



Unveiling Antibiotic Resistance, Virulence, and Molecular Detection of Enteric Bacterial Infections in Broilers: A Study in Egypt

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THIS study aimed to investigate the most prevalent enteric pathogens that infect broilers and pose a crucial threat in Egyptian farms. A total of 84 farms in some Egyptian governorates were investigated. Samples of liver and intestine were used for the isolation and identification of possible pathogens using traditional and molecular techniques. Antimicrobial resistance testing was adopted against 11 antibiotics frequently used in the poultry field. Moreover, polymerase chain reaction (PCR) was used for the detection of isolates' virulence genes. Results showed that mixed infections predominated over a single infection. The isolated bacteria species were *Salmonella* spp. *E. coli* and *Clostridia* with a percentage of 55%, 72% and 80%, respectively. Regarding *Salmonella* isolates, serotypes *S. Enteritidis*, *S. Typhimurium*, and *S. Kentucky* were the most common serotypes with a percentage of 12.9%, 11.8%, and 9.67%, respectively. On the other hand, *E. coli* serotyped into 16 different serotypes. One hundred percent of *Salmonella* strains were positive for *invA* and *ompA* genes, and 50% were positive for *stn* and *hlyA* genes. All *E. coli* strains were positive for *iss*, *papC*, and *iutA* and negative for *tsh* gene. The isolated strains of *Clostridia* were positive for Alpha - toxin only. The antibiotic profile showed nearly resistance to the most commonly used antibiotics in this study. This study sheds light on some serious enteric infections of broiler chickens by providing a simple picture of their virulence and antibiotic profile, emphasizing the critical need for proactive measures to effectively manage and minimize this problem.

Keywords: Broilers, *Clostridium perfringens*, *E. coli*, PCR, *Salmonella* spp.

Introduction

Poultry, especially broiler chickens, represents an important and fundamental source of protein in Egypt [1]. Thus, the broilers industry has been gaining interest in recent years. Consequently, to achieve high economics and meet human protein demands, great attention should be directed toward gastrointestinal tract integrity [1]. A healthy gastrointestinal tract is a main issue for good feed conversion, good-quality meat production, and a maximal degree of growth and performance [1; 2]. Enteropathy is a great problem

facing the broiler industry due to damage of GIT, which in turn affects digestion and absorption and leads to severe economic losses. These economic losses include mortalities, loss of weight, poor feed conversion, and the high cost of medication [1; 2]. The gastrointestinal tract may be affected by bacteria, viruses, parasites, fungi, and their mycotoxins, and non-infectious management causes. Mostly, diseases in poultry are multifactorial [3]. Bacterial diseases have a great impact on the poultry industry in Egypt. Some of these serious pathogens are (*Salmonella*, *E. coli*, *Clostridia*). Salmonellosis is a severe disease that

affects broilers, especially newly hatched chicks, mainly due to vertical transmission [4; 5]. *S. Typhimurium* and *S. Enteritidis* are considered of the most threatening *Salmonella* spp. spreading globally [6]. *Salmonella* causes high mortality in chicks, low body weight, diarrhea, and pasty vent. The postmortem lesions in GIT are Hepatitis, splenitis, typhlitis, and omphalitis [7]. *E. coli* infection associated with signs similar to *salmonella* [3]. Necrotic enteritis in broilers is a severe disease that causes severe economic losses and is caused mainly by *Clostridium perfringens* type A [2]. Severe enteritis, ballooning with offensive watery fluid, and diphtheroid membranes are the PM lesions observed in this disease [2]. In poultry flocks that are reared in large numbers, where antibiotics are often used to enhance growth in all flocks, prophylaxis and control of these infections lead to the antibiotic resistance in these bacteria being exaggerated and producing more resistant bacteria in their fecal microflora [2]. Now, molecular identification is very important for accurate specification of different criteria of bacteria. This study was conducted to illustrate the common enteric diseases circulating in Egyptian poultry farms.

Material and Methods

Ethical Approval

This work was approved by the Institutional Animal Care and Use Committee (Vet. Cu. IACUC) of the Faculty of Veterinary Medicine, Cairo University, by code No. Vet CU 08072023673.

Sample collection

A total of 84 broiler farms suffering from enteric problems were examined. The investigated farms were located in Dakahlia, Sharkia, Qalyubia, Gharbia, Fayoum, and Menia governorates, with 15 farms from each governorate except Menia, which had 9 farms in Egypt. Liver tissue and intestinal samples were collected from clinically diseased and freshly dead birds of different ages for further investigation. Five samples were taken from each farm and pooled into one sample.

Isolation of enteric pathogens

For *Salmonella* and *E. coli* isolation, liver tissue, and intestinal samples were cultured on Rappaport Vassiliadis Soya Broth and Nutrient Broth, respectively. Then both were incubated at 37°C for 24 h, then subcultured on Xylose Lysine Deoxycholate and Eosin Methylene Blue media, respectively. The cultured plates were incubated

at 37 °C for 24 hours. The suspected colonies were picked up after purification and preserved for complete identification [9; 10].

For *Clostridia perfringens*, samples from the intestine were cultured on cooked meat media and incubated at 37°C for 24 hr. anaerobically, then streaked on 10% Neomycin sheep blood agar and incubated at 37°C for 24 hrs. anaerobically. Suspected colonies were picked up after purification and preserved in 16% glycerol with Reinforced Clostridia broth [2; 11] for further identification.

Serological identification

Serological identification of Salmonella:

Serological identification of *Salmonella* was carried out according to Kauffman-White scheme [12] by slide agglutination technique for the determination of Somatic (O) and flagellar (H) antigens using *Salmonella* antiserum (DENKA SEIKEN Co, Japan).

Serological identification of E. coli:

Serogrouping of Congo red positive *E. coli* applied by slide agglutination test, using *E. coli* antisera according to [13] to determine somatic (O) and capsular (K) antigens.

In vitro pathogenicity assay of E. coli

Pure *E. coli* isolates were cultured on Congo red media and incubated at 37°C for 24 hr. The reaction was observed after incubation, and then the plates were left at room temperature for an additional 2 days (not to exceed 4 days) [14].

Antimicrobial susceptibility of Salmonella and E. coli

Antimicrobial susceptibility was tested for *salmonella* and *E. coli* isolates by the disk diffusion method according to [15]. The test was evaluated to determine the susceptibility of the isolated strains against Nalidixic acid (NA) (30 µg), Penicillin G (P) (10 iu), Clindamycin (CL) (10 µg), Amoxyclav (AMk) (30 µg), Sulpha & Trimethoprim (SXT) (25 µg), Cefotaxim (CF) (30 µg), Tetracycline (T) (30 µg), Kanamycin (K) (30 µg), Ciprofloxacin (CP) (5 µg), Meropenem (M) (10 µg), Gentamicin (G) (10 µg), (Oxoid Limited, Hampshire, UK). The antimicrobial susceptibility testing was applied according to the guide lines stipulated by CLSI [16].

Multiple Antimicrobial Resistance (MAR) Index

The MAR was determined as MAR index = Number of antimicrobials that show resistance/ Number of total antibiotics that used [17]. The

interpretation of results was conducted by MAR index ≤ 0.2 , which indicated a low risk, while ≥ 0.2 referred to a high risk of antimicrobial contamination [18].

Molecular Identification of Salmonella, E. coli, and Clostridia isolates

For extraction of DNA, QIA amp DNA Mini Kit (Catalogue No.51304) and ethanol 96% according to QIA amp DNA Mini Kit instructions were used. PCR Master Mix was used following Emerald Amp GT PCR Master Mix (Takara) Code No. RR310A.

Preparation of four Clostridium toxins multiplex PCR Master Mix for each component and its volume/reaction according to Emerald Amp GT PCR master mix were Emerald Amp GT PCR Master Mix (2x premix- 25 μ l), PCR grade water (12 μ l), forward primer (20 pmol-1 μ l each), reverse primer (20 pmol – 1 μ l each), template DNA (5 μ l), and total (50 μ l).

Preparation of uniplex PCR Master Mix for each component and its volume/reaction: Emerald Amp GT PCR Master Mix (2x premix-12.5 μ l), PCR grade water (5.5 μ l), forward primer (20 pmol-1 μ l), reverse primer (20 pmol -1 μ l), template DNA (5 μ l), and total (25 μ l). Agarose gel electrophoreses was applied as [19; 20] with modification. The source of primers was Midland Certified Reagent Company Oilgos (USA). Sequence, temperature, and time conditions of the primers during PCR are shown in **Table 1, 2**.

Results

The observed clinical signs included poor body condition, ruffled feathers, diarrhea, dehydration, and decreased body weight. The surveillance study of the investigated enteric problems is summarized in **Table 3**. In brief, the total isolates of *Salmonella* and *E. coli* were 93 and 121 positive samples from 168 liver and intestinal samples, respectively, that were isolated from some Egyptian governorates. A surveillance study of isolated *clostridia* isolates showed a total of 68 *clostridia perferingens* positive samples from a total of 84 intestinal samples for the same governorates.

Serological Identification

Serotyping of *salmonella* isolates illustrated that serotypes with high prevalence were *S. Enteritidis* (12.9%), *S. Typhimurium* (11.8%), and *S. kentucky* (9.67%), and serotypes with low prevalence were *S. Tamale* (7.5%), *S. Labadi*

(7.5%), *S. Alfort* (6.5%), *S. Apeyeme* (5.4%), *S. Inganda* (5.4%), *S. Rissen* (5.4%), *S. Heidelberg* (5.4%), *S. Larochele* (5.4%), *S. Haifa* (5.4%) and untypable (11.8%). As in table 4. Serotyping of 96 Congo red- positive *E. coli* isolates illustrated that serotypes with high prevalence were O142 (9.4%), O126 (7.3%), O128 (6.3%), O44 (6.3%), O86 (6.3%) and serotypes with low prevalence were O111 (5.2%), O118 (5.2%), O191 (5.2%), O127 (5.2%), O78 (4.2%), O125 (4.2%), O164 (4.2%), O91 (4.2%), O25 (4.2%), O103 (4.2%), O55 (4.2%) and untypable (14.6%) (Table 5).

Antimicrobial susceptibility of Salmonella and E. coli

The results of antibiotic susceptibility testing of *salmonella* and *E. coli* isolates show resistance against Nalidixic acid (100%-100% respectively), Penicillin G (95.1%-100% respectively), Clindamycin (75.6%-86.4% respectively), Amoxyclav (64.6%-93.9% respectively), Sulpha & Trimethoprim (56%-80.4% respectively), Cefotaxim (41.5%-70.4% respectively), tetracycline (40.2%-93.9% respectively), Kanamycin (35.4%-68.3% respectively), Ciprofloxacin (30.5%-71.9% respectively), Meropenem (25.6%-24.6% respectively), Gentamicin (8.5%-72% respectively) as mentioned in (Tables 6, 7).

The analysis of health risk of the MAR index found that 100% of each *Salmonella* and *E. coli* showed a MAR index ≥ 0.2 , which indicated a high risk of antimicrobials. So, most of *Salmonella* and *E. coli* isolates showed a multidrug resistance profile.

In-vitro pathogenicity testing of E. coli by Congo red binding assay:

Ninety-six of *E. coli* isolates 96/121 (80%) were pathogenic and gave red colonies as Congo red positive, and 25/121 (20.6%) were nonpathogenic and gave white colonies. Whereas Congo red positive gave red colonies, while Congo red colonies did not bind the dye and gave white colonies.

Molecular Identification of Salmonella, E. coli, and Clostridia Isolates

All identified *clostridial* isolates have alpha-toxin gene (*cpa*) by multiplex PCR, and (100%) of *clostridial* isolates were *clostridium perfringens* type A, as illustrated in (Fig.1.).

All identified *E. Coli* isolates were confirmed by the presence of *papC*, *iss* and *iutA* virulence genes and negative for *tsh* gene as shown in (Fig.2.).

All *Salmonella* isolates were identified by *invA* as a common gene for the identification of *Salmonella* and *ompA* genes, and fifty percent of *Salmonella* isolates carry *stn* and *hila* virulence genes, as shown in (fig.3.).

Discussion

Enteropathogens are an important problem that threaten the poultry industry, especially broilers. This paper covered some common enteric complications that affect broilers to provide a current view of this problem. This study was conducted on 84 broiler farms. Samples of liver and intestine were collected for the isolation of *Salmonella* and *E. coli*. Intestinal samples were only collected for *Clostridium perfringens* pathogen isolation. Our findings showed that most enteric infections are a mixed infection rather than a single infection. This can be explained by the opportunistic activity of some bacteria [8]. *E. coli*, *Salmonella*, and *clostridium* bacteria were isolated. The rate of *Salmonella* isolation was 55.35% of total samples, with 21.4% and 33.9% from the liver and intestine, respectively. These results were in contrast to those of [29], who showed 6.6% positive *salmonella* samples, and [30], who reported 2.28% positive *Salmonella* from liver (3.36%) and intestinal content (1.44%). This may be due to the different localities, investigated farms, and types of samples. *E. coli* prevalence was 72.02% of total samples, with 30% and 42.2% from the liver and intestine, respectively. Our result is counteracting with [31], who detected a 53.2% isolation rate, which may be due to sampling in this study from the intestine. Our results are clockwise with [32], who isolated *E. coli* with a percentage of 63.6% from broilers. On the other hand, *Clostridium perfringens* isolation percentage was 80.9% in diseased birds. This finding is concurred with [11], who recorded 75% positive *clostridium* from diseased birds. Contrariwise, [2] found 51 positive samples out of 184 samples (27.71%). In this work, the total percent of mixed infections out of isolated microorganisms was 59.5% for mixed *Salmonella* and *E. coli*. These results were counteracted with [9] who detect 37.6% mixed infection for *Salmonella* and *E. coli*. It is crucial to note that the prevalence rates of *Salmonella* and *E. coli* in this study were notably higher than those reported in some previous studies, perhaps reflecting differences in geographical locations, farm management practices, and sampling methodologies. besides, the upsurge in virulence of these bacteria

due to their circulation in poultry farms [33]. However, it is essential to interpret these differences with caution, considering the dynamic nature of bacterial populations.

Serogrouping of the isolated *salmonella* revealed *S. Enteritidis* (12.9%), *S. Typhimurium* (11.8%), *S. Kentucky* (9.67%), *S. Tamale* (7.5%), *S. Labadi* (7.5%), *S. Alfort* (6.5%), *S. Apeyeme* (5.4%), *S. Inganda* (5.4%), *S. Rissen* (5.4%), *S. Heidelberg* (5.4%), *S. Larochelle* (5.4%), *S. Haifa* (5.4%) and untypable (11.8%). Other work stated a high prevalence of *S. Enteritidis* that corresponds to our results [30]; however, he recorded different *Salmonella* serotypes, which may be due to the vast majority of *Salmonella* species that are widely spread in Egypt and all over the world [34]. Clockwise, [35] showed the same three most prevalent serotypes and shared some of the rest of the serotypes. Salmonellosis is one of the food-transmitted diseases that has a human public health concern [36]. Poultry products were considered the main source of human infections by *Salmonella*. The most prevalent serotypes were detected in diarrhetic patients, as follow *S. Enteritidis* (58.33%), *S. Typhimurium* (41.66%) were recorded in [37] and *S. Kentucky* (100%) according to [38]. Different *E. coli* serotypes were detected as O142 (9.4%), O126 (7.3%), O128 (6.3%), O44 (6.3%), O86 (6.3%), O111 (5.2%), O118 (5.2%), O191 (5.2%), O127 (5.2%), O78 (4.2%), O125 (4.2%), O164 (4.2%), O91 (4.2%), O25 (4.2%), O103 (4.2%), O55 (4.2%), and untypable (14.6%). These findings were nearly close to [39] while disagreeing in prevalence with [31] with *E. coli* O78 (27.8%), then O44 and O55 (16.7%) in Egypt. This may be due to different serotypes of *E. coli* [40]. The antibiotic resistance results of *Salmonella* and *E. coli* showed a high resistance against nalidixic acid and Penicillin G 100% for each, while *Salmonella* isolates showed high sensitivity to gentamycin (8.5%) and *E. coli* isolates were more sensitive to Meropenem (24.6%). Other antibiotics showed a variable degree of resistance against *Salmonella* and *E. coli*. Briefly, Clindamycin showed 75.6% and 86.4% respectively, Amoxiclav showed 64.6% and 93.9% respectively, Sulpha & Trimethoprim showed 56% and 80.4%, respectively; Cefotaxime showed 41.5% and 70.4%, respectively; tetracycline showed 40.2% and 93.9%, respectively; Kanamycin showed 35.4% and 68.3%, respectively; Ciprofloxacin showed 30.5% and 71.9%, respectively; gentamycin

showed 8.5% and 72%, respectively. Our data is nearly similar to [29] in tetracycline, Sulpha & Trimethoprim and Kanamycin and disagrees in Ciprofloxacin (4.8%-23.8% respectively), Nalidixic acid (9.6%-60.7% respectively) and Gentamicin (0%-20.2% respectively). Our result of antibiotic resistance in *Salmonella* is in contrast with [35] at which Gentamycin and Cefotaxime showed 100% resistance and 65% resistance against Ciprofloxacin. This difference might be due to the misuse of antibiotics in different locations. *E. coli* resistance result was nearly similar to that of [39], who showed 100% resistance to tetracycline, and 80.92% for Amoxicillin-Clavulanic acid, 75% for Trimethoprim/ Sulphamethoxazole, and 50% for Gentamycin. Health risk assessment was determined by MAR index, and 100% of each *Salmonella* and *E. coli* showed a MAR index ≥ 0.2 . This result is nearly similar to [29], who stated that the majority of *Salmonella* and *E. coli* isolates had a MAR index > 0.2 . This is a sequela to the misuse of antibiotics and antibiotic prophylactic treatment that increase the resistance of bacteria to antibiotics.

The results of *E. coli* pathogenicity by Congo red testing showed that ninety-six (80%) of *E. coli* isolates were pathogenic as Congo red -positive (red colonies), while 25 isolates (20.6%) were Congo red- negative (white colonies), which were considered non-pathogenic isolates. These results were confirmed by virulence gene testing that clarified the relationship between the phenotypic and genotypic characters of *E. coli*. The results of Congo red were in contrast with those of [41], who reported a higher prevalence of Congo red negative (64.3%) than Congo red positive (35.7%). Molecular detection of virulence genes in *Salmonella* isolates was applied through detection of certain genes as follows (*invA* gene that is responsible for adhesion and invasion of the host cell; *stn*: enterotoxin gene; *ompA*: outer membrane protein that is responsible for adhesion and *hila*: hyperinvasive locus that helps in bacterial invasion). The results showed 100% detection of *invA* and *ompA* genes and 50% detection of *stn* and *hila* genes throughout *Salmonella* isolates. These results coincide with those of [35], who recorded 100% detection of *invA* gene in all tested samples. This gene is considered a specific virulence marker for *Salmonella*. On the contrary, [35] detected *hila*

gene in percent 88.24% and 100% respectively, and was nearly close to [42], who detected the prevalence of *stn* gene in percent 58.82% and 40%, respectively. Our results were matched with those of [43], who detected *invA* and *ompA* genes in 100% of the examined isolates but not the same result in *stn* gene, which has a prevalence 100%.

The result of molecular detection of *E. coli* virulence genes showed 100% positivity for *papC*, *iss*, and *iutA* genes and 0% detection for *tsh* gene. *papC* gene (P fimbriae) this gene was encoded by pyelonephritis associated pili gene clusters (*pap*) that promote the attachment of the bacteria to cell receptors, and were very important for development of septicemia), *iss* (increased serum survival: Serum resistance VGs allow the bacteria to survive exterior to the gastrointestinal tract, and overcome defense mechanisms of the host involving complement and antimicrobial peptides) and *iutA* (*iutA* that encodes for an outer membrane protein, which serves as a receptor, that regulates aerobactin siderophore receptor) virulence genes and negative for *tsh* gene (adhesion gene ,temperature-sensitive hemagglutinin (*tsh*) gene is an auto transporter protein with double functions of proteolytic and adhesive activities). Our data shows the same prevalence as [44] that reported *iss* (100%) *iutA* (98.81%). Our findings were matched with those of [39], who found that the percentage of *iss*, and *papC* was 100% in all isolates. On the other hand, [45] detected *tsh* gene with a prevalence of 60%-45.5%, respectively. Molecular detection of *Clostridia perfringens* showed that 100% of *clostridial* isolates have the alpha- toxin gene using multiplex PCR, which refers to all isolates being *C. perfringens type A*, which contain the *cpa* gene that encodes for alpha-toxin that mainly causes necrotic enteritis in poultry. These findings concurred with [46], which reported 100% of isolates were positive for alpha- toxin. On the other hand, our results do not match with those of [47], who found that 87.94% of isolates contain alpha -toxin.

Conclusion

This study provides valuable insights into the virulence and antibiotic resistance profiles of enteric pathogens in broiler chickens, with a particular focus on *Salmonella* spp., *E. coli*, and *Clostridia*. The prevalence of mixed infections, the diverse serotypes of *Salmonella* and *E. coli*, as

well as the detection of specific virulence genes, underscore the complex nature of these infections. The concerning levels of antibiotic resistance emphasize the urgent need to explore alternative strategies for develop strategies beyond antibiotics to mitigate this issue in Egyptian farms.

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Conflict of interest

Authors have no conflict of interest.

TABLE 1. Oligonucleotide primers sequences for virulence genes of *Salmonella* Spp and *E. coli* and toxins genes of *Clostridium perfringens*.

Agent	Toxin	Sequence	Amplified product	Reference
<i>C. perfringens</i>	<i>Alpha toxin</i>	GTTGATAGCGCAGGACATGTTAAG CATGTAGTCATCTGTTCCAGCATC	402 bp	[21]
	<i>Beta toxin</i>	ACTATACAGACAGATCATTCAACC TTAGGAGCAGTTAGAACTACAGAC	236 bp	
	<i>Epsilon toxin</i>	ACTGCAACTACTACTCATACTGTG CTGGTGCCTTAATAGAAAGACTCC	541 bp	
	<i>Iota toxin</i>	GCGATGAAAAGCCTACACCACTAC GGTATATCCTCCACGCATATAGTC	317 bp	
<i>E. coli</i>	<i>Tsh</i>	GGT GGT GCA CTG GAG TGG AGT CCA GCG TGA TAG TGG	620 bp	[22]
	<i>papC</i>	TGATATCACGCAGTCAGTAGC CCGGCCATATTCACATAA	501 bp	[23]
	<i>Iss</i>	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATACCC	266 bp	[24]
	<i>iutA</i>	GGCTGGACATGGGAAGTGG CGTCGGGAACGGGTAGAATCG	300 bp	
	<i>Stn</i>	TTG TGT CGC TAT CAC TGG CAA CC ATT CGT AAC CCG CTC TCG TCC	619 bp	[25]
<i>Salmonella</i> Spp.	<i>invA</i>	GTGAAATTATCGCCACGTTCCGGCAA TCATCGCACCGTCAAAGGAACC	284 bp	[26]
	<i>Hila</i>	CATGGCTGGTCAGTTGGAG CGTAATTCATCGCCTAAACG	150 bp	[27]
	<i>ompA</i>	AGT CGA GCT CAT GAA AAAGAC AGC TAT CGC AGT CAA GCT TTT AAG CCT GCG GCT GAG TTA	1052 bp	[28]

TABLE 2. Cycling conditions of the different primers during conventional PCR

Agent	Toxin	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
<i>C. perfringens</i>	<i>Alpha, Beta, Iota and Epsilon</i>	94°C 5 min.	94°C 30 sec.	54°C 40 sec.	72°C 45 sec.	35	72°C 10 min.
	<i>Tsh</i>	94°C 5 min.	94°C 30 sec.	54°C 40 sec.	72°C 45 sec.	35	72°C 10 min.
	<i>papC</i>	94°C 5 min.	94°C 30 sec.	59°C 40 sec.	72°C 45 sec.	35	72°C 10 min.
<i>E. coli</i>	<i>Iss</i>	94°C 5 min.	94°C 30 sec.	54°C 30 sec.	72°C 30 sec.	35	72°C 7 min.
	<i>iutA</i>	94°C 5 min.	94°C 30 sec.	63°C 30 sec.	72°C 30 sec.	35	72°C 7 min.
	<i>Stn</i>	94°C 5 min.	94°C 30 sec.	59°C 40 sec.	72°C 45 sec.	35	72°C 10 min.
	<i>invA</i>	94°C 5 min.	94°C 30 sec.	55°C 30 sec.	72°C 30 sec.	35	72°C 7 min.
<i>Salmonella</i>	<i>Hila</i>	94°C 5 min.	94°C 30 sec.	60°C 30 sec.	72°C 30 sec.	35	72°C 7 min.
	<i>ompA</i>	94°C 5 min.	94°C 30 sec.	55°C 40 sec.	72°C 1 min.	35	72°C 10 min.

TABLE 3. Incidence of Isolated *Salmonella*, *E. coli* and *Clostridia* organisms from broiler chickens

Governorate	Salmonella				E. coli total No.				Clostridia	
	(total No. 93\168 (55.35%)				(72.02%)168\121				Total 68\84(80.95%)	No.
	(Intestine)	%	(Liver)	%	(Intestine)	%	(Liver)	%	(Intestine)	%
Dakahlia	8	4.8	5	2.97	13	7.7	9	5.4	11	13.1
Sharkia	12	7.1	7	4.2	14	8.3	8	4.8	12	14.3
Qalyubia	6	3.6	4	2.4	13	7.7	9	5.4	15	17.9
Gharbia	11	6.5	11	6.5	12	7.14	9	5.4	9	10.7
Fayoum	11	6.5	3	1.8	11	6.5	8	4.8	12	14.3
Menia	9	5.4	6	3.6	8	4.8	7	4.2	9	10.7
Total	57	33.9	36	21.4	71	42.2	50	30	68	80.9

TABLE 4. Serotyping of *Salmonella* isolates.

	S	S. Kentucky	S. Tamale	S. Labadi	S. Alfort	S. Apeyeme	S. Inganda	S. Rissen	S. Heidelberg	S. Larochelle	S. Haifa	untypable
Daqahliya	2	1	-	-	2	2	-	2	-	-	-	-
Sharqiya	1	1	2	2	2	-	-	-	2	1	1	-
Qalioubiya	2	2	2	-	-	1	-	-	-	-	-	-
Gharbiya	-	1	1	3	2	1	2	-	3	4	1	-
Fayum	2	2	2	2	-	-	1	1	-	-	-	-
Menia	4	2	-	-	-	1	2	2	-	-	3	-
Total\ Isolates	12(12.9%)	11(11.8%)	7(7.5%)	7(7.5%)	6(6.5%)	5(5.4%)	5(5.4%)	5(5.4%)	5(5.4%)	5(5.4%)	5(5.4%)	11(11.8%)

TABLE 5. Illustrate Serotyping of *E. coli* isolates.

	O125	O164	O111	O142	O128	O44	O91	O25	O118	O86	O126	O127	O78	O119	O103	O55	Untypable
Daqahliya	2	1	2	1	1	-	3	-	3	2	3	3	1	1	-	-	-
Sharqiya	1	1	-	1	-	2	1	-	-	2	1	1	-	2	1	1	-
Qalioubiya	1	-	-	2	3	-	-	3	1	1	2	1	1	-	-	1	Untypable
Gharbiya	-	2	1	-	-	-	-	1	-	-	-	-	2	1	-	-	-
Fayum	-	-	2	3	2	4	-	-	-	-	-	-	-	-	2	1	-
Menia	-	-	-	2	-	-	-	-	1	1	1	-	-	1	1	1	-
Total\ isolate	4(4.2%)	4(4.2%)	5(5.2%)	9(9.4%)	6(6.3%)	6(6.3%)	4(4.2%)	4(4.2%)	5(5.2%)	6(6.3%)	7(7.3%)	5(5.2%)	4(4.2%)	5(5.2%)	4(4.2%)	4(4.2%)	14(14.6%)

TABLE 6. Antibiotic resistance profile of *Salmonella* isolates with their MAR index.

<i>Salmonella</i> Strains (No.of isolates)	Antimicrobial Resistance Profile	MAR Index (No.of isolates)
<i>S.Enteritidis</i> (3)	NA,P,CL,Amk,SXT,CF,T,K,CP,M,G	1(3)
<i>S.Enteritidis</i> (3)	NA,P,CL,Amk,SXT,CF,T,K,CP,M	0.9(3)
<i>S.Enteritidis</i>	NA,P,CL,Amk,SXT,CF,T,K,CP,M	0.9
<i>S.Enteritidis</i>	NA,P,CL,Amk,SXT,CF,T,K	0.7
<i>S.Enteritidis</i>	NA,P,CL,Amk,SXT,CF,T	0.6
<i>S.Enteritidis</i>	NA,P,CL,Amk	0.4
<i>S.Enteritidis</i>	NA,P,CL	0.3
<i>S.Enteritidis</i>	NA	0.09
<i>S. Typhimurium</i> (4)	NA,P,CL,Amk,SXT,CF,T,K,CP,M,G	1(4)
<i>S. Typhimurium</i> (2)	NA,P,CL,Amk,SXT,CF,T,K,CP	0.8(2)
<i>S. Typhimurium</i>	NA,P,CL,Amk,SXT,CF,T	0.6
<i>S. Typhimurium</i>	NA,P,CL,Amk,SXT,CF	0.5
<i>S. Typhimurium</i>	NA,P,CL	0.3
<i>S. Typhimurium</i>	NA,P,CL	0.3
<i>S. Typhimurium</i>	NA,P	0.2
<i>S.Kentucky</i> (3)	NA,P,CL,Amk,SXT,CF,T,K,CP,M	0.9(3)
<i>S.Kentucky</i> (2)	NA,P,CL,Amk,SXT,CF,T,K,CP	0.8(2)
<i>S.Kentucky</i> (2)	NA,P,CL,Amk,SXT,CF,T	0.6(2)
<i>S.Kentucky</i>	NA,P,CL,Amk	0.4
<i>S.Kentucky</i>	NA	0.09
<i>S.Tamale</i> (3)	NA,P,CL,Amk,SXT,CF,T,K,CP,M	0.9(3)
<i>S.Tamale</i> (3)	NA,P,CL,Amk,SXT,CF,T,K	0.7(3)
<i>S.Tamale</i>	NA,P,CL,Amk,SXT	0.5
<i>S.Labadi</i> (4)	NA,P,CL,Amk,SXT,CF,T,K,CP,M	0.9(4)
<i>S.Labadi</i> (2)	NA,P,CL,Amk,SXT	0.5(2)
<i>S.Labadi</i>	NA,P,CL	0.3
<i>S.Alfort</i> (4)	NA,P,CL,Amk,SXT	0.5(4)
<i>S.Alfort</i> (2)	NA,P	0.2(2)
<i>S.Apeyeme</i> (5)	NA,P,CL,Amk,SXT	0.5(5)
<i>S.Inganda</i> (5)	NA,P,CL,Amk	0.4(5)
<i>S.Rissen</i> (5)	NA,P,CL	0.3(5)
<i>S.Heidelberg</i> (5)	NA,P	0.2(5)
<i>S.Larochelle</i> (5)	NA,P	0.2(5)
<i>S.Haiifa</i> (5)	NA,P	0.2(5)
Average	0.530	

NA:Nalidixic acid – P:Penicillin G –CL:Clindamycin –Amk: Amoxyclav –SXT: Sulpha &Trimothoprim CF: Cefotaxim –T:tetracycline- K: Kanamycin –CP:Ciprofloxacin-M:Meropenem-G:Gentamicin.

TABLE 7. Antimicrobial resistance of *E. coli* isolates with their MAR index

E. coli strains (No.of isolates)	Antimicrobial resistance profile	MAR Index (No.of isolates)
(O111:K58(2	NA,P,CL, Amk,SxT, T, K,CP, M,G	0.9(2)
O142:K86	NA,P, Amk,SxT, T, K,G	0.6
(O128:K67(3	NA,P,CL, Amk , T,CP	0.5(3)
O44:K74	NA,P, Amk, T,CP, G	0.5
(O91(4	NA,P, Amk,SXT ,T	0.4(4)
O25:K11(4)	NA,P,CL, Amk,SxT,CF,T,CP,M, G	0.9(4)
(O118:K(3	NA,P, Amk,SXT,T, G	0.5(3)
(O128:K67(2	NA,P,CL, Amk,SXT,CF,T, K,M	0.8(2)
O142:K86	NA,P, Amk,SXT,K	0.4
O86:K61	NA,P,CL,CF,T, K,G	0.6
(O126:K71(2	NA,P,Cl,Amk,SXT,CF, T,K,CP,M, G	1(2)
O142:K86	NA,P,CL, Amk,SXT,T, K,G	0.7
(O127:K63(2	NA,P, Amk,SXt,T, K	0.5(2)
O126:K71	NA,P,CL, Amk,SXT,CF,T,K,CP,M,G	1
(O78:K80(4	NA,P,CL,CF,CP	(4)0.4
O86:K61(5)	NA,P,CL, Amk,SXT,CF,T,K,CP,M,G	1(5)
(O111:K58(3	NA,P,CL, Amk,SXT,CF,T,K,CP,G	(3)0.9
(O119:K69(2	NA,P,CL, Amk,SXt CF,T,CP ,G	(2)0.8
(O118:K(2	NA,P,CL, Amk,SXT,T,K,CP	(2)0.7
O126:K71	NA,P,CL, Amk,SXT,T,K,CP	0.7
(O142:K86(2	NA,P,CL, Amk,SXT,CF,T,K,CP,G	0.9(2)
(O103:K(4	NA,P,CL, Amk,SXT,CF,T,K,CP,M,G	(4)1
(O142:K86(2	NA,P,CL, Amk,SXT,CF,T,K,CP,M,G	1(2)
(O119:K69(3	NA,P,CL, Amk,SXT,CF,T,K,CP,G	(3)0.9
(O142:K86(2	NA,P,CL, Amk,CF,T,K,CP,G	(2)0.8
(O55:K59(4	NA,P,CL, Amk,SXT,CF,T, K,G	(4)0.8
O128:K67	NA,P,Cl, Amk,CF,T,CP, G	0.7
(O44:K74(4	NA,P,CL, Amk,SXT,CF,T,K,CP,G	0.9(4)
O44:K74	NA,P,CL, Amk,SXt,CF,T,K,CP,M,G	1
(O126:K71(3	NA,P,CL, Amk,SXt,CF,T,K,CP,G	(3)0.9
(O125:K70(4	NA,P,CL, Amk,SXT,CF,T,K,CP,G	0.9(4)
(O164:K(4	NA,P,CL, Amk ,T	(4)0.4
(O127:K63(3	NA,P,CL, Amk,SXt,CF,T,K,CP,G	0.9(3)
		Average 0.763

NA: Nalidixic acid – P: Penicillin G -CL: Clindamycin –Amk: Amoxyclav -SXT: Sulpha&Trimothoprim CF: Cefotaxim -T:tetracycline- K: Kanamycin-CP:Ciprofloxacin-M:Meropenem-G:Gentamicin.

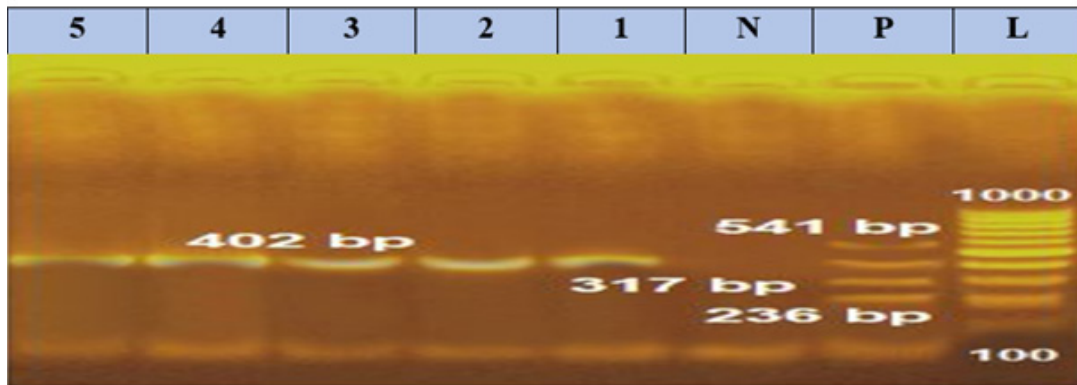


Fig. 1. Representative agar gel electrophoresis of multiplex PCR of *clostridia* showing alpha toxin (402 bp), Beta toxin (236bp), Epsilon toxin (541bp), and Iota toxin (317bp)

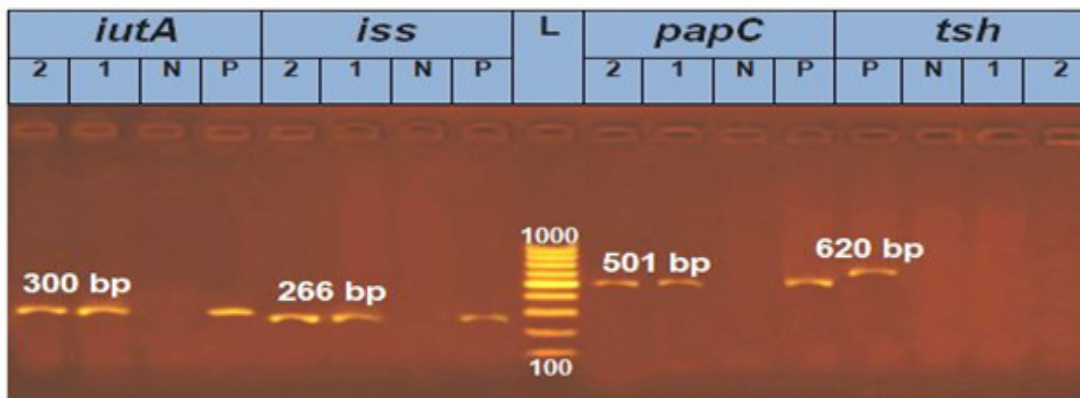


Fig. 2. Representative agar gel electrophoresis of PCR of *E. coli* showing *iss* gene (266bp), *papC* gene (501bp), *tsh* gene (620bp), and *iutA* gene(300bp).

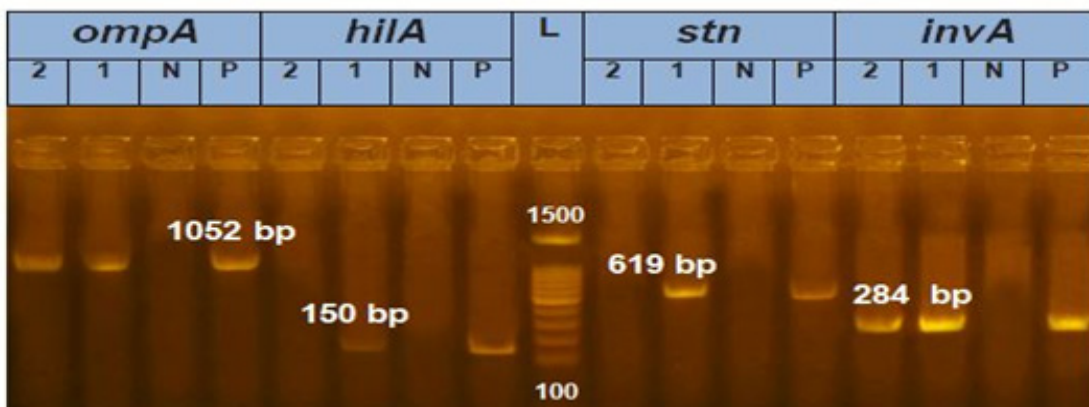


Fig. 3. Representative agar gel electrophoresis of PCR of *Salmonella* showing *ompA* gene (1052bp), *hilA* gene (150bp) *stn* gene (619bp), and *invA* gene(284 bp).

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الكشف عن مقاومة المضادات الحيوية، و الضراوة، والكشف الجزيئي للعدوى البكتيرية المعوية في دجاج التسمين: دراسة في مصر

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هذه الدراسة هدفت إلى التعرف على مسببات الأمراض المعوية الأكثر انتشارًا والتي تصيب دجاج التسمين وتشكل تهديدًا خطيرًا في المزارع المصرية. تم إجراء التحري على ٨٤ مزرعة في بعض المحافظات المصرية. تم استخدام عينات من الكبد والأمعاء لعزل وتحديد مسببات الأمراض المحتملة باستخدام التقنيات التقليدية والجزيئية. تم اعتماد اختبار مقاومة مضادات الميكروبات ضد ١١ مضادًا حيويًا تستخدم بشكل متكرر في حقل الدواجن. علاوة على ذلك، تم استخدام تفاعل البلمرة المتسلسل (PCR) للكشف عن جينات ضراوة العزلات. وأظهرت النتائج أن العدوى المختلطة هي السائدة على عدوى واحدة. وكانت أنواع البكتيريا المعزولة هي *Salmonella spp.* و *E. coli* و *Clostridia* بنسبة ٥٥٪، ٧٢٪ و ٨٠٪ على التوالي. وفيما يتعلق بعزلات السالمونيلا، كانت الأنماط المصلية *S. Enteritidis* و *S. Typhimurium* و *S. Kentucky* هي الأنماط المصلية الأكثر شيوعًا بنسبة ١٢,٩٪، ١١,٨٪ و ٩,٦٧٪ على التوالي. من ناحية أخرى، تم تصنيف الإشريكية القولونية إلى ١٦ نمطًا مصليًا مختلفًا. كانت مائة بالمائة من سلالات السالمونيلا إيجابية لجينات *invA* و *ompA*، وكانت ٥٠٪ إيجابية لجينات *stn* و *hilA*. كانت جميع سلالات الإشريكية القولونية إيجابية بالنسبة لجين *iss* و *papC* و *iutA* وسلبية بالنسبة لجين *tsh*. وكانت السلالات المعزولة من كلوستريديا إيجابية بالنسبة لسم ألفا فقط. أظهر ملف المضادات الحيوية مقاومة تقريبًا للمضادات الحيوية الأكثر استخدامًا في هذه الدراسة. تسلط هذه الدراسة الضوء على بعض الانتهاكات المعوية الخطيرة للدجاج اللحم من خلال تقديم صورة بسيطة عن مدى فوعتها وخصائصها المضادة للمضادات الحيوية، مع التأكيد على الحاجة الماسة لاتخاذ تدابير استباقية لإدارة هذه المشكلة بشكل فعال وتقليلها.

الكلمات الدالة: دجاج التسمين، الكلوستريديا بيرفيرنجينز، البكتيريا القولونية، تفاعل البوليميراز المتسلسل، السالمونيلا.