
O164	+	-	-	-	1.39
O157	+	-	-	+	1.39
O91	-	-	+	-	1.39
O152	-	+	-	+	.69
O128	-	-	+	-	.69
O153	+	-	-	+	.69
O127	-	+	-	+	.69

(+) Positive, (-) Negative

Table 5: Antibiotic susceptibility of isolated *Salmonella* and *E. coli* serotypes.

Antimicrobial agent	***Salmonella***						***E. coli***					
	S		I		R		S		I		R	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
Oxytetracycline (O_{30})	1	14.3	1	14.3	5	71.4	1	10	0	0	9	90
Doxycycline (DO_{30})	0	0	0	0	7	100	1	10	0	0	9	90
Enrofloxacin (EX_5)	4	57.1	0	0	3	42.9	5	50	2	20	3	30
Norfloxacin (NX_{10})	4	57.1	2	28.6	1	14.3	6	60	2	20	2	20
Flumequine (UB_{30})	2	28.6	2	28.6	3	85.7	4	40	2	20	4	40
Ciprofloxacin(CIP_5)	0	0	5	71.4	2	28.6	5	50	2	20	3	30
Ampicillin (AMP_{10})	0	0	0	0	7	100	0	0	1	10	9	90
Amoxicillin(AMX_{10})	0	0	0	0	7	100	1	10	0	0	9	90
Cefotaxime (CTX_{30})	3	42.9	3	42.9	1	14.3	2	20	8	80	0	0
Ceftriaxone (CTR_{30})	4	57.1	1	14.3	2	28.6	7	70	1	10	2	20
Gentamicin (GEN_{10})	7	100	0	0	0	0	8	80	2	20	0	0

Serological identification of Enterobacteriaceae: The positive isolates of *Salmonella* were serologically identified according to Kauffman's white scheme (Kauffman, 1974), by using *Salmonella* antiserum according to (Cruickshank et al., 1975) for the detection of Somatic (O) and flagellar (H) antigens. Meanwhile, the positive *E. coli* were serologically identified according to (Kok et al., 1996) by using rapid diagnostic *E. coli* antisera sets for detection of somatic (O) and capsular (K) antigens.

Antibiotic Resistance of Enterobacteriaceae: The disk diffusion method was done according to (CLSI, 2015) to test the sensitivity of isolated Enterobacteriaceae by using eleven antibiotics. The concentrations of antimicrobial discs and the diameters of the inhibition zone of the tested strains were demonstrated in (Table 2). MAR index for each strain was determined according to the following formula, isolates classified as intermediate were considered sensitive to MAR index (Cusack et al., 2019).

MAR index = No. of resistance / total No. of tested antibiotics.

Conventional Polymerase Chain Reaction (cPCR)

Molecular detection of antibiotic resistance genes by using cPCR was carried out after (Momtaz et al., 2012). The isolated *Salmonella* and *E. coli* serotypes were subjected to cPCR for detection of two resistant genes, these genes were the bla_{SHV} resistance gene for β lactams (amoxicillin and ampicillin) and tet_A resistance genes for tetracyclines (oxytetracycline and doxycycline). The genomic DNA extraction was carried out using a QIAamp DNA mini kit (Catalogue No.51304). The master mix was carried out according to the Emerald Amp GT PCR master mix (Takara, Code No. RR310A kit). The Oligonucleotide primers for tet_A gene F, $^{5'}$GGTTCACTCGAACGACGTCA$^{3'}$ and R, $^{5'}$CTGTCCGACAAGTTGCATGA$^{3'}$ with 576 bp according to (Dipineto et al., 2006). While primers of the bla_{SHV} gene were F, $^{5'}$AGGATTGACTGCCTTTTTG$^{3'}$ and R, $^{5'}$ATTTGCTGATTTCGCTCG$^{3'}$, with 392 bp according to (Bisi-Johnson et al., 2011). The cycling conditions of the primers during cPCR were carried out according to a specific Emerald Amp GT PCR master mix (Takara kit).

Statistical Analysis

The statistical analyses were carried out following (Mahmoud and Abd Abd El-Hamed, 2018) to analyze the prevalence of *Salmonella* and *E. coli* in collected samples and swabs from different poultry farms by using General Linear Models (GLM) of SPSS Statistics 25 (IBM Corp., Somers, NY, USA). Studied trials were subjected to a two-way ANOVA. Multiple comparisons were carried out by applying the Duncan test. The significance level was set at a P value < 0.05.

RESULTS

Enterobacteriaceae prevalence

Salmonella and *E. coli* had the highest prevalence in the duck farms (36.2% and 54.3% respectively). In contrast, the lowest prevalence of *Salmonella* and *E. coli* were in the breeder chicken farms (10.19 % and 29.28 % respectively). The prevalence of *Salmonella* was the highest in pen litter (49.45%), while it was the lowest in stored feed and hand swabs (1.67% per each). On the other hand, the prevalence of *E. coli* was the highest in droppings (85%), while it was the lowest in stored feed (10%), as shown in (Table 3).

Identification of *Salmonella* and *E. coli*

Seven serotypes of *Salmonella* were isolated; the most isolated *Salmonella* serotype was *S.* Agona (39.76%). On the other hand, fifteen *E. coli* serotypes were isolated; the most isolated *E. coli* serotype was O26 (22.9%), as shown in (Table 4).

Antibiotic Susceptibility of *Salmonella* and *E. coli* Serotypes

The different *Salmonella* serotypes showed 100% resistance against doxycycline, ampicillin, and amoxicillin. In contrast, the serotypes of *Salmonella* showed 100% sensitivity to gentamicin. Moreover, the different serotypes of *E. coli* showed the highest resistance (90%) against oxytetracycline, doxycycline, ampicillin, and amoxicillin. In contrast, all serotypes of *E. coli* were susceptible to gentamicin and cefotaxime, as shown in (Table 5). The highest MAR index (.727) was shown in *S.* Enteritidis followed by *S.* Kentucky (.545) and *E. coli* O164 had the highest MAR index (.909), followed by O114 (.727), as shown in (Table 6).

Molecular characterization of *Salmonella* and *E. coli* isolates using conventional PCR (cPCR)

The resistant gene tet_A was detected in *S.* Typhimurium and *S.* Enteritidis serotypes, in addition to O26, O119, O124, O114, O55, O164, and O157 serotypes. While the resistant gene bla_{SHV} was detected in *S.* Agona, *S.* Kentucky *S.* Typhimurium, and *S.* Enteritidis, in addition, O26, O119, O124, O114, O164, and O157 serotypes *of E.coli* as shown in (Tables 7 and 8) and (Figures 1 and 2).

Table 6: Antimicrobial resistance profile of isolated *Salmonella* and *E. coli* serotypes.

Strains	Antimicrobial resistance profile	MAR index
S. Agona	O, DO, EX, AMP, AMX	.454
S. Kentucky	O, DO, EX, UB,AMP, AMX,	.545
S. Derby	O, DO, AMP, AMX	.364
*S.*Typhimurium	O, DO, AMP, AMX,	.364
S. Enteritidis	O, DO, EX, CIP, AMP, AMX, CTX,CTR	.727
S. Molade	O,DO, AMP, AMX,	.364
S. Virchow	O, DO, AMP, AMX	.364
O26	O, DO, EX , UB, AMP, AMX	.454
O44	AMP	.090
O119	O, DO, AMP, AMX	.364
O86	O, DO, AMP, AMX	.364
O124	O, DO, UB, AMP, AMX	.454
O114	O,DO, EX, NX, UB,CIP, AMP, AMX	.727
O55	O, DO,CIP, AMP, AMX	.454
O112	O, DO, AMP, AMX	.364
O164	O,DO, EX, NX, UB,CIP, AMP, AMX, CTX,CTR	.909
O157	O, DO, AMP	.273

Table 7: The tet_A and bla_{SHV} resistant genes in isolated *Salmonella* from different poultry farms.

Salmonella isolate	tet_A	bla_{SHV}
S. Agona	-	+
S. Kentucky	-	+
S. Derby	-	-
S. Typhimurium	+	+
S. Enteritidis	+	+
S. Molade	-	-
S. Virchow	-	-
Total	28.5%	57.1%

(+) Positive, (-) Negative

Table 8: The tet_A and bla_{SHV} resistant genes in isolated *E. coli* from different poultry farms.

E. coli sample	tet_A	bla_{SHV}
O26	+	+
O44	-	-
O119	+	+
O86	-	-
O124	+	+
O114	+	+
O55	+	-

O112	-	-
O164	+	+
O157	+	+
Total	70%	60%

(+) Positive, (-) Negative

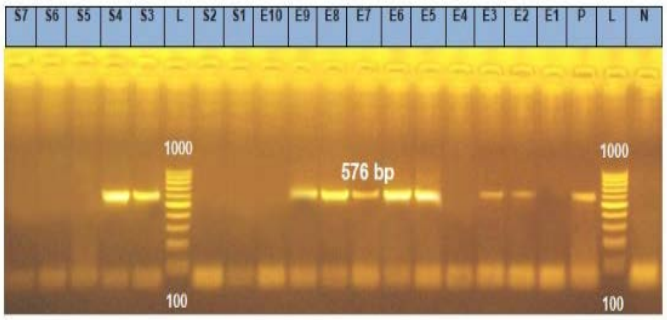

Figure 1:

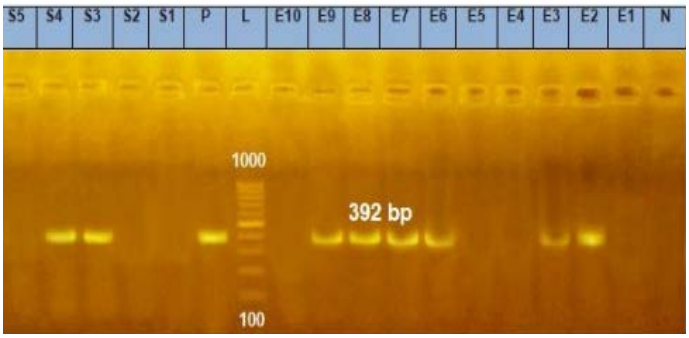

Figure 2:

DISCUSSION

Enterobacteriaceae are serious contamination facing poultry industries as they are one of the most important groups of bacteria which can infect the poultry and cause dangerous diseases. Improper biosecurity measures and poor hygiene in poultry farms are the main cause of the introduction and spreading of Enterobacteriaceae (Khan et al., 2016).

Our results showed that there was a negative relationship between the poultry farms' hygiene and the prevalence of Enterobacteriaceae. The duck farms were the most contaminated farms with *Salmonella* and *E. coli*; in contrast, the breeder chicken farms recorded the lowest prevalence. This is might be due to the variation in the level of hygienic measures of each farm as breeder chicken farms were the highest, unlike the duck farms (Noha and Halla, 2019). Moreover, the variation in the survival capabilities of *Salmonella* and *E. coli* in poultry farms is affected by many factors such as poultry husbandry systems, antibiotic use, environmental temperature, stress factors, in addition, age, type, immune status, and physiological status of birds (Rukambile et al., 2019).

Salmonella and *E. coli* were isolated from litter, droppings, birds' cloaca, drinkers, feed, dust, vehicles' wheels, farms' walls, and workers' hands, but *Salmonella* prevalence was the highest in pen litter (49.45%). The main source of *Salmonella* spreading in poultry farms was the contaminated litter that could be contaminated by the surrounding environment, dust, insects, free-living animals, and rodents (Noha and Halla, 2019). On the other hand, the contamination of *E. coli* was the highest in birds' droppings (85%), birds' droppings were the main source of *E. coli* contamination in different poultry farms (Blaak et al., 2015).

The isolated *Salmonella* serotypes were *S*. Agona, *S*. Kentucky, *S*. Derby, *S* Typhimurium, *S*. Enteritidis, *S*. Molade, and *S*. Virchow. While, the isolated *E. coli* serotypes were O26, O44, O119, O86, O124, O114, O55, O112, O164, O157, O91, O152, O128, O153, and O127. The appropriate management practices, proper hygiene, and biosecurity measures in poultry farms are very essential for the control of Enterobacteriaceae (Rukambile et al., 2019).

Our results indicated that all examined samples were free from *Shigella* species and this result may be due to the high sensitivity of *Shigella* species to unfavorable macroclimatic environmental conditions. It is destroyed by dryness and direct sunlight and is sensitive to different antibiotics (Ibrahim and Abo El-Makarem, 2021).

The routine prophylactic use of antibiotics in different poultry farms leads to an increase in the prevalence of an antibiotic-resistance against many bacterial species which is considered one of the most important public health hazards (Omara et al., 2017). Our result demonstrated that *Salmonella* species showed 100% resistance against doxycycline, ampicillin, and amoxicillin. Moreover, *Salmonella* species showed 85.7% resistance against flumequine. In previous studies, this high *Salmonella* resistance was reported against the same antibiotics in Egypt (Al-baqir et al., 2019), India (Waghamare et al., 2018), Pakistan (Kamboh et al., 2018), Bangladesh (Parvin et al., 2020), western Algeria (Yahya et al., 2021) and Côte d'Ivoire (Assoumy et al., 2021).

While, the *E. coli* species showed 90% resistance against oxytetracycline, doxycycline, ampicillin, and amoxicillin (Kamboh et al., 2018). This resistance may be attributed to the prolonged use of these antibiotics (Diab et al., 2019). On the other hand, *E. coli* isolates showed relatively low resistance against norfloxacin and cefotaxime (20% each). A previous incompatible study in Pakistan reported that *E. coli* isolates showed high resistance against flumequine, enrofloxacin, ciprofloxacin, and norfloxacin (76.7%, 79.6%, 82.5%, and 73.7% respectively), it demonstrated that this

high resistance was due to extensive use of these antibiotics as feed additives in tested poultry farms for diseases prevention (Kamboh et al., 2018).

Gentamicin and cefotaxime antibiotics had the highest effect against *E. coli* species (Harakeh et al., 2005). In addition, Gentamicin had the highest effect on *Salmonella* species. This result was previously reported in, Egypt (El-Sharkawy et al., 2017). A previous study in Egypt reported that the isolated *Salmonella* showed complete resistance against gentamicin and ceftriaxone (100 %). It demonstrated that these antibiotics were commonly used by poultry producers as a preventive tool (Al-baqir et al., 2019). In contrast, these antibiotics weren't commonly used in the tested poultry farms in our study and this proved the strong relationship between prophylactic use of the antibiotics and the development of antimicrobial resistance in poultry farms.

The results revealed that multidrug resistance was observed in all *Salmonella* isolates against four antibiotics or more and in all *E. coli* isolates against three antibiotics or more (except in O44). This high prevalence of multidrug resistance in *Salmonella* and *E. coli* has been previously reported in Egypt (Amer et al., 2018), Bangladesh (Matin et al., 2017), Pakistan (Kamboh et al., 2018), western Algeria (Yahya et al., 2021) and in Côte d'Ivoire (Assoumy et al., 2021).

The tet_A gene was detected in (28.5%) of *Salmonella* and (70%) of *E. coli* serotypes, while the bla_{SHV} gene was detected in (57.1%) of *Salmonella* and (60%) of *E. coli* serotypes. These genes were previously detected in *Salmonella* and *E. coli* isolates from poultry farms by (El-Sharkawy et al., 2017).

Our results showed that the serotypes of *Salmonella* and *E. coli* which had the highest MAR index also had the two resistant genes (tet_A and bla_{SHV}). In contrast, the serotypes which had the lowest MAR index didn't have the two resistant genes (tet_A and bla_{SHV}). The results of the disk diffusion test were confirmed by the results of cPCR (Phagoo and Neetoo, 2015).

CONCLUSION

Improving the hygiene practices and enforced application of the maximum biosecurity measures in different poultry farms can reduce the prophylactic use of antibiotics and as a result, the drug residues can be minimized in eggs and poultry meat. In addition; minimize the anti-microbial resistance and reduce the cost of using antibiotics.

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CONFLICT OF INTEREST

The authors declared that they had no competing interests.

AUTHORS CONTRIBUTION

All authors contributed equally to this study.

NOVELTY STATEMENT

This study was the first study in Qalubia governorate, Egypt aimed to detect resistance genes of the field Enterobacteriaceae strains already present in the poultry farm environment and isolated from the environmental samples as feeders, drinkers, dust, walls, and wheels of vehicles.

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