Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Molecular Identification of Resistance and Pathogenicity Genes of E. coli Isolated From Broiler Chicken Farms

Ahmed A. Ahmed[1,2], Heba M. Salem[3*], Mohamed M. Hamoud[3,4] and Mohamed M. Amer[3]
[1] *MVSc student, Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, P.O. 12211, Giza, Egypt*
[2] *Quality Assurance - Cairo Poultry Co., Egypt.*
[3] *Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, P.O. 12211, Giza, Egypt.*
[4] *General manager of Cairo 3A Poultry Co., Egypt.*

Abstract

Escherichia coli (*E. coli*) is a widespread avian pathogen and usually is considered as a secondary pathogen for different infectious agents causing huge financial losses in the poultry industry. Thus, the current works aim to molecular detection of resistance and pathogenicity genes of *E. coli* isolated from broiler chicken farms. A total of 18 flocks out of 30 broiler flocks (60%) were positive for *E. coli* infection. On the base of the Congo red (CR) binding assay, 15 flocks out of 18 were identified as pathogenic *E. coli* (83.3%) and 3 flocks appeared as non-pathogenic *E. coli* (16.6%). All pathogenic *E. coli* were subjected to *in vitro* antibiotic sensitivity testing to select the most resistant isolates. Ten pathogenic *E. coli* isolates representing ten different broiler flocks were subjected for molecular identification via polymerase chain reaction (PCR) for both resistance and virulence genes. The most predominant isolated serotypes were O91, O128, O78, O124, O_2 and O44. These strains were related to EHEC, EPEC, ETEC, and EIEC. These *E. coli* isolates are multidrug resistant (MDR) to extensively drug-resistant (XDR). The virulence genes tsh, papC, iss, iutA, and hlyF were detected in 50% of isolates having 5 genes and 50% having 4 genes. All the tested isolates showed MDR genes 60% of isolates were positive for 5 resistance genes and 20% were positive for 4 resistance genes and 20% were positive for resistance 3 genes. We can conclude that *E. coli* continues to threaten poultry industry and further studies are recommended to found safe antibiotic natural alternatives to overcome *E. coli* MDR existing strains side by side with strengthening the bird's immunity and application of strict hygienic measures.

Keywords: *E. coli*, Antibiotic sensitivity, Multidrug resistance, PCR, Resistant genes, Virulence genes.

Introduction

The pathogenic *E. coli* infection is known as colibacillosis, which has significant economic impacts on the poultry industry [1,2]. *E. coli* commonly resides in the intestinal tract of healthy chickens, but under certain conditions, it can become pathogenic and cause disease [3]. Colibacillosis in broiler chickens can manifested with different forms, including respiratory, intestinal, localized, or systemic infections [1]. The primary route of infection is through the oral-faecal route, where birds become infected by consuming food or water contaminated with pathogenic strains of *E. coli* also, false vertical transmission through eggshell contamination were reported due to penetration of the pathogen to the fertile eggs in the contaminated hatcheries [1,4].

Symptoms of *E. coli* infection in broiler chickens may vary depending on the virulence of the pathogen, bird immunity and surrounding environment [5]. Numerous factors can increase the risk of *E. coli* infection in broiler chickens including overcrowding, poor ventilation, high stocking density, inadequate sanitation practices, and immunosuppressive diseases [6]. Stress conditions such as transportation, temperature changes, and

*Corresponding author: Heba M. Salem, E-mail dr.hebasalem@cu.edu.eg , Tel.: +201018489282
ORCID ID https://orcid.org/0000-0002-4453-7884. Scopus Author ID: 55561340300
(Received 31/12/2023, accepted 04/03/2024)
DOI: 10.21608/EJVS.2024.252030.1691

concurrent diseases can also make chickens more susceptible to infection [4,6].

Respiratory infections can cause respiratory distress, coughing, sneezing, and nasal discharge, while, intestinal infections may result in diarrhoea, loss of appetite, and poor growth, and systemic infections can lead to septicaemia, with symptoms such as depression, swollen joints, and death [1].

Isolates of Avian Pathogenic *E. coli* (APEC) have specific virulence factors that enable them to colonize and cause disease in the avian host [7]. Dependent on the tissues in which APEC usually express their virulence, they are divided into two major groups: intestinal and extra intestinal pathogenic *E. coli* [8]. Frequent antigenic and genotypic properties of APEC in broilers involve serotypes O1, O2 and O78, phylogroups B2 and D [9].

Virulence factors expressed by APEC strains can vary between different isolates and strains [3,10, 11]. According to Kathayat et al. [3], and Yallow et al. [12] the virulence factors linked to APEC strains are: a) Adhesins (type 1 fimbriae, P fimbriae, F1C fimbriae, and curli fimbriae) are surface proteins that enable *E. coli* to adhere to specific receptors on host cells (respiratory and intestinal epithelial cells; b) specific iron uptake systems, including aerobactin, bactin, salmochelin and, yersinia which scavenge iron from host proteins and enhance bacterial survival and growth; c) a capsule that helps them evade host immunity and inhibits phagocytosis by macrophages and provides resistance against complement-mediated killing, thereby enhancing bacterial survival within the host; d) several exotoxins including cytotoxic necrotizing factor 1 (CNF1), which disrupts host cell signaling and impairs immune responses, some APEC strains produce hemolysin, a toxin that causes damage to red blood cells and other host cells; e) type III Secretion System (T3SS) is a main virulence determinant in APEC strains, it is a specialized protein secretion system used by APEC strains to inject effector proteins directly into host cells, the effector proteins modulate host cell signaling and immune responses, facilitating bacterial colonization and survival; f) biofilms, which are communities of microbe embedded in a self-produced matrix, biofilms provide protection versus host immune reaction and antimicrobials and allows APEC strains to persist in the avian environment and serve as a reservoir for infection [3]. APEC strains accompanied with colibacillosis in broiler chickens have been found to exhibit varying levels of antibiotic resistance [11]. This poses challenges in the treatment and control of diseases [3].

Diagnosis of *E. coli* infection in broiler chickens involves a combination of detection of clinical signs, and post-mortem (PM) lesions, as well as laboratory testing [3]. Isolation and identification of bacteria from infected organs or tissues, such as the respiratory tract or intestines, can be done using bacterial culture and biochemical tests [11]. Molecular techniques, such as PCR, can also be employed for more accurate identification and characterization of the pathogenic strains [1]. Also, clinical APEC isolates, which are specific strains of *E. coli* associated with avian diseases, can exhibit significant genetic and phenotypic variations [1]. This diversity can be observed not only between different countries but also within the same flock or during a disease outbreak [2]. Such diversity poses challenges in terms of diagnosing and preventing the disease promptly [2,13,14]. Research recommended that eight VGs donated to the virulence of APEC including aerobactin (iucD); VGs–P-fimbriae (papC); iron repressible protein (irp2); vacuolating autotransporter toxin (vat); temperature-sensitive hemagglutinin (tsh); increased serum survival protein (iss); enteroaggregative toxin (astA); and colicin V plasmid operon genes (cva/cvi) [11]. The researchers suggested that the existence of 4 of these 8 VGs could recognize to be APEC [15, 16].

Effective control and prevention strategies for *E. coli* infections in broiler chickens focus on maintaining good management practices [17]. Understanding the specific virulence factors of APEC strains is crucial for developing targeted strategies for prevention and control [3]. By targeting these factors, such as through the development of vaccines or therapies that disrupt adhesion or inhibit toxin production, it may be possible to decrease the impact of APEC infections in broiler chickens [3,18]. Thus, this work was done to isolate APEC from clinically infected and freshly dead broiler chickens, with molecular detection of some antibiotic and virulence genes.

Material and Methods

Broiler chicken flocks

Broiler chicken flocks aged 19-33 days located in Giza, Behaira and El-Sharqiyah governorates- Egypt were investigated from January 2022 to December 2023. Chicken shows clinical signs and PM lesions suggestive to colibacillosis. Clinical signs and PM lesions were recorded [19, 20]. Samples were aseptically collected and rapidly transported to the laboratory for further investigations.

Sample collection for bacteriological examination and Transportation

A total of 30 flocks from each flock 3 freshly dead or clinically diseased were necropsied for collection of liver, heart blood, spleen, air sacs and unabsorbed yolk sac. The collected tissue samples from each bird were pooled. Tissue samples from each bird were collected in individual bags,

preserved in ice box, and instantly transferred to the lab.

Bacteriological examination

From each organ a loopfuls was inserted into nutrient broth and kept under aerobic circumstances at 37ºC for 12 hours. After inoculation a loopful from inoculated nutrient broth was streaked onto Eosin methylene blue (EMB) agar and incubated for 24 hours at 37ºC. The colony suspected to be relevant was re-streaked onto MacConkey's agar plates and incubated for an additional 24-48 hours at 37ºC. The colonies showing potential lactose fermentation were then picked up and preserved in semi-solid agar for further testing [21].

***In Vitro* virulence detection**

All *E. coli* isolates pathogenicity examined on Congo red dye binding test as per the technique of Berkhoff and Vinal [22]. Each isolate was cultivated on Trypticase soy agar (TSA) with 0.003% Congo red dye (Sigma) and 0.15% bile salts. The presence of deep brick red colour post incubation at 37°C for 24 hours was considered pathogenic.

Serological identification of *E. coli*

The acquired 14 biochemically and Congo red positive *E. coli* isolates were exposed to serological characterization via the somatic and flagellar antigen according to Gruenewald et al. [23] and Ørskov [24] using slide agglutination test.

Antibiotic discs

The subsequent 17 antibiotic discs were applied including Aminocyclitol (Spectinomycin, Gentamycin10 µg/ml (CN), & Neomycin), Cephalosporin (Cephradin), Chloramphenicol), fluoroquinolone (Enrofloxacin, Ciprofloxacin, Ofloxacin & pfloxacine), Macrolide (Spiramycin), Penicillin like (Amoxicillin), Phosphonic (Fosfomycin), Polymyxin (Colistin), Quinolone (Norfloxacin), Rifamycins (Rifampicine) & Tetracyclines (Doxycycline, Oxytetracycline), respectively. The choice of both disk concentrations and interpretations of inhibition zone diameters were fulfilled following the guides of Difco Laboratories, Detroit, MI, USA) [25].

Antibiotic sensitivity testing

Antibiotic sensitivity testing of the characterized isolates was assessed applying disc agar diffusion test following to CLSI [25] & Weinstein and Lewis [26]. Single and similar colonies on solid media plate were inserted into 3 ml of normal saline and the turbidity was matched with 0.5 McFarland standard. Utilizing sterile swabs, the Muller Hinton agar plates, 9 cm-diameter, were kept with bacterial suspension via streaking on agar surface and rotating the plate to confirm uniform distribution then the plates were permitted to dry for 10 minutes, the antibiotic discs were inserted on the agar surface, and they were left for the pre-diffusion period prior aerobic kept at 37°C for 16-18 hours. Growth inhibition zones were evaluated to the nearest millimetre and isolates categorized as sensitive, intermediate, and resistant based following CLSI [25].

Assessment of MAR indices

The MAR indices were decided via the formula MAR= The count of antibiotics to which the test isolate depicted resistance/ The total count of antibiotics to which the test isolate was estimated for susceptibility [27,28]. Following standardized international terminology designed by European Centre for Disease Control and Prevention, Atlanta, the MDR bacteria was distinct as non-susceptible to at least one agent in 3 or more antimicrobial categories, Extensively Drug-Resistant (XDR) bacteria was distinct as non-susceptibility to at least one agent in all but 2 or fewer antimicrobial groups (i.e., bacterial isolates stay susceptible to only one or 2 antimicrobial groups), and Pan Drug-Resistant (PDR) bacteria was distinguished as non-susceptible to all agents in all antimicrobial groups [29].

Polymerase chain reaction (PCR)

The virulence genes (tsh, papC, iss, iutA, and hlyF) and antibiotic resistance gene to 5 classes of antibiotics (blaTEM, ereA, TetA(A), qnrA, and aac(3)-Ia) were detected by PCR.

Bacterial DNA extraction

DNA isolation from samples was adopted via the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with little changes from the production's guides. In a few words, 200 µl of the culture broth was kept with 10 µl of proteinase K and 200 µl of lysis buffer at 56 °C for 10 min. Post incubation, 200 µl of absolute ethanol (100%) was inserted to the lysate. Then sample was centrifuged then rinsed following to the producer's guided and nucleic acid was eluted with 100 µl of elution buffer specified in the kit.

Oligonucleotide primers

The utilized Primers were obtained from Metabion (Germany) and presented in Table 1.

PCR procedures

Primers were used in a 25 µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer (20 pmol concentration), 4.5 µl of water, and 6 µl of DNA template. The reaction was adopted in an applied biosystem 2720 thermal cycler, Amplification requirements and amplified product are scheduled in Table 2.

Analysis of the PCR products

The products of PCR were split by electrophoresis on 1.5% agarose gel (AppliChem,

Germany, GmbH) in 1x TBE buffer at room condition applying gradients of 5V/cm. For gel analysis, 15 µl of the amplified products was inserted in each gel slot, and gel pilot 100 bp plus DNA ladder (Qiagen, Germany, GmbH) was applied to find out the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) [38].

Results

Recorded signs including low conversion rate, diarrhea, increased mortality 7-11% among the different investigated farms while lesions were airsacculitis score varied from score 1 (10%-), score 2 (25 %), score 3 (25%) and score 4 (40 %), enteritis 20% - 60%, nephrosis (100%), pericarditis (20-100%), perihepatitis (10-100%), ballooning and thinning of intestine in 10- 50%, and hepatitis (25%).

Colonial morphology are pink colonies on MacConkey, orange colonies on Congo red, TSI is Acid to Acid and negative urea test.

Serological identification of 10 *E. coli* Congo red positive isolates (Table 3) includes Two isolate from O91: H21 & O128:H2; 3 isolates from O78; and 1 from each O124, O2: H6, and O44:H18. Regarding the strain character, two strains are Enterohemorrhagic *E. coli* (EHEC), two strains are Enteropathogenic *E. coli* (EPEC), five strains are Enterotoxigenic *E. coli* (ETEC), & one strain is Entero-invasive *E. coli* (EIEC).

Regarding the tested antibiotics in disc diffusion, test results indicate that all isolates were sensitive to Doxycycline and 10 % resistant to both Neomycin and Ciprofloxacin. Moreover, 40 %- 100% of isolates are resistant, the resistance of isolates to the antibiotic it is notice that rate resistance is ranged from 47.1% to 76.5%. The used 17 antibiotic discs related to 11 antibiotic classes including Aminocyclitol (Spectinomycin, Gentamycin, & Neomycin), Cephalosporin (Cephalin), Chloramphenicol, fluoroquinolone (Enrofloxacin, Ciprofloxacin, Ofloxacin & Pfloxacine), Macrolide (Spiramycine), Penicillin like (Amoxicillin), Phosphonic (Fosfomycin), Polymyxin (Colistin), Quinolone (Norfloxacin), Rifamycins (Rifampicine) and Tetracycline (Doxycycline, Oxytetracycline). The obtained *E. coli* isolates are MDR bacteria to Extensively Drug-Resistant (XDR) (Table 4).

The virulence genes tsh, papC, iss, iutA, and hlyF were observed in 50% of isolates showing 5 On the base of PCR, the ten examined strains were classified 5 strains as ETEC (50%), 2 were EPEC (20%), 2 were EHEC (20%) and one strain was EIEC (10%) (Table 5). One EIEC strain, two ETEC, and two EPEC strains carry the five virulence genes (tsh, papC, iss, iutA, & hlyF). Two EHEC strains carry different pattern of virulence genes as the 1st strain has papC, iss, iutA, and hlyF, while the 2nd strain has tsh, iss, iutA, and hlyF, as both has 4 genes (Tabe 5 and Figure 1).

Genes blaTEM, ereA, TetA(A), qnrA, and aac(3)-Ia indicate resistance to beta-lactamase, erythromycin, tetracycline, quinolone, and aminoglycoside, respectively. Antibiotic resistance gene blaTEM, ereA, TetA(A), qnrA, and aac (3)-Ia to five classes of antibiotics mainly used in therapy of *E. coli* in infected birds (ampicillin, erythromycin, tetracycline, quinolone, and aminoglycoside), respectively. The result showed that all the tested isolates showed multiple Antibiotic resistance genes 60% of isolates showed 5 genes and 20% showed 4 genes and 20% showed 3 genes (Tabe 6 and Figure 2).

Discussion

E. coli is a serious avian pathogen that contributes a significant hazard to all avian species [5]. For *E. coli* identification, both phenotypic and genotypic approaches are essential [5]. In this study, pathogenic *E. coli* was recovered from 30 different broiler chicken flocks located in Giza, Behaira and El-Sharqiyah governorates in Egypt with a recovery rate 50% (15/30).

In this work, the investigated farms were suffered from different clinical signs including mortalities, reduced body weight, respiratory and enteric signs also, PM exanimation revealed the existence of different degrees of pericarditis, perihepatitis, airsacculitis, pneumonia, nephritis, and enteritis. Similar clinical signs and PM lesions have been recorded by Hussein et al. [39] because of *E. coli* infection in broilers.

Ali et al. [40] found that several virulence genes of *E. coli* in Egypt were examined, and it was discovered that the differences were location specific nevertheless, beside the inconsistent nature of these screened genes, all research were restricted to a limited set of screened virulence genes. From our data the most predominant serotypes were O91, O128, O78, O124, O2 and O44 and these strains were related to EHEC, EPEC, ETEC, and EIEC. In old studies, most avian pathogenic *E. coli* strains linked to colibacillosis outbreaks were O1, O2, O15, O35, and O78 serotypes [41], but recently new serotypes have been emerged as APEC [42] and other authors reported different serotypes in Egypt as O78, O1, O2, O91, and O8 by Younis et al.[43] from Mansoura governorates, serotypes O78, O24, O44, O55, O86, O124, O158 and O127 by Amer et al.[44] from Giza and Kaluobia governorates and serotypes O169, O115, and O29 by Ellakany et al.[45] from Alexandria governorates.

It has been investigated how pathogenic APEC was in connection to specific virulence gene patterns, also several patterns were proposed as quick diagnostic tools for APEC, numerous virulence genes

were screened in Egypt; however, the primary restriction on all the studies was restricted count of screened virulence genes [40]. In the current investigation, the ten tested *E. coli* strains showed the existence of virulence genes tsh, papC, iss, iutA, and hlyF in five strains while the rest five strains having 4 genes. On the same way different virulence genes of APEC have been recorded from different localities in Egypt by different authors as Ahmed et al. [46] reported the existence of ompA, papC, eaeA, and tsh virulence genes in APEC recovered from birds in Qena governorate, also AbdEl-Tawab et al. [47] notices the presence of iss and ompA virulence genes in APEC recovered from birds in Gharbia governorate. The incidence of different *E. coli* serotypes in chickens varies across Egypt and other countries. Individual virulence genes did not cause *E. coli* pathogenicity; rather, the presence of specific traits resulting from these genes [40].

Antibiotics have been used for decades to manage APEC; but, due to the rise of multi-drug resistant *E. coli* and the challenge of developing novel antimicrobial medicines, vaccination has emerged as the most effective means of controlling *E. coli* infections on poultry farms [5, 44]. In this study, the antibiotic sensitivity testing exposed that all isolates were sensitive to Doxycycline and 10 % resistant to both Neomycin and Ciprofloxacin. Moreover, 40 %-100% of isolates are resistance, the resistance of isolates to the antibiotic it is notice that rate resistance is ranged from 47.1% to 76.5%. They used 17 antibiotic discs related to 11 antibiotic classes including Aminocyclitol, Cephalosporin, Chloramphenicol, fluoroquinolone, Macrolide, Penicillin, Phosphonic, Polymyxin, Quinolone, Rifamycins and Tetracycline. These results concur with Amer et al. [44] who reported MDR of avian *E. coli* strains with a percentage of 85% to kanamycin and oxytetracycline; 80% to clindamycin, ampicillin, and streptomycin. Resistance was 75%, 65%, 55%, 45%, 35% and 30% to enrofloxacin, chloramphenicol, gentamicin, and cefotaxime; sulfamethoxazole-trimethoprim; erythromycin; and oxacillin; respectively.

Antibiotic resistances on the base of antibiotic resistance test were confirmed via the detection of five different resistant genes using PCR. On the base of PCR, the tested *E. coli* strains in the current work showed that the antibiotic resistance gene blaTEM, ereA, TetA(A), qnrA, and aac (3)-Ia representing 5 categories of antibiotics (ampicillin, tetracycline, erythromycin, quinolone, and aminoglycoside), usually utilized as a medication for *E. coli* infection in broiler chickens and the data of this work noted that all the tested isolates showed multiple antibiotic resistance genes 60% of isolates showed 5 genes and 20% showed 4 genes and 20% showed 3 genes. On the same way, Amer et al. [44] recorded that from twenty strains examined for the existence of MDR genes, fourteen were +ve to CITM, twelve for ere and aac (3) -(IV) genes, eight for tet(A), eleven for tet(B), eight for dfr(A1), and nine for aad(A1). Studies of the phenotypical and genotypical alterations of antibiotic-resistant *E. coli* strains indicate that phenotype-genotype mapping is complicated and involves a variety of mutations that result in comparable phenotypic changes [48]. The current investigation shows that broiler chickens in Egypt harbour pathogenic MDR *E. coli* and most of the isolates had antibiotic resistance and virulence genes, however some did not express the genes. It is a fact that the poultry industry. In fact, there are many pathogens that high mortalities of birds and cause huge economic losses [49, 50], and the widespread use of antibiotics has led to the emergence of antibiotic-resistant strains [51], so the world has recently turned to applying strict hygienic measures side by side with usage of save antibiotic alternatives to overcome MDR problem via the usage of natural safe products such as prebiotics, probiotics, symbiotic, postbiotics, hyperimmune serum [52, 53], herbal extracts, organic acids, essential oils, nano-preparations and other safe products to improve avian gut microbiome to compete pathogens [54], and finally enhance birds' productivity.

Conclusion

In this investigation APEC were recovered from broiler chicken flocks in Giza, Behaira and El-Sharqiyah governorates with a detection rate of (60%) and from these isolates ten strains were selected and they were classified serologically as O91, O128, O78, O124, O2 and O44 and these strains were related to EHEC, EPEC, ETEC, and EIEC also, these stains showed multidrug resistant pattern against most common commercial antibiotics on the base of antibiotic sensitivity and molecular PCR testing. Regular molecular *E. coli* monitoring for both virulence and resistant genes, biosecurity precautions at the farm and hatchery levels, and increasing the immunity of birds especially by vaccination were suggested to reduce the hazard of *E. coli* infection in broiler chickens also, the usage of safe natural antibiotic alternatives is essential to limit the hazard of MDR in the avian and human levels.

Acknowledgments

The authors acknowledge all members of the Poultry Disease Department, Faculty of Veterinary Medicine, Cairo University.

Authors' contributions

A.A.A and H.M.S. collected samples, experimental and laboratory investigations. M.M.H. and M.M.A. supervised the work. All team members wrote, revised the original draft, and approved the final manuscript.

Funding statement

This work was done by Fund supplied by Quality Assurance - Cairo Poultry Co., Egypt.

Availability of data and materials

Not applicable

Declarations

All data included in this paper is an original obtained from our work-by-work team.

Ethics approval and consent to participate

This work follows the regulations of IACUC, Faculty of Veterinary Medicine, Cairo University.

Competing interests

The authors declare that they have no competing interests.

Author details

Ahmed A. Ahmed: 1. MVSc student, Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, P.O. 12211, Giza, Egypt. 2.Quality Assurance - Cairo Poultry Co., Egypt. E-mail address: drahmedaliahmed.2016@gmail.com

Heba M. Salem: BVSc, MVSc, PhD, Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, P.O. 12211, Giza, Egypt. dr.hebasalem@cu.edu.eg mobile: +201018489282; ORCID ID https://orcid.org/0000-0002-4453-7884

Mohamed M. Hamoud: 3. Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, P.O. 12211, Giza, Egypt. General manager of Cairo 3A Poultry Co., Egypt. e-mail address: mohamed.hamoud@cairo3 apoultry.com

Mohamed M. Amer: BVSc, MVSc, PhD, Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, P.O. 12211, Giza, Egypt. Email: profdramer@yahoo.com. Mobile +201011828228. ORCID: 0000-0001-8965-7698

TABLE 1. Oligonucleotide primers sequences of target *E. coli* genes & amplicon sizes.

Gene	Sequence	Amplified product	Reference
hlyF	GGCCACAGTCGTTTAGGGTGCTTACC GGCGGTTTAGGCATTCCGATACTCAG	**450 bp**	**Johnson et al. [30]**
Tsh	GGT GGT GCA CTG GAG TGG AGT CCA GCG TGA TAG TGG	**620 bp**	**Delicato et al. [31]**
papC	TGA TAT CAC GCA GTC AGT AGC CCG GCC ATA TTC ACA TAA	**501 bp**	**Wen-jie et al. [32]**
Iss	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATACCC	**266 bp**	**Yaguchi et al., [12]**
iutA	GGCTGGACATGGGAACTGG CGTCGGGAACGGGTAGAATCG	**300 bp**	
TetA(A)	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	**570 bp**	**Randall et al. [33]**
qnrA	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	**516 bp**	**Robicsek et al. [34]**
ereA	GCCGGTGCTCATGAACTTGAG CGACTCTATTCGATCAGAGGC	**420 bp**	**Nguyen et al. [35]**
blaTEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	**516 bp**	**Colom et al. [36]**
aac(3)-Ia	TTGATCTTTTCGGTCGTGAGT TAAGCCGCGAGAGCGCCAACA	**150 bp**	**Frana et al. [37]**

TABLE 2. Cycling environment of the various primers during PCR at recommended temperature & time.

Gene	°C & Time	Primary denaturation °C/min	Secondary denaturation °C/sec	Annealing °C/sec	Extension °C/sec	Final extension °C/min
hlyF	**˚C**	94	94	63	72	72
	Time	5	30	40	45	10
Tsh	**˚C**	94	94	54	72	72.
	Time	5	30	40	45	10
papC	**˚C**	94	94	58	72	72
	Time	5	30	40	45	10
Iss	**˚C**	94	94	54	72	72.
	Time	5	30	30	30	7
iutA	**˚C**	94	94	63	72	72
	Time	5	30	30	30	7
TetA(A)	**˚C**	94	94	50	72	72
	Time	5	30	40	45	10
qnrA	**˚C**	94	94	55	72	72
	Time	5	30	40	45	10
ereA	**˚C**	94	94	60	72	72
	Time	5	30	40	45	10
blaTEM	**˚C**	94	94	54	72	72
	Time	5	30	40	45	10
aac(3)-Ia	**˚C**	94	94	55	72C	72
	Time	5	30	30	30	7

TABLE 3. Serological identification of 10 Congo red positive isolates.

Isolate No	Serodiagnosis	Strain characterization
1	O91: H21	EHEC
2	O124	EIEC
3	O78	ETEC
4	O78	ETEC
5	O91: H21	EHEC
6	O2: H6	EPEC
7	O128: H2	ETEC
8	O44: H18	EPEC
9	O78	ETEC
10	O128: H2	ETEC

TABLE 4. Data of antibiotic sensitivity test for 10 Congo red positive isolates

Strain No	Serotype	Colistin	Cephradine	Fosfomycin	Gentamycin	Chloramphenicol	Neomycin	Enrofloxacin	Ciprofloxacin	Norfloxacin	Amoxicillin	Doxycycline	Spiramycine	Spectinomycine	Pfloxacine	Rifampicine	Oxytetracyclin	Ofloxacin	No of resistant	%
1	**O91: H21**	+	+	+	-	-	+	+	+	+	-	+	-	-	+	-	-	-	8	47.1
2	**O124**	-	-	+	-	-	+	-	+	+	-	+	-	-	-	-	-	-	12	70.6
3	**O78**	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	13	76.5
4	**O78**	-	+	-	-	-	+	+	+	-	-	+	-	-	-	+	-	-	11	64.7
5	**O91: H21**	-	-	-	-	-	+	+	+	-	-	+	-	-	-	+	-	-	12	70.6
6	**O2: H6**	-	-	+	-	-	+	-	+	+	-	+	-	-	+	-	-	-	11	64.7
7	**O128: H2**	-	-	+	-	+	+	-	-	-	-	+	-	+	-	-	-	-	12	70.6
8	**O44: H18**	+	-	-	-	-	+	+	+	-	-	+	-	-	+	+	-	-	10	58.8
9	**O78**	+	-	+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	12	70.6
10	**O128: H2**	+	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	13	76.5
Total resistant strain		6	8	4	10	9	1	6	1	7	10	0	9	9	7	7	10	10		
%		60	80	40	100	90	10	60	10	70	100	0	90	00	70	70	100	100		

+: Sensitive **-: Resistant**

TABLE 5. Showing the distribution of virulent genes in *E. coli* isolates.

***E. coli* isolate**	***Class***	***Virulence genes***					***No of +ve gens***
		tsh	***papC***	***iss***	***iutA***	***hlyF***	
1	EHEC	-	+	+	+	+	4
2	EIEC	+	+	+	+	+	5
3	ETEC	+	-	+	+	+	4
4	ETEC	+	+	+	+	+	5
5	EHEC	+	-	+	+	+	4
6	EPEC	+	+	+	+	+	5
7	ETEC	+	+	+	+	+	5
8	EPEC	+	+	+	+	+	5
9	ETEC	-	+	+	+	+	4
10	ETEC	+	-	+	+	+	4

+: Positive **- :Negative**

TABLE 6. Distribution of Antibiotic resistance Genes in *E. coli* isolates.

***E. coli* isolate**	**Antibiotic resistance genes**					**No of positive genes**
	blaTEM	***ereA***	***TetA(A)***	***qnrA***	***aac(3)-Ia***	
1	+	+	+	+	+	5
2	+	-	+	+	+	4
3	+	-	+	+	-	*3*
4	+	+	+	+	+	5
5	+	+	+	+	+	5
6	+	+	+	+	+	5
7	+	+	+	+	+	5
8	+	-	+	+	+	4
9	+	+	-	-	+	3
10	+	+	+	+	+	5

+: Positive **-: Negative**

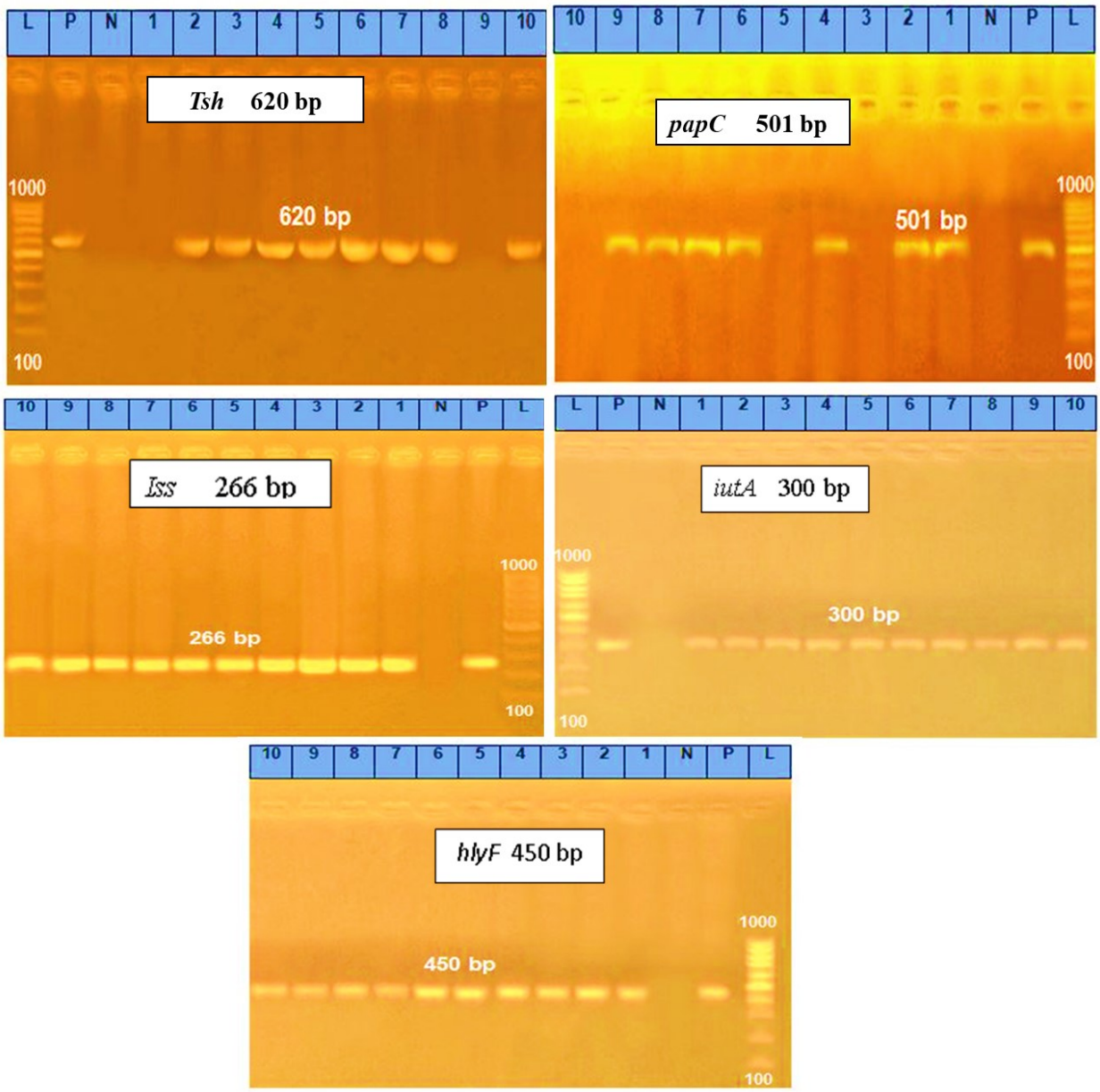

Fig. 1. The detected virulence genes tsh, papC, iss, iutA, and hlyF. L: ladder, P: positive control, N: negative control 1:10: tested isolates.

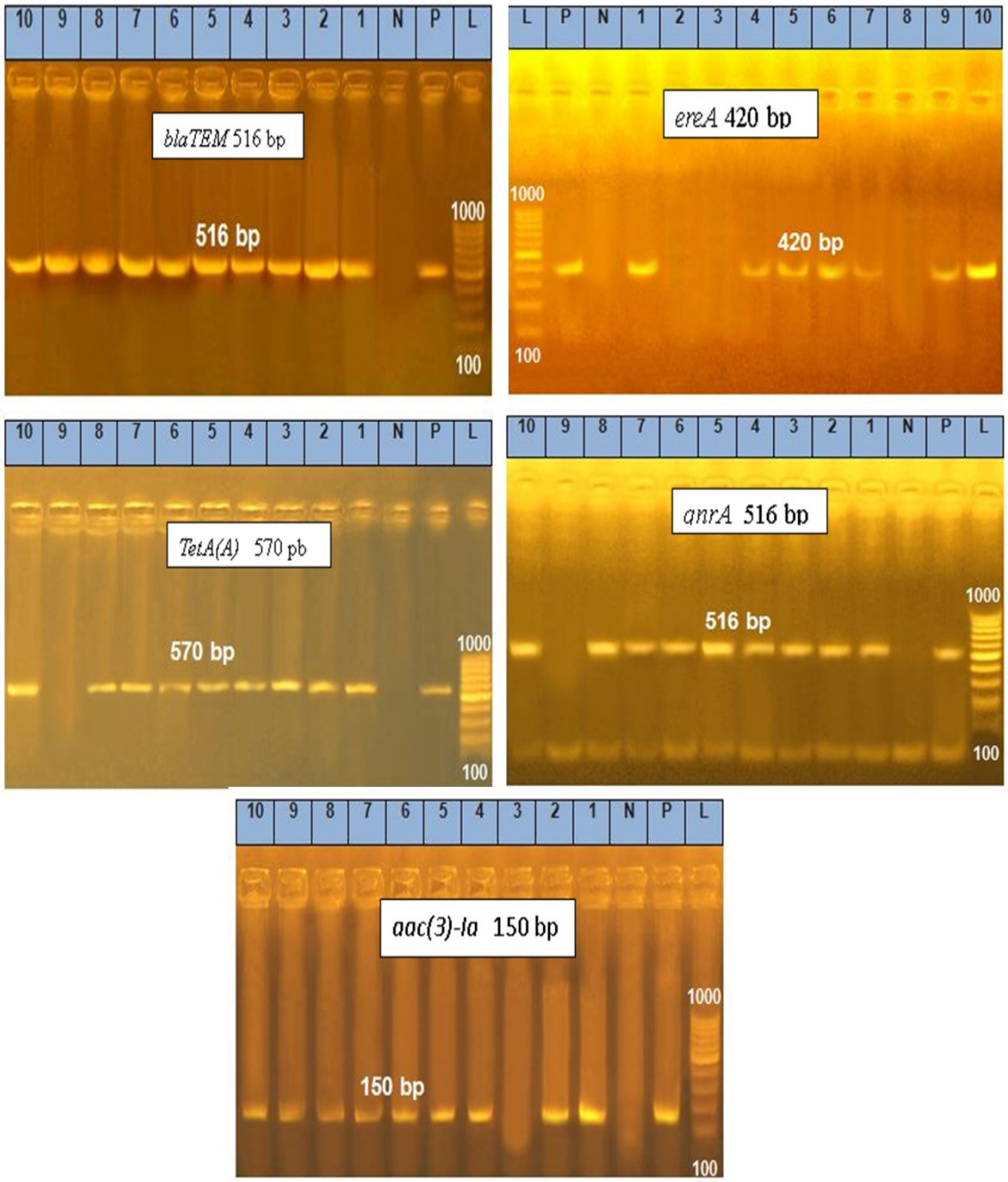

Fig. 2. Detection of antibiotic resistance genes in *E. coli* isolates, L: ladder, P: positive control, N: negative control 1:10: tested isolates.

References

1. Barnes H. J., Nolan L. K. and Vaillancourt J. P. Colibacillosis. In Diseases of Poultry,12th Ed., pp. 691-734 (2008) Wiley-Blackwell.

2. Apostolakos I., Laconi A., Yapicier Ö. Ş. and Piccirillo A. Occurrence of Colibacillosis in Broilers and Its Relationship with Avian Pathogenic Escherichia coli (APEC) Population Structure and Molecular Characteristics. *Front. Veterinary Science*, **8**, 737720 (2021). https://doi.org/10.3389/fvets.2021.737720

3. Kathayat, D., Lokesh, D., Ranjit, S. and Rajashekara, G. Avian Pathogenic Escherichia coli (APEC): An Overview of Virulence and Pathogenesis Factors, Zoonotic Potential, and Control Strategies. *Pathogens (Basel, Switzerland)*, **10**(4), 467 (2021). https://doi.org /10.3390

4. Amer M.M., Bastamy, M., Elbayoumi, Kh.M. and Mervat Salem. Isolation and characterization of avian pathogenic *Escherichia coli* from broiler chickens in some Governorates of Egypt. *Veterinary Medical Journal –Giza* (VMJG), **61** (1), 1-7 (2015).

5. Yousef, H. M., Hashad, M. E., Osman, K. M., Alatfeehy, N. M., Hassan, W. M., Elebeedy, L. A., Salem, H.M., Shami, A., Al-Saeed, F.A., El-Saadony, M.T., El-Tarabily, K.A. and Marouf, S. Surveillance of *Escherichia coli* in different types of chicken and duck hatcheries: One health outlook. *Poultry Science.*, **102**(12),103108(2023). https://doi:10.1016 /j.psj.2023.103108.

6. Fancher, C. A., Zhang, L., Kiess, A. S., Adhikari, P. A., Dinh, T. T. N. and Sukumaran, A. T. Avian Pathogenic *Escherichia coli* and Clostridium perfringens: Challenges in No Antibiotics Ever Broiler Production and Potential Solutions. *Microorganisms*, **8**(10), 1533 (2020). https://doi.org/10.3390/microorganisms8101533

7. Wilczyński, J., Stępień-Pyśniak, D., Wystalska, D. and Wernicki, A. Molecular and Serological Characteristics of Avian Pathogenic *Escherichia coli* Isolated from Various Clinical Cases of Poultry Colibacillosis in Poland. *Animals: an open accessJournal from MDPI*, **12**(9), 1090 (2022). https://doi.org/10.3390/ani12091090

8. Kaper, J.B., Nataro, J.P. and Mobley, H.L.T. Pathogenic *Escherichia coli*. *Nature Reviews Microbiology,* **2**,123– 140 (2004). https://doi:10.103 8/nrmicro818

9. Mellata, M. Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trends. *Foodborne Pathogen Disease,* **10**,916–32 (2013). https://doi:10.1089/fpd.2013.1533

10. Schouler, C., Schaeffer, B., Brée, A., Mora, A., Dahbi, G., Biet, F., Oswald, E., Mainil, J., Blanco, J. and Moulin-Schouleur, M. Diagnostic Strategy for Identifying Avian Pathogenic Escherichia coli Based on Four Patterns of Virulence Genes. *Journal of Clinical Microbiology,* **50**(5), 1673-1678(2012). https://doi.org/10.1128/JCM.05057-11

11. Amer, M.M., Mekky, H.M., Fedawy, H.S., El-Shemy, A., Bosila ,M.A. and Elbayoumi, K.M. Molecular identification, genotyping of virulence-associated genes, and pathogenicity of cellulitis-derived Escherichia coli. *Veterinary World*, **13**(12), 2703-2712 (2020). doi:www.doi.org/10.14202/vetworld.2020.2703-2712.

12. Yaguchi, K., Ogitani, T., Osawa, R., Kawano, M., Kokumai, N., Kaneshige, T., Noro, T., Masubuchi, K. and Shimizu, Y. Virulence Factors of Avian Pathogenic *Escherichia coli* Strains Isolated from Chickens with Colisepticemia in Japan. *Avian Diseases*, **51** (3),656-662 (2007). https://doi:10.16 37/0005-2086(2007)51[656: VFOAPE]2.0.CO;2

13. Nolan, L.K., Vaillancourt, J.P., Barbieri, N.L. and Logue, C.M. Colibacillosis. In: *Diseases of Poultry.* John Wiley and Sons, Inc. p 770–830 (2020). https://doi:10.1002/ 9781119371199.ch18

14. Ovi, F., Zhang, L., Nabors, H., Jia, L. and Adhikari, P. A compilation of virulence-associated genes that are frequently reported in avian pathogenic *Escherichia coli* (APEC) compared to other *E. Coli. Journal of Applied Microbiology*, **134**(3), lxad014 (2023). https://doi.org/10.1093/jambio/lxad014

15. Ewers, C., Janssen, T., Kiessling, S., Philipp, H.C. and Weiler, L.H. Rapid detection of virulence-associated genes in avian pathogenic *Escherichia coli* by multiplex polymerase chain reaction. *Avian Disease*, **49**(2), 269-273(2005). https://doi:10.1637/7 293-102604R

16. De Carli, S., Ikuta, N., Lehmann, F.K., da Silveira, V.P., de Melo Predebon, G., Fonseca, A.S. and Lunge, V.R. Virulence gene content in *Escherichia coli* isolates from poultry flocks with clinical signs of colibacillosis in Brazil. *Poultry Science*, **94**(11), 2635-2640 (2015). https://doi:10.3382/ps/pev256 .

17. Swelum, A. A., Elbestawy, A. R., El-Saadony, M. T., Hussein, E. O. S., Alhotan, R., Suliman, G. M., Taha, A. E., Ba-Awadh, H., El-Tarabily, K. A. and Abd El-Hack, M. E. Ways to minimize bacterial infections, with special reference to *Escherichia coli,* to cope with the first-week mortality in chicks: an updated overview. *Poultry Science*, **100**(5), 101039 (2021). https://doi.org/10.1016/j.psj.2021.101039

18. Joseph, J., Zhang, L., Adhikari, P. and Evans, J.D., Ramachandran R. Avian Pathogenic *Escherichia coli* (APEC) in Broiler Breeders: An Overview. *Pathogens*, **12**(11), 1280 (2023). https://doi.org/10.3390/pathogens12111280/pathogens10040467

19. Peighambari, S. M., Julian, R. J. and Gyles, C. L. Experimental *Escherichia coli* Respiratory Infection in Broilers. *Avian Diseases*, **44** (4),759-769 (2000).

20. Stipkovits, L., Glavits, R., Palfi, V., Beres, A., Egyed, L., Denes, B., Somogyi, M. and Szathmary, S. Pathologic lesions caused by coinfection of *Mycoplasma gallisepticum* and H3N8 Low Pathogenic Avian Influenza virus in chickens. *Veterinary Pathology*, **49**(2), 273-283 (2012). https://doi:10.1177/0300985811415702

21. Singh, V. K. Clinical veterinary microbiology. 4th ed., Mosby Yearbook Europe limited (2017). https://vetbooks.ir/clinical-veterinary-microbiology/

22. Berkhoff, H.A. and Vinal, A.C. Congo red medium to distinguish between invasiveness and non-invasiveness *E. coli* pathogenic for poultry. *Avian Disease*, **30**, 117 – 121 (1986).

23. Gruenewald, R., Dixon, D.P,. Brun, M., Yappow, S., Henderson, R., Douglas, J.E., Backer, M.H. Identification of Salmonella somatic and flagellar antigens by modified serological methods. *Applied Environmental Microbiology,* 56(1):24-30 (1990). https://doi:10.1128/ aem.56.1.24-30.1990

24. Ørskov, F. and Ørskov, I. Serotyping of Escherichia coli. In: *Methods in microbiology*. 14th ed., Bergan T. London, UK: Academic Press. p. 43-112 (1984).

25. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI supplement M100S. Wayne, PA: *Clinical and Laboratory Standards Institute* (2016). http://ljzx.cqrmhospital. com/upfiles/ 201601/20160112155335884.pdf

26. Weinstein, M. P. and Lewis, J. S., 2nd. The Clinical and Laboratory Standards Institute Subcommittee on Antimicrobial Susceptibility Testing: Background,

Organization, Functions, and Processes. *Journal of Clinical Microbiology*, **58**(3), 10-1128 (2020). https://doi.org/10.1128/JCM.01864-19

27. Paul, S., Bezbaruah, R.L., Roy, M.K. and Ghosh, A.C. Multiple antibiotic resistance (MAR) index and its reversion in Pseudomonas aeruginosa. *Letters in Applied Microbiology*, **24**, 169-171(1997). https://doi.org/10.1046/j.1472-765X.1997.00364.x

28. Krumperman, P.H. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Applied Environmental Microbiology*, **46**, 165-170 (1983). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC239283/

29. Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T. and Monnet, D.L. Multidrug-resistant, extensively drugresistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, **18**(3), 268- 281(2012). https://doi.org/10.1111/j.1469-0691.2011.03570.x

30. Johnson, T.J., Wannemuehler, Y., Doetkott, C., Johnson, S.J., Rosenberger, S.C. and Nolan, L.K. Identification of Minimal Predictors of Avian Pathogenic *Escherichia coli* Virulence for Use as a Rapid Diagnostic Tool. *Journal of Clinical Microbiology*, **46** (12), 3987–3996 (2008). https://doi:10.1128/JCM.00816-08

31. Delicato, E.R., de Brito, B.G., Gaziri, L.C.J. and Vidotto, M.C. Virulence-associated genes in *Escherichia coli* isolates from poultry with colibacillosis. *Veterinary Microbiology*, **94**, 97-103 (2003). https://doi:10.1016/s0378-1135(03)00076-2

32. Wen-jie, J., Zhi-ming, Z., Yong-zhi, Z., Ai-jian, Q., Hong-xia, S., Yue-long, L., Jiao, W. and Qian-qian, W. Distribution of Virulence-Associated Genes of Avian Pathogenic *Escherichia coli* Isolates in China. *Agricultural Sciences in China*, **7**(12),1511-1515 (2008). https://doi.org/10.1016/S16712927(08) 60410-1

33. Randall, L.P., Cooles, S.W., Osborn, M.K., Piddock, L.J.V. and Woodward, M.J. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. *Journal of Antimicrobial Chemotherapy,* **53**, 208–216 (2004). https://doi:10.1093/jac/dkh070 .

34. Robicsek, A., Strahilevitz, J., Jacoby, G.A., Macielag, M., Abbanat, D., Park, C.H., Bush, K. and Hooper, D.C. Fluoroquinolone modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Natural Medicine*, **12,** 83-88 (2006). https://doi:10. 1038/nm1347

35. Nguyen M.C.P., Woerther, P., Bouvet, M., Andremont, A., Leclercq, R. and Canu, A. *Escherichia coli* as reservoir for macrolide resistance genes. *Emerging Infectious Diseases,* **15**(10), 1648–1650(2009). https://doi.org/10.3201/eid1510.090696.

36. Colom, K., Pèrez, J., Alonso, R., Fernández-Aranguiz, A., Lariño E. and Cisterna, R. Simple and reliable multiplex PCR assay for detection of bla_{TEM},bla_{SHV} and bla_{OXA-1} genes in Enterobacteriaceae. *FEMS Microbiology Letters,* **223** , 147-151 (2003). https://doi.org /10.1016/S0378-1097(03)00306-9

37. Frana, T. S., Carlson, S. A. and Griffith, R. W. Relative distribution and conservation of genes encoding aminoglycoside-modifying enzymes in *Salmonella enterica* serotype Typhimurium phage type DT104. *Applied and Environmental Microbiology,* **67**, 445–448(2001). https://doi:10. 1128/AEM.67.1.445-448.2001

38. Sambrook, J., Fritscgh, E.F. and Mentiates, T. Molecular coloning. A laboratory manual. Vol 1, 4th Ed., Cold spring Harbor Laboratotry press, New York (2014).

39. Hussein, A. H., Ghanem, I. A., Eid, A. A., Ali, M. A., Sherwood, J. S., Li G., Nolan, L.K. and Logue, C.M. Molecular and phenotypic characterization of *Escherichia coli* isolated from broiler chicken flocks in Egypt. *Avian diseases*, **57**(3), 602-611 (2013). https://doi:10.1637/10503-012513-Reg.1

40. Ali, A., Abd El-Mawgoud, I. A., Dahshan, M. A. H., EL-Sawah, A. A. and Nasef, A. S. *Escherichia coli* in broiler chickens in Egypt, its virulence traits and vaccination as an intervention strategy. *Novel Research in Microbiology Journal*, **3**(4), 415-427 (2019). https://api.semanticscholar.org/CorpusID:202007129

41. Dho-Moulin, M. and Fairbrother, J.M. Avian pathogenic *Escherichia coli* (APEC). *Veterinary Research*, **30** (2-3), 299-316 (1999).

42. El-Sawah, A.A., Dahshan, A.L.H.M., El-Nahass, E.-S. and El-Mawgoud, A.I.A. Pathogenicity of *Escherichia coli* O157 in commercial broiler chickens. *Beni-Suef University Journal of Basic and Applied Sciences*, **7**(4),620-625 (2018). https://doi.org/10.1016/j.bjbas.2018.07.005

43. Younis, G., Awad, A. and Mohamed, N. Phenotypic and genotypic characterization of antimicrobial susceptibility of avian pathogenic *Escherichia coli* isolated from broiler chickens. *Veterinary World,* **10**(10), 1167-1172 (2017). http://doi:10.14202/vetworld.2017.1167-1172

44. Amer, M.M., Mekky, H.M., Amer, A.M. and Fedawy, H.S. Antimicrobial resistance genes in pathogenic *Escherichia coli* isolated from diseased broiler chickens in Egypt and their relationship with the phenotypic resistance characteristics. *Veterinary World*, **11**(8),1082-1088 (2018). http://www.veterinaryworld.org/Vol.11/August-2018/10.pdf

45. Ellakany, H., Elhamid, H., Mostafa, N., Elbestawy, A. and Gado, A. Isolation, Serotyping, Pathogenicity and Antibiotic Sensitivity Testing of *Escherichia coli* from Broiler Chickens in Egypt. *Alexandria Journal of Veterinary Sciences*. **61**(2), 45-45 (2019). http://dx.doi.org/10.21608/EJVS.2021.93246.1274

46. Ahmed, S.A.M., Ahmed, I.A., Osman, N. and El-Hamd, D.M. Problem of *Escherichia coli* infection in broilers in Qena province. *Assiut Veterinary Medical Journal*, **63**(153), 252-264 (2017). http://dx.doi.org/10.21608/AVMJ.2017.170679

47. Abd El-Tawab, A.A., El-Hofy, F.I., Alekhnawy, k.I., Talaie, A.T. and Ahmed, A. Insights into virulence and antimicrobial resistance plasmid associated genes of ESBL *Escherichia coli* associated with arthritis in chickens in Egypt. *International Journal of Advanced Research*, **7**(1), 174-182 (2018). http://dx.doi.org/10.21474/IJAR01/8310

48. Sundin, G. W. Examination of base pair variants of the strA–strB streptomycin resistance genes from bacterial pathogens of humans, animals and plants. *Journal of Antimicrobial Chemotherapy*, **46**(5), 848-849 (2000). https://doi.org/10.1093/jac/46.5.848

49. Qoraa, A. M., Salem, H. M., and Shakal, M. The Current Status of *Mycoplasma synoviae* in Broilers and Laying Chicken Farms in some Egyptian Governorates. *Egyptian Journal of Veterinary Sciences*, **54**(5), 805-813 (2023). https://doi.org/10.21608/ejvs.2023.206857.1491

50. Mosa, M. I., Salem, H. M., Bastamy, M. A., and Amer, M. M. Pathogenic and Non-pathogenic Factors; Especially Infectious Bursal Disease Viruses; Affect Chicken Digestive System Microbiota and Methods of Its Evaluation and Recovery: A review. *Egyptian Journal of Veterinary Sciences*, **54**(4), 733-760 (2023).https://doi.org/10.21608/EJVS.2023.203480.1476

51. Salem, H.M., Saad, A.M., Soliman, S.M., Selim, S., Mosa, W.F., Ahmed, A.E., Al Jaouni, S.K., Almuhayawi, M.S., Abd El-Hack, M.E., El-Tarabily, K.A. and El-Saadony, M.T. Ameliorative avian gut environment and bird productivity through the application of safe antibiotics alternatives: A comprehensive review. *Poultry Science*, p.102840 (2023). https://doi.org/10.1016/j.psj.2023.102840

52. Radwan, F.M., El-Shemy, A.A., Bastamy, M.A. and Amer, M.M. Chicken Immunoglobulin IgY Preparation, and Its Applications in Prevention and/or Control of Some Microbial Affections. *Egyptian Journal of Veterinary Sciences*, **54**(4), p.653-668 (2023). https://doi.org/10.21608/ EJVS.2023.200854.1464

53. Radwan, F.M., EL-Shemy, A., Bosila, M.M., Bastamy, M.A. and Amer, M.M. Evaluation of the efficacy of Purified Egg Yolk Immunoglobulin (IgY) in Preventing and Controlling Newcastle Disease Virus Infection in Broiler Chickens. *Egyptian Journal of Veterinary Sciences*, **55**(4), p.931-943 (2024). https://doi.org/10.21608/EJVS .2023.246553.1663

54. Mosa, M. I., Salem, H. M., Bastamy, M. A., and Amer, M. M. The Potential Diversity of Intestinal Enterobacteriaceae in Broiler Chickens is Associated with Infectious Bursal Disease Virus Infection. *Egyptian Journal of Veterinary Sciences*, **55**(4), 917-930 (2024). https://doi.org/10.21608 /EJVS.2023.246007.1662

التحديد الجزيئي لمقاومة وضراوة جينات الايشريكيا القولونية المعزولة من قطعان الدجاج اللاحم

أحمد علي احمد [1،2]، هبة محمد سالم [*3]، محمد ممدوح حمود [4،3] ومحمد محروس عامر [3]

[1].طالب ماجستير بقسم أمراض الدواجن- كلية الطب البيطري - جامعة القاهرة - ص.ب. 12211 - الجيزة - مصر

[2].مراقبة الجودة شركة القاهرة للدواجن - مصر

[3]. قسم أمراض الدواجن - كلية الطب البيطري - جامعة القاهرة - ص.ب. 12211 – الجيزة - مصر.

[4]. مدير عام شركة القاهرة 3 أ للدواجن - مصر.

تعتبر الايشيريا كولاي (*E. coli*) من مسببات أمراض الطيور واسعة الانتشار وعادة ما تعتبر ممرضًا ثانويًا لمختلف أنواع الأمراض التي تسبب خسائر اقتصادية فادحة في قطاع صناعة الدواجن. لذلك، كان إجمالي 18 قطيعًا من أصل 30 قطيعًا من الدجاج اللاحم (60٪) إيجابيًا لعدوى الليكروب القولوني. وعلى أساس نمو علي منبت الأحمر الكونغولي (CR)، تم تحديد 15 عتره من أصل 18 على أنها بكتيريا إي كولاي المسببة للأمراض (83.3%) وظهرت 3 على أنها بكتيريا غير ممرضة (16.6%). تم إخضاع جميع عزلات الممرضة لاختبار الحساسية للمضادات الحيوية في المختبر وكانت العزلات اكثر مقاومة، وكانت الأنماط المصلية الأكثر شيوعا هي O91، O128، O78، O124، O2 وO44. كما تم إخضاع عشر عزلات من الميكروب القولوني من 10 قطعان مختلفة من دجاج اللاحم للتعرف الجزيئي علي جينات المقاومة والامراضيه باستخدام تفاعل البلمره المتسلسل (PCR). كانت هذه العترات مرتبطة بـEHEC ، وEPEC، وETEC، و EIEC. كما إن عزلات الميكروب القولوني كانت جميعا تحمل خاصيه المقاومة المتعددة للمضادات الحيويه (MDR) إلى المقاومة واسع النطاق (XDR). تم اكتشاف جينات الضراوه ومنها tsh وpapC وiss وiutA وhlyF في 50% من العزلات التي تحتوي على 5 جينات و50% منها تحتوي على 4 جينات. أظهرت جميع العزلات المختبرة جينات MDR، 60% من العزلات أظهرت 5 جينات و 20% أظهرت 4 جينات و 20% أظهرت 3 جينات. و بناء علي الدراسه الحاليه لا تزال بكتيريا *E. coil* تهدد صناعة الدواجن ويوصى بإجراء المزيد من الدراسات لإيجاد بدائل طبيعية آمنة للمضادات الحيوية للتغلب على سلالات MDR *E. coli* الموجودة جنبًا إلى جنب مع تقوية مناعة الطيور وتطبيق إجراءات صحية صارمة.

الكلمات المفتاحية: الايشيرشيا كولاي؛ حساسية المضادات الحيوية ؛ مقاومة الأدوية المتعددة PCR ؛ الجينات المقاومة؛ جينات الضراوة.

المؤلف المسؤل: هبه محمد سالم، البريد الإلكتروني: dr.hebasalem@cu.edu.eg