



## Multidrug Resistance of *Escherichia coli* From Poultry and Humans in contact: Serotyping, Resistance Genes, and Their Public Health Importance

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### Abstract

**C**HICKEN intestinal microbiome naturally includes *Escherichia coli*. However, only a small subset, known as avian pathogenic *E. coli* (APEC), may be harmful and zoonotic to humans. The study primary goal was to examine *E. coli* isolates from poultry and humans, serotype them, and find genes that confer resistance to popular antibiotics as well as drug sensitivity. The study tested 160 meat and organ samples from chickens and ducks, along with 40 human stool samples, finding *E. coli* in 15 % of samples, with the highest rates in human stool (40 %) and lower rates in duck (11.7 %), chicken organs (3.3%) and their meat (12.5 %). Thirty tested *E. coli* showed high resistance to several antibiotics, especially erythromycin, but were more sensitive to colistin and fosfomycin, with many strains showing resistance to multiple drugs. The isolates were analysed for some antibiotic resistance genes of *E. coli*. Duck and chicken organ isolates were found to be positive to *aadA1*, *sul1*, *tetA*, and *msr-1* genes. Whereas their meat possessed *aac(3)Iv*, *aadA1*, *sul1*, *tetA*, *msr-1* and *aac(6)Ib* genes. Human isolates carried *ereA*, *aadA1*, *tetA*, *sul1*, *msr-1*, and *aac(3)Iv* genes. In conclusion, the detection of shared antimicrobial resistance genes among *E. coli* isolates from both poultry and human sources strongly suggests a potential zoonotic transmission pathway. This highlights the substantial public health importance of these findings, indicating that avian *E. coli* contributes not only to economic losses in poultry production but also to the increasing burden of multidrug-resistant infections in both animal and human populations.

**Keywords:** *E. coli*, poultry, chicken and duck meat, human stool, multidrug resistance genes.

### Introduction

Humans depend upon poultry as a major source of protein, and Middle Eastern consumption of poultry is rising yearly [1]. The intestinal microbiota of

poultry naturally contains *E. coli*, which has the potential to be harmful. Avian pathogenic *E. coli* (APEC) is the term used to describe pathogenic strains in birds and is reportedly the most common

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bacterial pathogen that infects avian [2] which, frequently after the immune system is weakened, produce avian colibacillosis, which results in large financial losses [3]. The overuse of antimicrobial agents has led to a global problem known as antimicrobial resistance (AMR) in bacteria, which impacts the economy and human health [4]. The outbreak of *E. coli* from poultry samples in Egypt is a significant public health concern, primarily due to the prevalence of antibiotic-resistant strains. A study in Gharbia and Menofeya, indicate that 30% prevalence of APEC in broiler farms, with alarming rates of antimicrobial resistance observed across various regions [5].

Bacteria are considered resistant to a class of antibiotics if they do not respond to at least one of the products in that class [6]. Serotyping is crucial for defining these strains of *E. coli*, since it can be challenging to isolate and accurately identify pathogenic strains, especially in poultry where non-pathogenic types coexist [7,8]. Additionally, the diversity in O-antigen gene clusters among strains of *E. coli* that are part of the similar sequence type, like ST678, emphasizes the importance of serotyping for accurate identification of highly pathogenic strains, as seen in the STEC O104:H4 outbreak strain [9]. Furthermore, research on STEC strains from various animal sources underscores the value of serotyping in understanding the prevalence and distribution of different serogroups, aiding in epidemiological studies and disease management [10].

AMR in avian *E. coli* poses significant public health risks, particularly as these pathogens can spread from chickens to human beings. Since humans and animals share the same environment, they potentially share resistant bacteria and resistant genes [11]. Research indicates a concerning incidence of *E. coli* in poultry farms that is resistant to many antibiotics, with implications for both animal and human health. The grouping of *E. coli* isolates from poultry and human samples suggests a potential zoonotic transmission route, emphasizing the need for enhanced surveillance [12]. Most avian pathogenic *E. coli* isolates (56%) from broiler chickens in Egypt showed high resistance to common antibiotics, with all samples (100%) resistant to streptomycin [13] and ampicillin [14], and the multi-antimicrobial resistance index values of all the identified *E. coli* serogroups were high (>0.2) indicating a serious problem with antibiotic effectiveness in these animals [13].

In the Czech Republic, 20.5% of multidrug-resistant *E. coli* isolates from one-day-old chicks show the danger of human transmission via contaminated chicken products [15]. Multidrug resistance was found in 66.7 % of pathogenic *E. coli* isolates from poultry in South Africa, specifically to aminoglycosides (61.1 %) and tetracycline (41.7 %) [12]. According to a Chinese study, 96.4 % of *E. coli*

bacteria from chicken farms were multidrug-resistant, and significant resistance genes, such as those for colistin and carbapenem resistance, were detected [16]. The presence of multidrug-resistant *E. coli* strains in poultry highlights the need for improved biosecurity and antibiotic stewardship in the poultry industry to mitigate risks to human health [14,17].

The pathogenicity *E. coli* in avian species is primarily attributed to a few serotypes, notably O1, O2, and O78, representing 15-61% of isolates which have been consistently identified as significant contributors to avian colibacillosis [8]. Due to the negative economic impact of *E. coli* and the increased risk of emergence of antibiotic-resistant *E. coli* isolates in both poultry and human, the current study's objectives were to isolate *E. coli* from poultry and humans, perform serotyping, identify antibiotic sensitivity, and find *E. coli* resistance genes to common antibiotics used in the field to combat *E. coli*.

### **Material and Methods**

#### *Samples collection:*

From chicken and duck farms in the Dakahlia and Gharbeya provinces of Egypt, 160 samples of chicken and duck organs (heart, liver, spleen, and meat) additionally, 40 stool samples from farm workers were randomly selected. They were then transported to the laboratory in an ice container under aseptic conditions for additional bacteriological investigation.

#### *E. coli isolation and identification:*

All samples were incubated at 37°C/24h for pre-enrichment. Approximately, 50 mL of each pre-enriched sample was mixed with 50 mL of double-strength MacConkey broth (Oxoid, USA) and incubated at 37 °C/24 h. After incubation, 10 µL of each sample was streaked onto Eosin-Methylene Blue (EMB) agar plates (Oxoid, USA) and incubated at 37 °C/24 h. Blue/black with a greenish metallic Isolates were identified as presumptive *E. coli* which randomly selected and confirmed using the API 20E identification system (bioMerieux, USA). Presumptive colonies were inoculated onto nutrient agar plates and incubated at 37 °C/24 h for further investigations [48,49].

#### *E. coli identification by serology:*

The isolates were recognized serologically following standard procedures [50]. Rapid diagnostic techniques were used to identify the enteropathogenic isolates using of *E. coli* antisera (DENKA SEIKEN Co., Japan).

#### *PCR assay:*

#### *DNA extraction:*

The QIAamp DNA Mini Kit (Qiagen GmbH, Germany) was used to extract DNA from bacterial isolates. The collected genomic DNA was subsequently amplified using PCR with definite primers to determine whether antibiotic resistance genes were present in *E. coli*. These primers included ciprofloxacin (*aac(6)-Ib*), gentamicin (*aac-3-IV*), erythromycin (*ereA*), streptomycin (*aadA1*), tetracycline (*tetA*), sulfonamide (*sul1*), colistin (*Msr-1*), and gentamicin (*aac-3-IV*) as shown in Table 4.

*Amplification of 5 antibiotic resistance genes (aac-3-IV, ereA, aadA1, tetA and sul1):*

A thermal cycler was used for amplification. Five antibiotic-resistant genes, including *aac-3-IV*, *ereA*, *aadA1*, *tetA*, and *sul1*, were found using multiplex PCR amplification. 10X PCR buffer, 2.5 mM MgCl<sub>2</sub>, 200 mM dNTP, 2.0 U of Taq DNA polymerase, 0.5 mM of each primer set, and 3 µL of the DNA template were all included in the 50 µL mixture used for the PCR experiment. Denaturation at 94 °C/7m was followed by 32 amplification cycles of denaturation at 95 °C/60s, annealing at 55 °C/1m, extension at 72 °C/2m, and final extension at 72 °C/5m. Following electrophoresis on a 2 % agarose gel, the PCR results were stained with ethidium bromide and examined under a UV lamp. The size of the amplicons was established using a 100 bp DNA molecular marker.

*Amplification of msr-1 and aac(6)-Ib genes[33]:*

The multiplex PCR reaction mixture (12.5 µL), 8.5 µL nuclease-free water, 0.5 µL of each primer (10 µM), and 3 µL DNA template were used to identify colistin (*msr-1*) and ciprofloxacin (*aac(6)-Ib*). One cycle of denaturation at 94 °C/15m was followed by 25 cycles, each of which at 94°C/30s, 58 °C/90s, 72 °C/60s, and a final cycle at 72 °C/10 m. A 1.5 % agarose gel at 100 V was used to visualize the amplified product, and ethidium bromide (1 µg/mL) was used for staining. The fragment sizes were determined using a DNA ladder with a limit of 100 bp.

*E. coli antibiotic susceptibility test:*

All positive isolates for *E. coli* (n = 30) were tested for antimicrobial susceptibility by the agar disk diffusion method using disks as shown in Table 5, according to Clinical and Laboratory Standards Institute (CLSI) guidelines [54]. A set of 16 antibiotics (Ampicillin, Ceftiofur, Cefotaxime, Colistin, Ciprofloxacin, Erythromycin, Fosfomycin, Gentamicin, Levofloxacin, Ofloxacin, Streptomycin, Sulphamethoxazol, Tylosin, Tetracycline, Tilimicosin, Tylvalosin) was selected for susceptibility testing following the recommendations of the National Committee for Clinical Laboratory Standards, USA.

The inoculum was prepared in nutritive broth at a density adjusted to a 0.5 Mc Farland turbidity

standard for disc diffusion and diluted 1:10 for the dilution method. A final inoculum was placed on Müller-Hinton agar plates. The inoculated plates were incubated at 37°C under aerophilic atmosphere for 24 hours. MAR index for each strain was determined according to [55, 56]. To conduct quality control, the *E. coli* ATCC 25922 inhibitory zones were determined.

## Results

*E. coli prevalence in human stool, meat and organs from chickens and ducks:*

*E. coli* was isolated from 15 % (30/200) of total examined samples. The percentages of *E. coli* positive samples in duck organs, chicken organs, and their meat were 11.7 % (7/60), 3.3 % (2/60) and 12.5 % (5/40) respectively. For human stool samples, 16/40 (40 %) were found to be positive to *E. coli*, as shown in (Table 1).

*E. coli serotyping:*

In the current study, different serotypes were identified, from duck organs, O17:H18, O128:H2, O26:H11, O2:H6, O55:H7, O91:H21, O78 were serotyped, while from chicken organs, O128:H2, O159 were identified, moreover, O119:H6, O146:H21, O78 were identified in their meat. For human positive isolates, different serogroups were typed such as O128:H2, O78, O91:H21, O146:H21, O121:H7, O26:H11 as cleared in (Table 1).

*E. coli isolates' susceptibility to antibiotics*

Thirty positive isolates were tested against different antimicrobial agents. The tested isolates showed high resistance to erythromycin (100 %) followed by tylosin (96.7 %), tilimicosin and tylvalosin (90 %, per each) and streptomycin (80 %). However, the test showed high sensitivity to colistin (96.7 %), fosfomycin (93.3 %), ceftiofur (80 %) and gentamicin (76.7 %). Most recovered strains exhibited multidrug resistance as shown in Fig.1. The MAR index values showed multiple resistant patterns, revealing that the MAR index average of *E. coli* was 0.531 (Table 2.)

*Prevalence of resistance genes:*

All isolates from examined samples were possessed to *ereA* gene. Duck organs and chicken organs isolates were found to be positive to *aadA1*, *sul1*, *tetA* and *msr-1* genes. Whereas their meat was possessed to *aac(3)Iv*, *aadA1*, *sul1*, *tetA*, *msr-1* and *aac(6)Ib* genes. Moreover, human isolates were carried to *ereA*, *aadA1*, *tetA*, *sul1*, *msr-1* and *aac(3)Iv* genes as reviewed in Table 3 and Figure2 and 3.

## Discussion

*E. coli* is one of the most severe and common bacterial avian pathogens, it causes several diseases in birds and accounts for up to 30 % of chicken

mortality [18]. In the current study, 15 % of all the samples that were examined had *E. coli*. The percentages of *E. coli* positive samples in human stool, duck organs, chicken organs, and their meat were 40 %, 11.7 %, 3.3 % and 12.5 %, respectively, while, previous studies have reported a percentage of 37.7 % [19], 46.7 % [20] and 86.66 % [21]. These results were in disagreement with previous studies which found higher prevalence rates (89.4 %) for *E. coli* [22], additionally, 40 % of human samples tested positive for *E. coli*, indicating a high prevalence rate in humans as supported by several studies [23,24].

Avian pathogenic *E. coli* represents a significant health threat to poultry, with various serotypes classified based on somatic (O) and flagellar (H) antigens. A number of researchers have reported that O1, O2, O35, and O78 are the most dominant and widespread types [8], serotyping results showed that 32 % of isolates belonged to 3 serotypes (O1, O2, and O78) [21].

Thirty *E. coli* isolates were obtained, and all were subjected to antibiotic sensitivity test against 16 antibiotics. Multidrug resistance appears to be an absolute problem. There are a high ratio of *E. coli* strains showing resistance to multiple antibiotics, 98.7 % of the strains are resisted to at least 3 antibiotics, 92.81 % are resisted to at least 5 antibiotics, 43.13 % are resisted to 8 antibiotics, and 16.33 % are resisted to ten antibiotics [21]. Our study found that poultry, duck, and meat samples showed complete resistance to erythromycin (100 %) and high resistance to other antibiotics, with similar issues seen in human *E. coli*, highlighting a serious problem with antibiotic resistance, this results similarly with previous study [19]. In general, antibiotic resistance was found in poultry and humans, with erythromycin showing the highest resistance, this suggests that antibiotics used in poultry may pass resistance to humans, as seen in studies linking farmers and their chickens [25]. Gentamicin had the lowest resistance rate in our study (23.3 %), but other research showed higher resistance in backyard poultry. Resistance to ampicillin and tetracycline varied by location, with some areas reporting very high rates [26]. Increased resistance bacteria in chickens and their environment have been connected to the addition of small amounts of tetracycline to feed [27]. Tetracycline resistance was 63.3 % in our study, less than earlier reports of 100 % [21] and it is almost consistent with a previous study which showed that 70.8 % of the strains are resistant to tetracycline [28]. Ampicillin resistance was 53.3 %, also lower than earlier studies which reported higher rates, 83.01 % [21], 74.08 % [29] and 89 % [30]. Colistin resistance was very low at 3.3 %, as Gram-negative bacteria usually show little resistance to it. This resistance can change but develops slowly, making colistin a key treatment for serious infection [31–33]. In France, low colistin-

resistant *E. coli* was found in poultry [34], while China and Lebanon reported higher rates [35].

Because of their high levels of resistance, the most widely used antibiotics in chicken may not be useful in treating colibacillosis [21]. It is important to be concerned about antibiotic resistance and the potential for it to spread to human. Currently, there is a lack of evaluation and quantification of the transfer of resistance genes between human and animal bacterial populations [36]. Beta-lactam antibiotics face increasing resistance due to factors like beta-lactamase production [37], changes in target sites, and reduced drug entry [38,39]. Other antibiotics also show different resistance mechanisms, especially in developing countries where quinolones are overused [40]. The rise of *ESBL* in Enterobacteriaceae is alarming as they can destroy key antibiotics, posing a significant risk to public health [26].

A MAR index higher than 0.2 is one indicator of the spread of bacterial resistance in a given population [41], which indicates that the bacteria strains in concern originated from a habitat that abuse a variety of antibiotics [42]. Genetic exchange between MAR pathogens and other microorganisms allows for a high incidence of MAR [43]. The MAR values of the *E. coli* isolates in this study were 0.531. This could be because of the excessive and inappropriate use of antibiotics for therapeutic or growth-promoting objectives, leading to the rise of resistant strains. These findings indicate that the *E. coli* that initially colonized the poultry was multi-drug-resistant and that these isolates are still present in the chicken environment. As a result, these types of environments may serve as an ideal environment for antibiotic resistance.

A strong link exists between the resistance traits (phenotype) and genetic makeup (genotype) of *E. coli*, with qPCR being an effective method for diagnosis. Key virulence genes were identified in many isolates, indicating their classification as APEC. Recent studies found that specific genes like *bla*TEM and *tetA* are common in resistant *E. coli* strains. The spread of resistant bacteria from animals to human through zoonotic transmission enhances the risks to public health and increases the incidence of disease [44]. The most frequently detected resistance genes include *tetA*, *aac*(3)-VIa, *aadA*1, and *sul*1, highlighting the ongoing issue of antibiotic resistance [45]. In the current study, PCR results showed that all the tested *E. coli* isolates were found to carry *ereA* (11 isolates), *aadA*1 (10 isolates) and *sul*1 (9 isolates). Samy et al. [19] detected that all AML- and OT-resistant *E. coli* carry *bla*TEM and *tetA* genes. The same genes were found in other recent investigations on AML- and OT-resistant *E. coli* [46]. According to previous investigation, *tetA* (48%), *aac*(3)-VIa (40%), *aadA*1 (37.9%), and *sul*1 (34%) were the most prevalent resistance genes [47].

Due to the significant impact of *E. coli* on both poultry and humans, more research is needed to identify common *E. coli* types, their resistance genes, how these genes spread, and potential solutions to combat this resistance. Due to the research goal, facilities, and financial constraints, the study had limitations that hampered our understanding of *E. coli* resistance, such as a small sample size and in vitro analysis rather than in vivo. Future research should involve more samples from different areas and advanced techniques to study drug resistance. To improve findings, researchers should focus on understanding how *E. coli* spreads and its resistance genes, especially in poultry and humans. To lower the possibility of antimicrobial resistance, recommendations for preventing antibiotic overuse in chicken farms must be developed.

### Conclusion

The rise of multidrug-resistant strains of *E. coli* in both poultry and humans, which share the same resistance genes and serotypes, highlights a significant zoonotic connection. This situation poses a serious threat to public health, food safety, and the sustainability of the poultry industry. Immediate action is necessary, including strict handling of antibiotic, improved biosecurity measures on farms, and integrated One Health surveillance. These steps are essential to avoid the spread of resistance and protect both animal and human health. The presence of multidrug-resistant *E. coli* strains in poultry highlights the need for improved biosecurity, control measures and antibiotic stewardship in the poultry

industry to mitigate risks to human health. We recommend that the Egyptian authorities plan and implement a national one-health approach to combat antibiotic resistance, including widening the surveillance of antimicrobial resistance, enforcing the available regulations, and monitoring the production, storage, sale, and usage of veterinary drugs.

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### Declaration of Conflict of Interest

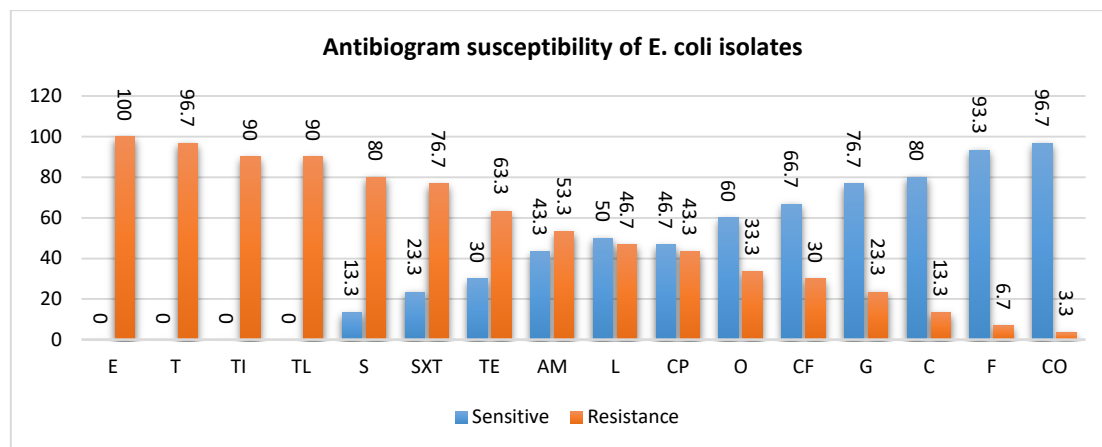
The authors state that none of the work described in this study could have been influenced by any known competing financial interests or personal relationships.

### Ethical of approval

The ethical approval has been given by Mansoura University's Animal Care and Use Committee, Code number: MU-ACUC (VM.R.24.11.195), and the samples were collected with the owners of poultry farms' consent.

**TABLE 1. Occurrence and serotypes of *E. coli* isolated from chicken and duck organs, their meat, and human stool samples.**

|                       | Source of samples | No. examined | +ve on MacConkey | %    | Serotypes identified                                                                              |
|-----------------------|-------------------|--------------|------------------|------|---------------------------------------------------------------------------------------------------|
| <b>Duck Organs</b>    | Heart             | 20           | 1                | 5    | O17:H18 (n=1)                                                                                     |
|                       | Liver             | 20           | 1                | 5    | O128:H2 (n=1)                                                                                     |
|                       | Spleen            | 20           | 5                | 25   | O26:H11(n=1), O2:H6 (n=1),                                                                        |
|                       | Total             | 60           | 7                | 11.7 | O55:H7 (n=1), O91:H21 (n=1), O78 (n=1)                                                            |
| <b>Chicken Organs</b> | Heart             | 20           | 0                | 0    | 0                                                                                                 |
|                       | Liver             | 20           | 1                | 5    | O128:H2 (n=1)                                                                                     |
|                       | Spleen            | 20           | 1                | 5    |                                                                                                   |
|                       | Total             | 60           | 2                | 3.3  | O159 (n=1)                                                                                        |
| <b>Meat</b>           | Duck meat         | 20           | 4                | 20   | O119:H6 (n=1), O146:H21(n=1), O78 (n=2)                                                           |
|                       | Chicken meat      | 20           | 1                | 5    | O119:H6 (n=1)                                                                                     |
|                       | Total             | 40           | 5                | 12.5 |                                                                                                   |
| <b>Human</b>          | Stool             | 40           | 16               | 40   | O91:H21 (n=3), O78 (n=3), O2:H6 (n=3), O146:H21(n=2), O128:H2 (n=3), O121:H7 (n=1), O26:H11 (n=1) |
| <b>Total</b>          |                   | 200          | 30               | 15   |                                                                                                   |



**Fig. 1. Antibiogram susceptibility of *E. coli* isolates.**

\*\* AM: Ampicillin, C: Ceftiofur, CF: Cefotaxime, CO: Colistin, CP: Ciprofloxacin, E: Erythromycin, F: Fosfomycin, G: Gentamicin, L: Levofloxacin, O: Ofloxacin, S: Streptomycin, SXT: Sulphamethoxazol, T: Tylosin, TE: Tetracycline, TI: Tilmicosin, TL: Tylvalosin.

**TABLE 2. Antibiotics resistance profile and MAR index**

| Organ   | <i>E. coli</i> strains | Antimicrobial resistance profile                        | MAR index |
|---------|------------------------|---------------------------------------------------------|-----------|
| DS      | O78                    | E, T, TI, TL, S, SXT, TE, AM, L, CP, O, CF, G, C, F, CO | 1         |
| ST      | O78                    | E, T, TI, TL, S, SXT, TE, AM, L, CP, O, CF, G, C        | 0.875     |
| DL      | O78                    | E, T, TI, TL, S, SXT, TE, AM, L, CP                     | 0.625     |
| DH      | O78                    | E, T, TI, TL, S, SXT, TE                                | 0.437     |
| PL      | O78                    | E, T, TI, TL, S, SXT, TE                                | 0.437     |
| DL      | O78                    | E, T, TI, TL, S                                         | 0.312     |
| DM      | O128: H2               | E, T, TI, TL, S, SXT, TE, AM, L, CP, O, CF, G, C, F     | 0.938     |
| DH      | O128: H2               | E, T, TI, TL, S, SXT, TE, AM, L, CP, O, CF              | 0.750     |
| ST      | O128: H2               | E, T, TI, TL, S, SXT, TE, AM                            | 0.500     |
| DH      | O128: H2               | E, T, TI, TL, S, SXT, TE                                | 0.437     |
| DM      | O128: H2               | E, T, TI, TL                                            | 0.250     |
| ST      | O91: H21               | E, T, TI, TL, S, SXT, TE, AM, L, CP, O, CF, G, C        | 0.875     |
| DL      | O91: H21               | E, T, TI, TL, S, SXT, TE, AM, L, CP                     | 0.625     |
| PM      | O91: H21               | E, T, TI, TL, S, SXT, TE, AM, L                         | 0.563     |
| DH      | O91: H21               | E, T, TI, TL, S, SXT                                    | 0.375     |
| DH      | O2: H6                 | E, T, TI, TL, S, SXT, TE, AM, L, CP, O, CF, G           | 0.812     |
| ST      | O2: H6                 | E, T, TI, TL, S, SXT, TE, AM, L, CP                     | 0.625     |
| DL      | O2: H6                 | E, T, TI, TL, S, SXT                                    | 0.375     |
| PM      | O2: H6                 | E, T                                                    | 0.125     |
| DL      | O146: H21              | E, T, TI, TL, S, SXT, TE, AM, L, CP, O, CF, G           | 0.812     |
| PH      | O146: H21              | E, T, TI, TL, S, SXT, TE, AM                            | 0.500     |
| DM      | O146: H21              | E, T, TI, TL, S, SXT                                    | 0.375     |
| ST      | O26: H11               | E, T, TI, TL, S, SXT, TE, AM, L, CP, O, CF, G           | 0.812     |
| DH      | O26: H11               | E, T, TI, TL                                            | 0.250     |
| DL      | O119: H6               | E, T, TI, TL, S, SXT, TE, AM, L, CP, O, CF              | 0.750     |
| DL      | O119: H6               | E, T                                                    | 0.125     |
| DH      | O55: H7                | E, T, TI, TL, S, SXT, TE, AM, L, CP, O                  | 0.688     |
| DH      | O17: H18               | E, T, TI, TL, S, SXT                                    | 0.375     |
| PL      | O121: H7               | E, T, TI, TL                                            | 0.250     |
| DL      | O159                   | E                                                       | 0.063     |
| Average |                        |                                                         | 0.531     |

\*\* DH: Duck heart, DL: Duck liver, DM: Duck meat, DS: Duck spleen, PL: poultry liver, PM: Poultry meat, ST: Stool

TABLE 3. Genes associated with antibiotic resistance in strains of *E. coli* (n= 11 strains).

| <i>E. coli</i> strains | Genes           |             |              |             |             |              |                  |
|------------------------|-----------------|-------------|--------------|-------------|-------------|--------------|------------------|
|                        | <i>aac(3)IV</i> | <i>ereA</i> | <i>aadA1</i> | <i>tetA</i> | <i>sul1</i> | <i>Msr-1</i> | <i>aac(6)-Ib</i> |
| O2: H6                 | -               | +           | +            | +           | +           | +            | -                |
| O17: H18               | -               | +           | +            | -           | +           | -            | -                |
| O26: H11               | +               | +           | +            | +           | +           | +            | -                |
| O55: H7                | -               | +           | +            | +           | +           | +            | -                |
| O78                    | +               | +           | +            | +           | +           | +            | +                |
| O91: H21               | -               | +           | +            | +           | +           | -            | -                |
| O119: H6               | -               | +           | +            | -           | +           | -            | -                |
| O121: H7               | -               | +           | +            | -           | -           | -            | -                |
| O128: H2               | -               | +           | +            | +           | +           | +            | -                |
| O146: H21              | -               | +           | +            | +           | +           | -            | -                |
| O159                   | -               | +           | -            | -           | -           | -            | -                |

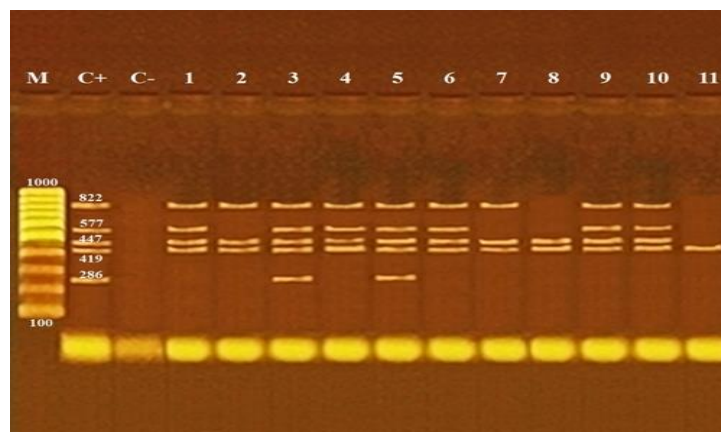


Fig. 2. Multiplex PCR using agarose gel electrophoresis of *aac(3)IV* (286 bp), *ereA* (419), *aadA1* (447 bp), *tetA* (577 bp) and *sul1* (822 bp) resistance genes for *E. coli* characterization. Lane M: As a molecular size marker for DNA, a 100 bp ladder. Lane C+: Control positive for *aac(3)IV*, *ereA*, *aadA1*, *tetA* and *sul1* genes. Lane C-: Control negative. Lanes 3 (O26) & 5 (O78): Positive strains for *aac(3)IV*, *ereA*, *aadA1*, *tetA* and *sul1* genes. Lanes 1 (O2), 4 (O55), 6 (O91), 9 (O128) & 10 (O146): Positive strains for *ereA*, *aadA1*, *tetA* and *sul1* genes. Lanes 2 (O17) & 7 (O119): Positive strains for *ereA*, *aadA1* and *sul1* genes. Lane 8 (O121): Positive strain for *ereA* and *aadA1* genes. Lane 11 (O159): Positive *ereA* gene strain.

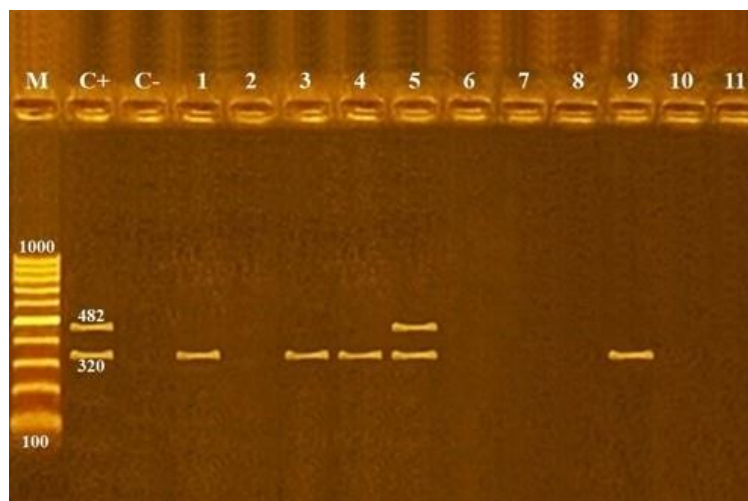


Fig. 3. Multiplex PCR using agarose gel electrophoresis of *Msr-1* (320 bp) and *aac(6)-Ib-cr* (482 bp) resistance genes for characterization of *E. coli*. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive for *Msr-1* and *aac(6)-Ib-cr* genes. Lane C-: Control negative. Lane 5: (O78): Positive strain for *Msr-1* and *aac(6)-Ib-cr* genes. Lanes 1: (O2), 3 (O26), 4 (O55) & 9 (O128): Positive strain for *Msr-1* gene. Lanes 2 (O17), 6 (O91) & 7 (O119), 8 (O121), 10 (O146) & 11 (O159): Negative strains for *Msr-1* and *aac(6)-Ib-cr* genes.

**TABLE 4. Primers of antibiotic resistance genes in *E. coli* isolates.**

| Antibiotics   | Target Gene          | Oligonucleotide sequence (5' → 3') | (bp) | References |
|---------------|----------------------|------------------------------------|------|------------|
| Gentamicin    | <i>aac(3)IV</i> (F)  | 5' CTTCAGGATGGCAAGTTGGT '3         | 286  | [51]       |
|               | <i>aac(3)IV</i> (R)  | 5' TCATCTCGTTCTCCGCTCAT '3         |      |            |
| Erythromycin  | <i>ereA</i> (F)      | 5' GCCGGTGCTCATGAACCTTGAG '3       | 419  | [51]       |
|               | <i>ereA</i> (R)      | 5' CGACTCTATTCGATCAGAGGC '3        |      |            |
| Streptomycin  | <i>aadA1</i> (F)     | 5' TATCCAGCTAAGCGCGAACT '3         | 447  | [52]       |
|               | <i>aadA1</i> (R)     | 5' ATTTGCCGACTACCTTGGTGTC '3       |      |            |
| Tetracycline  | <i>tetA</i> (F)      | 5' GGTTCACCTCGAACGACGTCA '3        | 577  | [52]       |
|               | <i>tetA</i> (R)      | 5' CTGTCCGACAAGTTGCATGA '3         |      |            |
| Sulfonamide   | <i>sul1</i> (F)      | 5' TTCGGCATTCTGAATCTCAC '3         | 822  | [51]       |
|               | <i>sul1</i> (R)      | 5' ATGATCTAACCCTCGGTCTC '3         |      |            |
| Colistin      | <i>msr-1</i> (F)     | 5' AGTCCGTTTGTCTTGTGGC '3          | 320  | [33]       |
|               | <i>msr-1</i> (R)     | 5' AGATCCTTGGTCTCGGCTTG '3         |      |            |
| Ciprofloxacin | <i>aac(6)-Ib</i> (F) | 5' TTGCGATGCTCTATGAGTGGCTA'3       | 482  | [53]       |

**TABLE 5. Antimicrobial discs used for *E. coli***

| Antimicrobial agent | Symbol | Concentration (µg) |
|---------------------|--------|--------------------|
| Levofloxacin        | L      | 5                  |
| Ofloxacin           | O      | 5                  |
| Streptomycin        | S      | 10                 |
| Colistin            | CO     | 10                 |
| Erythromycin        | E      | 15                 |
| Tilmicosin          | TI     | 15                 |
| Tylosin             | T      | 20                 |
| Tylvalosin          | TL     | 20                 |
| Sulphamethoxazol    | SXT    | 25                 |
| Tetracycline        | TE     | 30                 |
| Ampicillin          | AM     | 30                 |
| Ciprofloxacin       | CP     | 30                 |
| Cefotaxime          | CF     | 30                 |
| Gentamicin          | G      | 30                 |
| Ceftiofur           | C      | 30                 |
| Fosfomycin          | F      | 50                 |

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### مقاومة البكتيريا الإشريكية القولونية للأدوية المتعددة من الدواجن والبشر: التتميط المصلي، والجينات المقاومة، وأهميتها للصحة العامة

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- <sup>8</sup> قسم الصحة والأمراض المشتركة، كلية الطب البيطري، جامعة المنصورة، المنصورة، مصر.

### الملخص

يعتبر ميكروب الايشيريشيا كولاى من الميكروبات التى تعيش بشكل طبيعي في امعاء الانسان والطيور ولكن بعض هذه الميكروبات قد اكتسب مقاومة لمعظم المضادات الحيوية مما شكل خطوره علي صحة الانسان والطيور. ولذلك، كان الهدف الاساسي من هذه الدراسة هو عزل ميكروب الايشيريشيا كولاى من الدواجن والبشر، وتحديد النمط المصلي لها، وإيجاد الجينات التي تمنح مقاومة للمضادات الحيوية الشائعة وكذلك الحساسية الدوائية. اختبرت الدراسة 160 عينة من لحوم واعضاء الدجاج والبط، بالإضافة إلى 40 عينة براز بشري، ووجد ميكروب الايشيريشيا كولاى في 15% من العينات، وكانت أعلى المعدلات في البراز البشري (40%) ومعدلات أقل في البط (11.7%) وأعضاء الدجاج (3.3%) ولحومها (12.5%). وأظهرت ثلاثون ميكروب منها التي تم اختبارها مقاومة عالية للعديد من المضادات الحيوية، وخاصة الإريثروميسين، ولكنها كانت أكثر حساسية للكوليستين والفوسفوميسين، مع إظهار العديد من السلالات مقاومة لعدة أدوية. تم تحليل العزلات عن طريق تفاعل البلمرة المتسلسل لبعض جينات المقاومة للمضادات الحيوية للإشريكية كولاى. وقد وجد أن أعضاء البط وأعضاء الدجاج المعزولة كانت إيجابية لجينات *aadA1* و *sul1* و *tetA* و *msr-1*. في حين أن لحومها كانت تحتوي على جينات *aac(3)Iv* و *aadA1* و *sul1* و *tetA* و *msr-1* و *aac(6)ib*. وحملت العزلات البشرية جينات *ereA* و *aadA1* و *tetA* و *sul1* و *msr-1* و *aac(3)Iv*. في الختام، تم العثور على بعض العزلات في كل من عينات الدواجن والبشر التي تشترك في الجين المقاوم لمضادات الميكروبات، وبالتالي يمكن أن تسبب الايشيريشيا كولاى للطيور عدة مشاكل تتمثل في خسائر اقتصادية عالية في إنتاج الدواجن ومقاومة متعددة للأدوية في كل من الدواجن والبشر.

**الكلمات الدالة:** الإشريكية القولونية، الدواجن واللحوم، البراز البشري، جينات المقاومة.