Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Characterization of multiple antimicrobial-resistant patterns of *E. coli* isolated from broilers in Ismailia farms and markets.

Fadwa F. Mahmoud[*], Wafaa A. A. Ibrahim[**], Heba M. Hassan[***]

[*] Food Hygiene and Microbiology, Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agriculture Research Center, Ismailia 41511, Egypt.
[**] Biotechnology department, Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agriculture Research Center, Ismailia 41511, Egypt.
***Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agriculture Research Center, Giza, Egypt.

Received in 13/8/2024
Received in revised from 8/9/2924
Accepted in 25/9/2024

Keywords:

Broilers
E. coli
misuse of antibiotics
multi antimicrobial drug resistance
and Antibiogram.

ABSTRACT

Colibacillosis is a septicemic illness that affects poultry economically and usually results in numerous lesions in broiler chicken flocks. Previously published studies revealed increased *E. coli* resistance against a wide range of clinically used antimicrobials. This study was carried out to isolate and identify *E. coli* isolated from diseased broiler chicken farms and markets in Ismailia governorate, detect that *E. coli* isolates susceptibility to commonly utilized antibiotics, such as tetracycline, , ampicillin, amoxicillin, gentamicin, streptomycin, colistin, and co-trimoxazole, etc. as well as detect the aminoglycosides resistant genes *aacC* (Aminoglycoside acetyltransferase) and *Aada2* (Aminoglycoside adenyltransferases) by PCR in *E. coli*-resistant isolates.

From 70 diseased broilers chicken farms (5-7 birds/farm) aged (4-38 days) were received at the Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP) - Ismailia branch during the period from September 2019 to October 2022 and 70 chicken meat samples collected from Ismailia markets. The collected samples subjected to various examinations including isolation, phenotypic identification, antimicrobial drugs susceptibility and resistance assessments, and the aminoglycosides resistant genes *aacC* and *Aada2* identification by PCR.

Results: Showed that *E. coli* was isolated and phenotypically recognized from 12 out of 70 diseased broiler chicken farms with a per cent ratio of 17.14 % and from 8 out of 70 chicken breast meat samples collected from

Corresponding author: Fadwa F. Mahmoud, Food Hygiene and Microbiology, Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agriculture Research Center, Ismailia 41511, Egypt.
E-mail: Mohammed.fadwa@ahri.gov.eg
DOI: 10.21608/ejah.2024.390613

markets in Ismailia with a per cent ratio of 11.43 %. All of the isolates showed multidrug resistance and antimicrobial resistance genes *aacC* and *Aada2* genes were recorded in 13 out of 15 and 14 out of 15 of isolated phenotypically recognized *E. coli* isolates with a per cent ratio of 93.33% and 87.67%, respectively. Both *aacC* and *Aada2* genes were Aminoglycoside resistance genes against gentamicin and streptomycin, respectively.

Conclusion: Broiler chicken may constitute an important source of multi antimicrobial drugs resistant *E. coli*, which can be considered a potential threat to public health through the transmission of resistant bacteria via the food chain.

INTRODUCTION:

In the poultry production field, the control of infectious diseases causing significant financial losses is recognized as one of the primary challenges (**McKissick, 2006**). Colibacillosis is brought about by the avian pathogenic bacterial organism (*E. coli*) (**Barnes et al. 2008)**. They belong to the *Enterobacteriaceae* family and are responsible for both extra-intestinal and enteric infections in both humans and animals (**Percival and Williams, 2014)**. The avian pathogenic *E. coli* are one of the pathogenic and lethal bacteria in the majority of poultry products (broiler chickens, layers, breeding flocks, ducks, and geese). It is Gram-negative road bacteria. It displayed colibacillosis and colisepticemia symptoms such as swelling head syndrome, air vasculitis, cellulitis, omphalitis, pericarditis, and perihepatitis. On the other hand, they pose a risk to public health by storing and spreading antibiotic resistance genes globally (***Nolan* et al. 2013**). All ages of chickens are vulnerable to colibacillosis; however, young birds are more frequently infected than older hens (**Barnes et al. 2003**). The colisepticemia is considered the most prevalent form of colibacillosis and causes significant financial losses in poultry production in various regions all over the world (**Saif, 2003**).

The global production of chicken is significantly impacted financially by colibacillosis. The majorities of financial losses were caused by the impacted birds' deaths and decreased productivity. It is a prevalent illness in flocks of chicken, particularly in intensive breeding systems (**Otaki, 1995**). Birds suffering from colibacillosis can exhibit a variety of symptoms, such as abrupt death or an abnormal coloration with their necks dragged into their bodies (**Matin et. al. 2017**). Antimicrobials are frequently utilized in the production of animals to cure infectious illnesses and enhance growth. The considerable resistance to antimicrobial agents in the normal flora of poultry and pathogenic microorganisms is largely caused by the use of antimicrobials in poultry production industries for growth promotion (**Romanus et al. 2012)**. In practice, the employing of antimicrobials in the feed may alter the intestinal flora by exerting a selective pressure in favor of resistant bacteria populations such as resistant *E. coli* which may enter into the environment and food chain (**Furtula et al. 2010)**. An important public health concern is the use of antibiotics in animals raised for food and how they contribute to bacterial resistance. *E. coli* is one of the microorganisms that are frequently resistant to antibiotics as a result of its

widespread presence in humans and animals as well as its function as a pathogenic and commensal organism **(Zhao et al. 2012)**.

Bacteria become resistant to both single and multiple antimicrobials with repeated use over time, making it difficult to treat some infections (**Moustafa and Mourad, 2015).** Antimicrobial resistance, particularly multidrug resistance, has increased dramatically in recent years in clinical isolates, including *E. coli* isolates from animals (**Elsabet, 2011)**. It is a worldwide issue, and it has now been recognized as a global public health phenomenon (**Kaye et al. 2004**). Due to the risk of spreading these resistant bacteria to humans, antimicrobial resistance among *E. coli* in food animals like chicken is becoming a bigger issue **(Odwar et al. 2014)**. In veterinary practice, the antimicrobial sensitivity testing of pathogenic microorganisms *in vitro* is considered the best way for the veterinarian to select the suitable treatment (**Radwan et al. 2016**). Additionally, it helps identify the isolates that are multidrug resistant. As a result, the right antibiotic should be chosen based on the sensitivity that can be determined through laboratory testing. Poultry vets are concerned about the widespread resistance of *E. coli* species to antibiotics. Much attention has been paid to this growing resistance both in Egypt and globally. According to **Radwan et al.** (**2020**) Plasmids are the main vector used to disperse resistance genes across the bacterial community. Antimicrobial resistance genes in *E. coli* isolates can be found with PCR, and there is a large range of multidrug resistance *E. coli*.

Therefore, this study aimed to isolate and identify *E. coli* from diseased broiler chickens collected from 70 diseased broiler chicken farms received to the Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP) - Ismailia branch during the period from September 2019 to October 2022, and 70 chicken meat samples collected from Ismailia markets. Additionally, the *E. coli* isolates susceptibility to commonly utilized antibiotics, such as tetracycline, kanamycin, ampicillin, amoxicillin, gentamicin, and co-trimoxazole was tested. Moreover, the detection of *aacC* and *Aada2* genes by PCR in *E. coli*-resistant isolates was carried out.

MATEREIALS and METHODS

1- Sample collection:

The tested samples consisted of 70 diseased broiler chicken farms received to the Reference Laboratory for Veterinary Quality Control on poultry production (RLQP) - Ismailia branch during the period from September 2019 to October 2022 and 70 chicken meat samples were collected from Ismailia markets. The samples collected from 5 to 7 chickens per farm and the age of birds varied from 4 to 38 days) suffered from depression, ruffled feathers, diarrhea and loss of appetite were subjected to post-mortem examination under septic conditions, and the internal organs (liver, lung, spleen, and heart) were collected from birds showing colisepticemia, air vasculitis, perihepatitis, and pericarditis then pooled together for bacterial screening and isolation. 25 ± 0.5 gm, composite chicken meat sam-

ples (represented by breast) were aseptically excised and transferred into a high -duty sterile stomacher bag with mesh containing 225 ml 0.1% (w/v) sterile buffered peptone water, BPW (Oxoid) whereas homogenized using a lab blender for 2 minutes to obtain a homogenate fluid.

2-Isolation and identification:

The samples were incubated aerobically into buffer peptone water at 37° C for 24 h. A loopful from each incubated sample was streaked onto MacConkey's agar (HiMedia) and Eosin Methylene Blue agar (EMBA) (Lab M) plates were then incubated at 37° C for 24 hours. The suspected colonies were 1–2 mm in diameter and appeared as a pink color colony on MacConkey and metallic sheen colonies on EMBA. Suspected *E. coli* colonies were subjected to morphological and biochemical identification, including oxidase, urease, indole production, methyl red, Voges–Proskauer, hydrogen sulfide, and citrate tests along with glucose, lactose, sorbitol, sucrose, and mannitol fermentation **(Nolan et al. 2013 Islam et al. 2014**).

3-Antimicrobial susceptibility pattern of the isolated *E. coli:*

Antimicrobial Sensitivity Test (AST) was performed for all isolates against the most commonly used antibiotics by poultry farms in Ismailia. A pattern of 14 antibiotics discs (Oxoid) was used, which include, Tetracycline (Tetracycline 30 µg disk), Ampicillin (Ampicillin 10 µg disk), Amoxicillin (Amoxicillin 30 µg disk), Gentamicin (Gentamicin 10 µg disk), Kanamycin (Kanamycin 30 µg disk), Co-trimoxazole (Trimethoprim+Sulfamethoxazole 2.25/23.75 µg disk), Streptomycin (Streptomycin 10 µg disk), Ceftazidim (Ceftazidime 30 µg disk), Colistin (Colistin 10 µg disk), Levofloxacin (Levofloxacin 5 µg disk), Lincomycin (Lincomycin 5 µg disk), rifampicin (Rifampicin 5 µg disk), ofloxacin (Ofloxacin 5 µg disk) and Naldixic acid (Nalidixic acid 100 µg disk), using disk diffusion method as previously described (**WHO, CDC, 2013**). A bacterial suspension of 0.5 McFarland was prepared and streaked on Mueller-Hinton agar (Oxoid) plates using cotton swabs. Finally, antibiotic disks were placed on the surface of the plates followed by incubation at 37°C for 24 h. After incubation, the inhibition zones were measured (in millimeters) using a ruler and interpreted according to the guidelines of Clinical and Laboratory Standards Institute **(CLSI, 2021)**.

4- **Detection of *aacC* and *Aada2* genes by PCR in *E. coli*-resistant isolates:**

Fifteen *E. coli* were tested for *aacC* (aminoglycoside acetyltransferase) and Aada2 (aminoglycoside adenyltransferases) genes by PCR. Both genes are aminoglycoside resistance genes against gentamicin and streptomycin, respectively **(Lynne et al. 2008) and (Walker et al. 2001).**

DNA extraction:

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10

min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer:

Primers used were supplied from **Metabion (Germany)** are listed in table (1).

Table 1. Primers sequences, *aacC* and *Aada2* target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
aacC	GGCGCGATCAAC-GAATTTATCCGA CCATTCGATGCCGAAGG AAACGAT	448	94˚C 5 min.	94˚C 30 sec.	60˚ C 45 sec.	72˚ C 45 sec.	72˚ C 10 min.	Lynne *et al.*, (2008)
Aada2	TGTT-GGTTACTGTGGCCGTA GATCTCGCCTTTCACAA AGC	622	94˚C 5 min.	94˚C 30 sec.	50˚ C 45 sec.	72˚ C 45 sec.	72˚ C 10 min.	Walker *et al.*, (2001)

PCR amplification:

Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 5.5 µl of water, and 5 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

Analysis of the PCR Products:

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products were loaded in each gel slot. A general 100 bp ladder (Fermentas, thermos, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

RESULTS

Prevalence of *E. coli* Isolates in Broiler Chicken Samples from Diseased Farms and Markets in Ismailia, Egypt:

In a comprehensive study conducted in Ismailia, Egypt, the prevalence rates of *E. coli* isolates were investigated in broiler chicken samples collected from both diseased broiler chicken farms and markets. The findings revealed varying rates of *E. coli* occurrence in the two settings. Out of 70 samples collected from diseased broiler chicken farms, 12 isolates of *E. coli* were recovered, resulting in a prevalence rate of 17.14 %. In comparison,

samples obtained from markets exhibited a lower prevalence, with 8 isolates of *E. coli* recovered out of the 70 samples, corresponding to a prevalence rate of 11.43 %.

Antibiogram study of *E. coli* isolated from pooled internal organ samples of diseased broiler chickens collected from broiler farms and breast muscle samples collected from different markets in Ismailia:

In a comprehensive antibiogram study, 20 biochemically confirmed *E. coli* isolates from pooled internal organ samples of diseased broiler chickens from farms and breast muscle samples from various markets in Ismailia were evaluated for their resistance patterns against a range of selected antibacterial agents (N=14).

The results revealed a concerning trend of multidrug resistance among the tested isolates.

The antimicrobial resistance profile of the 20 biochemically confirmed *E. coli* isolates from broiler chicken samples in Ismailia revealed noteworthy patterns. Firstly, all isolates displayed 100% resistance against amoxicillin, rifampicin, lincomycin and nalidixic acid, indicating high resistance rates. Secondly, the isolates exhibited elevated resistance against ampicillin (95%), streptomycin (90%) and kanamycin (71.43%) representing the second-highest resistance rates. Conversely, ofloxacin (45.45%), and gentamicin (30%) recorded the lowest resistance rates. Notably, colistin demonstrated sensitivity across all tested isolates. Furthermore, the concerning observation of multidrug resistance patterns emerged, as all isolates exhibited resistance to three or more antimicrobial agents from different classes, underscoring the urgent need for comprehensive strategies to address the pervasive issue of multidrug-resistant *E. coli* strains in poultry samples.

Detection of *aacC* and *Aada2* resistance genes by PCR in *E. coli*-resistant isolates:

The antimicrobial resistance genes *aacC* and *Aada2* genes were recorded in 13 out of 15 and 14 out of 15 isolated phenotypically and biochemically recognized *E. coli* isolates with a per cent ratio of 93.33% and 87.67%, respectively. Both *aacC* and *Aada2* genes were Aminoglycoside resistance genes against gentamicin and streptomycin, respectively.

Table 2. Results of the antibiotic sensitivity test of *E. coli* isolates

Antibiotic disks	Total no. of tested *E. coli* isolates	Resistant		Sensitive		Intermediate	
		No.	%	No.	%	No.	%
Amoxy	20	20	100	0	0	0	0
Rifam	20	20	100	0	0	0	0
Naldix	13	13	100	0	0	0	0
Linko	13	13	100	0	0	0	0
Amp	20	19	95	1	0.05	0	0
Strep	20	18	90	1	0.05	1	0.05
Levo	14	12	85.72	1	7.14	1	7.14
Kana	14	10	71.43	0	0	4	26.57
Co tri/sul	20	14	70	6	30	0	0
Tetra	20	14	70	3	15	3	15
Ceftazi	20	14	70	0	0	6	30
Oflox	11	5	45.45	3	27.27	3	27,27
Genta	20	6	30	13	65	1	0.05
Colistin	20	0	0	20	100	0	0

Table 3. Detection of Aminoglycoside antimicrobial resistance genes aacC and Aada2 against gentamicin and streptomycin

Sample	Phenotype		Genotype	
	Gentamicin (CN10)	Streptomycin (S10)	Aada2	aacC
1	S	R	+	+
2	R	R	+	+
3	S	R	+	+
4	R	R	+	+
5	S	R	+	-
6	R	R	+	+
7	S	R	+	+
8	S	R	+	-
9	S	R	+	+
10	S	R	+	+
11	R	R	+	+
12	S	R	+	+
13	I	I	+	+
14	R	R	-	+
15	R	R	+	+
No. of +ve isolates	6/15	14/15	14/15	13/15
%	40%	93.33%	93.33%	86.67%

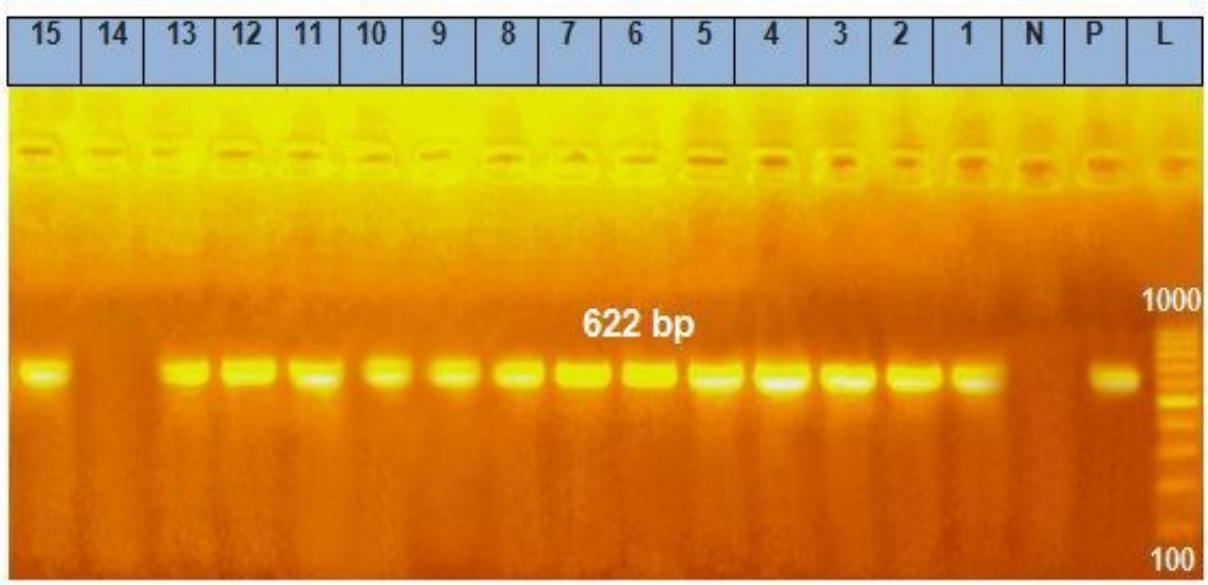

Figure 1. Amplification of *Aada2* resistance gene. All samples produced a band at 622 bp (positive to the *Aada2* gene) except in lane 14 (negative to the *Aada2* gene). Lane M: 1Kb DNA Ladder

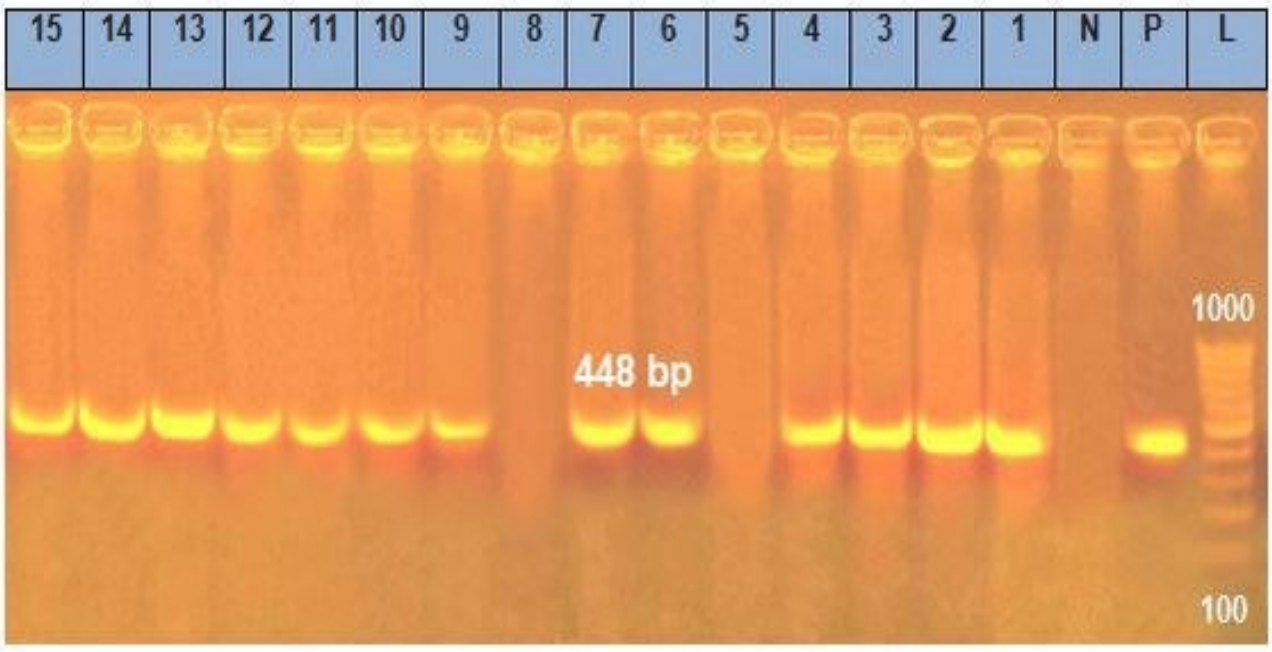

Figure 2.Amplification of aacC resistance gene. All samples produced a band at 448 bp (positive to *aacC* gene) samples, except in lanes 5, and 8 (negative to *aacC* gene). Lane M: 1Kb DNA Ladder.

DISCUSSION

The study involved the examination of 70 pooled internal organ samples from diseased broiler chicken farms in Ismailia, revealing that 12 of these samples (17.14%) were positive for *E. coli*. Additionally, 8 out of 70 samples of breast muscles from various markets in Ismailia were found to contain *E. coli*, resulting in an incidence of 11.43%. These findings underscore significant differences in the prevalence rates of *E. coli* isolates between broiler chicken samples from farms and those from markets. The higher prevalence in farm samples suggests a comparatively higher risk of *E. coli* contamination in these environments. Conversely, the higher prevalence in market samples indicates an increased potential for *E. coli* contamination in retail settings. Possible factors contributing to this difference include variations in hygiene practices, storage conditions, and transportation methods between the two sources. The study emphasizes the critical need for ongoing monitoring and targeted measures to improve food safety standards in both broiler chicken farms and marketplaces. Identifying and addressing specific risk factors associated with *E. coli* contamination is essential to ensure the overall safety of poultry products and safeguard public health. Continuous surveillance and collaboration between poultry producers, market stakeholders, and regulatory authorities are essential components for mitigating potential risks linked to *E. coli* contamination within the food supply chain.

These findings seemed somewhat compatible with **Moawad et al. (2018) and Shecho et al. (2017)** who recorded *E. coli* isolation from avian farms in both Egypt and Ethiopia at a very low rate (13.4% and 11%), respectively. In the same context, colibacillosis incidence was proven to be 0, 84% and 0, 8% in broiler chickens and layers (**Matin et al. 2017**).

On the other hand, laboratory investigation of 350 collected samples of poultry origin revealed that 132 samples were ensured to have *E coli* isolates with an incidence of 37.7%. These *E coli* isolates were segregated from chickens' internal organs with an incidence of 53.4% (**Ibrahim et al. 2019**). Furthermore, out of 270 examined whole chicken carcass samples, 216 isolates of *E coli* were segregated with an incidence of 80% **(Eltai, et al. 2020).**

In chicken farms, antibiotics are utilized for a variety of purposes, including prophylaxis, growth promotion, and medicinal uses **(Almofti et al. 2016 Mohamed-Noor et al. 2012)**. They include a large number of compounds of different types that can be given in chicken feed or drinking water. However, due to the existence of antibiotic residues and bacteria that are resistant to antibiotics, including *E. coli*, the careless administration of these medications may have unfavorable effects (**Singer et al. 2006 Almofti et al. 2016**). Furthermore, several scientific studies have shown a connection between the use of antibiotics in animals raised for food production and the development and evolution of bacteria resistant to antibiotics **(Singer et al. 2006, Mohamed-Noor et al. 2012** and **Almofti et al. 2016)**.

Recent studies in Egypt and worldwide have reported antimicrobial residues and antibiotic-resistant bacteria in food animal products such as chicken meat suggesting large-scale unregulated use of antibiotics by the poultry industry (**Samy et al. 2022**, **Brower et al. 2017**, **Mohamed-Noor et al. 2012**, and **Eckburg et al. 2005)**.

These seemed compatible with our findings in this study which revealed a marked predominance of antibiotic resistance among *E. coli* isolates obtained from different diseased broiler chicken farms and markets in Ismailia. Regarding the rising rate of *E. coli* isolates antimicrobial resistance in this study; these results were somewhat comparable to those published in other Egyptian publications (**Amer et al. 2018; El-Seedy et al. 2019; Qurani 2019)**. Furthermore, numerous reports from all around the world have confirmed this finding, including those from **Dou et al. (2016)** in China**, Rahman et al. (2017)** in Bangladesh**, (Danachi et al. 2018)** in Lebanon, and **Subedi et al. (2018)** in Nepal. These findings point to clear evidence of the indiscriminate and abusive use of certain antibiotics for infection prevention or control. These multidrug-

resistant bacteria eventually take the place of the drug-sensitive ones in an environment that is saturated with antibiotics (**Van den Bogaard et al. 2001**).

In this study, our obtained findings revealed that all isolates of *E coli* showed 100% résistance against amoxicillin, rifampicin, lincomycin and Nalidixic acid. The second-highest resistance rate was recorded against ampicillin (95%), streptomycin (90%) and kanamycin (71.43%). These results are nearly similar to that of **Abdel-Rahman et al. (2023)** who recorded that most *E. coli* isolates from diseased cases in broiler Egyptian farms showed the highest resistance percentage to ampicillin and nalidixic acid, **Samy et al. (2022)** who reported highest resistance against amoxicillin was found among *E coli* isolates from poultry samples with percentages of 83.3% and **Hamed et al. (2021)** who detected high resistance of *E. coli* isolates against ampicillin, tetracycline and nalidixic acid.

Meanwhile, the lowest resistance rate was recorded against ofloxacin (45.45%). This recorded result was somewhat in agreement with **Hamed et al. (2021)** who detected that *E. coli* isolates from some Egyptian poultry farms showed less resistance to ciprofloxacin and **Moawad et al. (2018)** who reported that *E. coli* isolates from healthy broilers in Egypt showed a low rate of resistance to fluoroquinolones ciprofloxacin (21.4%) and levofloxacin (14.3%). However, in the current study, none of the tested isolates exhibited resistance to colistin. Previous studies conducted in Egypt have found significant differences in *E. coli* isolates resistance to colistin. **Badr et al. (2022)** noted that *E. coli* isolates from broilers chicken farms in three Egyptian governorates displayed a low incidence rate of resistance (41%), **Awad et al. (2020)** found that 54 flocks of broilers in two North Delta governorates had a high incidence of 92.31%. However, 48 broiler farms spread across five governorates in northern Egypt expressed a very low incidence (7.9%) according to **Moawad et al. (2018)**.

In this study, all isolates showed resistance against 3 or more investigated antimicrobial agents of different class (multidrug resistance) patterns (100%). These obtained results were consistent with **Radwan et al. (2020)** who recorded that all *E. coli* isolates from broiler chickens in Beni-Suef, EL-Minia, El-Fayoum, Assiut and Sohag Governorates were 100% multidrug-resistant (MDR), **Hamed et al. (2021)** who found that all of *E. coli* isolates from some Egyptian poultry farms expressed resistance to at least three or more antimicrobials and **Abdel-Rahman et al. (2023)** who reported that all isolates of *E. coli* investigated for their sensitivity using the disk diffusion method against 18 antibiotics were described as multidrug-resistant strains.

Antimicrobial resistance (AMR) acquisition and spread are linked to several genetic pathways. Numerous mobile and mobilizable genetic components, such as integrons, transposons, insertion sequences, and plasmids, are included in the *E. coli* mobilome **(Gillings, 2014).**

It is commonly recognized that integrons have a significant role in the spread of antibiotic resistance in Gram-negative bacteria. Integrons are genetic structures that can transcribe, remove, and express genes. These genes are often found in mobile elements like plasmids permit their bacterial spread (**Fluit and Schmitz, 2004).** Integrons are genetic constructs that have been found in several studies to contain AMR genes in their variable region (as gene cassettes) in chicken farms (**Pérez-Etayo et al. 2018 and Kalantari et al. 2021**).

The polymerase chain reaction (PCR) is considered one of the most important molecular methods and has been extensively employed in recent years to investigate antibiotic resistance genes.

In the current study, the antimicrobial resistance genes *aacC* and *Aada2* genes were recorded by using PCR in 13 out of 15 and 14 out of 15 isolated phenotypically and biochemically recognized *E. coli* isolates with a per cent ratio of 93.33% and 87.67%, respectively. Both *aacC* and *Aada2* genes were Aminoglycoside resistance genes against gentamicin and

streptomycin, respectively. This result seemed in the same context as **Radwan et al. (2018)** who stated that antimicrobial resistance genes *Aada2* and *aacC* genes were the most prevalent found in all *E coli* isolates (100%). **Abd Elatiff et al. (2019)** by using the Aada2-specific primers, PCR screening for antibiotic resistance genes in *E. coli* revealed that 12 serogroup isolates were positive.

CONCLUSION

In conclusion, this study focus on the significant prevalence of antibiotic-resistant *E. coli* in broiler chickens, both in internal organ samples collected from diseased farms and in meat samples from markets in Ismailia. The high resistance rates observed against commonly used antibiotics, such as amoxicillin, rifampicin, and lincomycin, raise concerns about the indiscriminate use of these drugs in poultry farming. The detection of *aacC* and *Aada2* genes in the majority of the isolates highlights the genetic basis for resistance against aminoglycosides, further emphasizing the need for responsible antibiotic use in the poultry industry. The study reinforces the importance of ongoing surveillance efforts to monitor and address the emergence of multidrug-resistant bacteria, safeguarding both animal and public health.

REFERENCES:

Abd Elatiff A, El-Sawah AA, Amer MM, Dahshan AM, Salam H, Shany SAS. 2019. Serogrouping and resistance gene detection in avian pathogenic E.coli isolated from broiler chickens. Journal of Veterinary Medical Research, 26(1): 48-54.

Abdel-Rahman MAA, Hamed EA, Abdelaty MF, Sorour HK, Badr H, Hassan WM, Shalaby AG, Halem AAE, Soliman MA, Roshdy H. 2023. Distribution pattern of antibiotic resistance genes in *Escherichia coli* isolated from colibacillosis cases in broiler farms of Egypt, Veterinary World, 16(1): 1–11.

Almofti YA. 2016. Imprudent usage of antibiotics in dairy farms and antibiotics detection in milk. Scholars Research Library Annals of Biological Research, (7): 36-42.

Amer MM, Mekky HM, Amer AM. Fedawy HS. 2018. Antimicrobial resistance genes in pathogenic *escherichia coli* isolated from diseased broiler chickens in Egypt and their relationship with the phenotypic resistance characteristics. Vet. World, 11 (8).

Awad AM, El-Shall NA, Khalil DS, Abd El-Hack ME, Swelum AA, Mahmoud AH Ebaid H, Komany A, Sammour RH, Sedeik ME. 2020. Incidence, Pathotyping, and Antibiotic Susceptibility of Avian Pathogenic *Escherichia coli* among Diseased Broiler Chicks. Pathogens 2020, 9, 114.

Badr H, Samir A, El-Tokhi E, IShahein MA, Rady FM, SA, Hakim Fouad EA, El-Sady, Engy F, Ali Samah F. 2022. Phenotypic and Genotypic Screening of Colistin Resistance Associated with Emerging Pathogenic *Escherichia coli* Isolated from Poultry. Poultry Vet. Sci., 29, 282. https:// doi.org/10.3390/

Barnes HJ, Nolan LK, Vaillancourt JP. 2008. Colibacillosis, p691-732 In Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, Swayne DE, editors, Diseases of poultry,12th ed, Blackwell Publishing, Ames, IA.

Barnes HJ, Vaillancourt JP, Gross WB. 2003. Colibacillosis, p 631–652. In Saif YM, et al. (ed), Diseases of poultry, 11th ed. Iowa State University Press, Ames, IA.

Brower CH. 2017. "The prevalence of extended-spectrum Beta- lactamase-producing multidrug-resistant *Escherichia coli* in poultry chickens and variation according to farming practices in Punjab, India". Environmental Health Perspectives, (125): 1-10.

Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; CLSI: Wayne, PA, USA, 2021.

Dandachi I, Sokhn ES, Dahdouh EA, Azar E, El-Bazzal B, Rolain JM, Daoud Z. 2018. Prevalence and characterization of multidrug-resistant Gram-negative bacilli isolated from Lebanese poultry: A nationwide study. Front. Microbiol, (9): 550

Dou, X, Gong, J, Han, X, Xu, M, Shen, H,

Zhang, D, Zou, J. 2016.Characterization of avian pathogenic *Escherichia coli* isolated in eastern China. Gene, 576 (1): 244-248.

Eckburg, PB. 2005. "Microbiology: diversity of the human intestinal microbial flora". Science, (308): 1635-1638.

Elsabet ET. 2011. Characterization of *E. coli* Isolated from Village Chicken and Soil Samples. chicken meats sold in Nairobi, Kenya," BMC Research, vol. 7, article 627.

El-Seedy FR, Abed AH, Wafaa MMH, Bosila AS, Mwafy A. 2019. Antimicrobial resistance and molecular characterization of pathogenic *E. coli* isolated from chickens. J. Vet. Med. Res., 26 (2): 280-292.

Fluit AC, Schmitz FJ. 2004. Resistance integrons and super-integrons. Clinical Microbiology and Infection, (10): 272–288

Furtula V, Farrell EG, Diarrassouba F, Rempel H, Pritchard J, Diarra MS. 2010. Veterinary pharmaceuticals and antibiotic resistance of *Escherichia coli* isolates in poultry litter from commercial farms and controlled feeding trials, Poultry Science, vol. 89, no. 1, pp. 180–188.

Gillings MR. 2014. Integrons: Past, present, and future. Microbiol. Mol. Biol. Rev., (78): 257–277.

Hamed Basma M, RagabEman El-Enbaawy Mona IH. 2021. Antibiotic resistance pattern of avian pathogenic *Escherichia coli* in broilers belonging to some Egyptian farms. J. Egypt. Vet. Med. Assoc., 81, no (1): 243 – 253.

Islam NN, Nur SM, Farzana Z, Uddin I, Kamaruddin AM, Siddiki AMN. 2014. Rapid identification of eosin methylene blue positive *E. coli* by specific PCR from frozen chicken rinse in Southern Chittagong city of Bangladesh: Prevalence and antibiotic susceptibility. *Microbiol. J.*, 4(2): 27-40.

Kalantari M, Sharifiyazdi H, Asasi K, Abdi-Hachesoo B. 2021. High incidence of multidrug resistance and class 1 and 2 integrons in *Escherichia coli* isolated from broiler chickens in South of Iran. Vet. Res. Forum., (12): 101–107.

Kaye KS, Engemann JJ, Fraimow HS, Abrutyn E. 2004. "Pathogens resistant to antimicrobial agents: epidemiology, molecular mechanisms, and clinical management," Infectious Disease Clinics of North America, vol. 18, no. 3, pp.467–511. Livestock (20): 65-67.

Lynne AM, Rhodes-Clark BS, Kimberly Bliven Shaohua Zhao Foley SL. 2008. Antimicrobial Resistance Genes Associated with *Salmonella enteric* Serovar Newport Isolates from Food Animals. .Antimicrobial Agents and Chemotherapy, 353–356 Vol. 52, No. 1

Matin MA. 2017. Prevalence of colibacillosis in chickens in greater Mymensingh district of Bangladesh. Veterinary World, (10): 29-33.

Matin MA, Islam MA, Khatun MM. 2017. Prevalence of colibacillosis in chickens in greater Mymensingh district of Bangladesh. Vet. World, 10(1): 29-33

McKissick JC. 2006. Poultry Industry Outlook. The University of Georgia, Athens, USA.

Moawad AA, Hotzel H, Neubauer H, Ehricht R, Monecke S, Tomaso H, Hafez HM, Roesler U, El-Adawy H. 2018. Antimicrobial resistance in Enterobacteriaceae from healthy broilers in Egypt: Emergence of colistin-resistant and extended-spectrum β-lactamase-producing Escherichia coli. Gut Pathog., 10: 39.

Moawad Amira A, Hotzel H, Neubauer H, Ehricht R, Monecke S, Tomaso H, Hafez HM, Roesler U, ElAdawy H. 2018. Antimicrobial resistance in Enterobacteriaceae from healthy broilers in Egypt: emergence of colistin-resistant and extended-spectrum β-lactamase-producing Escherichia coliGut-Pathog., 10:(39): 1-12.

Mohamed-Noor SE. 2012. "Study of microbial contamination of broilers in modern abattoirs in Khartoum state". The Annals of the University Dunarea de Jos of Galati.

Moustafa S, Mourad D. 2015. Resistance to 3rd generation cephalosporin of *Escherichia coli* isolated from the faeces of healthy broilers chickens in Algeria. Journal of Veterinary Medicine and Animal Health, vol.

7, no. 8, pp. 290–295.

Nolan L, Barnes H, Vaillancourt J, Abdul-Aziz T, Logue C. 2013. Diseases of Poultry, 13th ed.; Swayne, D.E., Ed.; Wiley-Blackwell: Hoboken, NJ, USA.

Odwar JA, ikuvi GK, Kariuki JNS, Kariuki 2014."A cross-sectional study on the microbiological quality and safety of raw

Otaki Y. 1995. "Poultry disease control programme in Japan". Asian

Percival SL, Williams DW. 2014. Chapter Six—*Escherichia coli*. In Microbiology of Waterborne Diseases, 2nd ed.; Percival, S.L., Yates, M.V., Williams, D.W., Chalmers, R.M., Gray, N.F., Eds.; Academic Press: London, UK, 2014; pp. 89–117.

Pérez-Etayo L, Berzosa M, González D, Vitas AI. 2018. Prevalence of integrons and insertion sequences in ESBL-producing *E. coli* isolated from different sources in Navarra, Spain. Int. J. Environ. Res. Public Health, 15, 2308.

Quinn PJ, Markey BK, Carter ME, Donnelly, WJC, Leonard FC, Maguire D. 2002. VeterinaryMicrobiology and Microbial Diseases. 1st ed. Blackwell Science, New Jersey, United States.

Qurani RO. 2019. Phenotypic and genotypic characterization of Trypsin producing *Escherichia coli* isolated from broiler chickens. Ph. D. Thesis (Microbiology), Fact. Vet. Med., Beni-Suef Univ., Egypt.

Radwan IA, Mohamed MF, Ahmed AK. 2018. Bacteriological studies on bacterial pathogens isolated from broiler chickens with swollen head syndrome.Journal of Veterinary Medical Research, 25 (2): 191-198

Radwan I, Abd El-Halim M, Abed AH. 2020. Molecular Characterization of Antimicrobial-resistant *Escherichia coli* Isolated from Broiler Chickens. Journal of Veterinary Medical Research; 27 (2): 128 –142

Radwan IA, Abd El-Halim MW, Abed AH. 2020. Genotypic characterization of antimicrobial resistant *Escherichia coli* isolated from broiler chickens. J. Vet. Med. Res., 27 (2): xxx-xxx.

Radwan IA, Hassan HS, Abd-Alwanis SA, Yahia MA. 2014. Frequency of some virulence -associated genes among multidrug-resistant Escherichia coli isolated from septicemic broiler chicken. Int. J. Adv. Res., 2(12): 867 -874.

Rahman MA, Rahman AKMA, Islam MA, Alam MM. 2017. Antimicrobial resistance of Escherichia coli isolated from milk, beef and chicken meat in Bangladesh. Bang. J. Vet. Med., 15 (2): 141-146

Romanus II, Chinyere OE, Amobi NE. 2012 . Antimicrobial resistance of *Escherichia coli* isolated from animal and human clinical sample. Global Research Journal of Microbiology, vol. 2, no. 1, pp. 85–89.

Saif YM, Barnes HJ, Glisson JR, Fadly AM, Dougland LR, Swayne DE. 2003. Diseases of Poultry, 11th ed. Pp: 562-566. Press Iowa State, USA.

Samy AA, Mansour AS, Khalaf DD, Khairy EA. 2022. Development of multidrug-resistant *Escherichia coli* in some Egyptian veterinary farms, Veterinary World, 15(2): 488-495.

Shecho M, Thomas N, Kemal J, Muktar, Y. 2017. Cloacael carriage and multidrug-resistant Escherichia coli O157:H7 from poultry farms, Eastern Ethiopia. J. Vet. Med., 2017: 8264583.

Singer RS. 2006. Potential impacts of antibiotic use in poultry production. Avian Diseases (50): 161-172.

Subedi M, Luitel H, Devkota B, Bhattarai RK, Phuyal S, Panthi P, Chaudhary DK. 2018. Antibiotic resistance pattern and virulence genes content in avian pathogenic *Escherichia coli* (APEC) from broiler chickens in Chitwan, Nepal. BMC Vet. Res., 14 (1): 113

Van den Bogaard AE, London N, Driessen C, Stobberingh EE. 2001. Antibiotic resistance of faecal Escherichia coli in poultry, poultry farmers and poultry slaughterers. J Antimicrobe Chemother.(47):763-71

Walker RA, Lindsay E, Woodward MJ. 2001.Variation in clonality and antibiotic-resistance genes among multi-resistant *Salmonella enterica* serotype Typhimurium

phage-type U302 (MR U302) from humans, animals, and foods. Microbiological Research (7): 13–21.

World Health Organization 2003. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World: *Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Neisseria gonorrhoea*, *Salmonella serotype Typhi, Shigella, and Vibrio cholerae* / Principal authors: Mindy J. Perilla [et al.]; World Health Organization: Geneva, Switzerland,

Zhao S, Blickenstaff K, Bodeis-Jones S, Gaines SA, Tong E, McDermott PF. 2012. Comparison of the prevalences and antimicrobial resistance of Escherichia coli isolates from different retail meats in the United States, 2002 to 2008. Applied and Environmental Microbiology; 78(6):1701–1707.