**MINAR** International Journal of Applied Sciences and Technology
ISSN: 2717-8234
Article type: Research Article

**Received:** 16/09/2023 **Accepted:** 01/11/2023 **Published:** 01/12/2023

# MOLECULAR IDENTIFICATION OF VIRULENCE PROPERTIES AND VIRULENCE-ASSOCIATED GENE (ISS) OF E. COLI ISOLATED FROM BROILER CHICKS

**Rawaa Banay ZUBAIRI**[1]

Southern Technical University– Iraq

**Zainab A. MUTTER**[2]

University of Basrah – Iraq

**Abstract**

The present study identified virulence genes for existence of virulence genes increased the serum survival (Iss) for determining the pathogenicity regarding E. coli in broiler chicks. The results indicated that 45 (36.7%) of the 120 samples collected from dead and morbid broiler chicks that have been infected with E. coli, and the difference between non-pathogenic and pathogenic was investigated. Hemolysin production, motility, and the agglutination test have been also shown to be positive. In vitro tests revealed that three isolates have been classed as moderately, highly, and slightly virulent, based on their pathogenicity features. Broiler chicks have been typified by the monovalent antisera had indicated that (31.74%) have been identified as E. coli serotype O2. Slightly and highly pathogenic isolates have been responsive to chloramphenicol, penicillin, gentamicin, and streptomycin, while moderately pathogenic isolates have been only susceptible to the gentamicin and chloramphenicol. The use of the PCR as a valid approach for detecting virulence genes resulted in an increase in serum survival (Iss) in 15 (65.2%) of the total sample**.**

**Keywords**: *Extraintestinal Disease, Poultry Health, Virulence Gene, Serotype, Fimbrial Adhesions.*

http://dx.doi.org/10.47832/2717-8234.17.15

[1] rawa@stu.edu.iq

[2] Zainb.mutter@uobasrah.edu.iq

## Introduction

*E. coli* can be defined as a bacterium found in both human and animal digestive systems. *E. coli* differs from the normal microbiota in the digestive tract of poultry, but colonization regarding the respiratory tract through pathogenic strains of *E. coli* is linked to the extra-intestinal disease. A few *E. coli* strains have virulence properties that are related to host tissue. Serogroups, colonization, toxin production, iron uptake systems, serum resistance, O antigene, defensins, LPS, some fimbrial adhesions, and the K capsule are only a few examples (3). Those strains, known as Avian Pathogenic *E. coli* (APEC), were found in retail foods such as pork, chicken, and turkey(1).

Colibacillosis can be defined as a serious bacterial disease that affects the poultry industry (5). In poultry farms, APEC is the most common cause of colibacillosis (1) . The contamination regarding the avian respiratory system with APEC produces respiratory tract sores and septicemia. Septicemia, airsacculitis, pneumonitis, and coli septicemia or colibacillosis are all symptoms of this infection (6).In these contaminations, the microscopic organisms can enter the circulation system and become fundamental bringing about colibacillosis (7). These days, avian colibacillosis is one of fundamental driver prompting mortality and morbidity in poultry (8,9)

## LITERATURE REVIEW

APEC is responsible for considerable financial losses in poultry productions (10) and was linked to the extra-intestinal disorders in the humans because UPEC and APEC share typical characteristics of virulence (11). The explicit DNA locales of APEC and the human extra-intestinal pathogenic *E. coli* (ExPEC) have shown grouping homology in subtractive hybridization experiments (12). Furthermore, existence of similar virulence genes in the ExPEC as well as APEC strains suggested that APEC strains could be acting like zoonotic pathogens and a repository of disease-causing bacteria(1).

On blood agar plates, hemolysin, released by most pathogenic strains of *E. coli*, generated cell-related lysin, which was recognized as an acceptable zone of lysis (13). The significance regarding hemolysin criterions, notably a-hemolysin, stems from the fact that it's important for inflammatory chief to IL-6 and chemotaxins emission, setting the tone for renal infection pathogenesis. Clinical researches have shown that *E. coli* virulence factors like the hemolysin production and capability to test germicidal activity of serum impact pathogenesis(14)

Two types of pilus which defined as virulent factor associated with pathogenic *E. coli*. Type I pili are found predominant in chicken infected with pathogenic *E. coli* rather than P pili (15). Early research linked 10 to 15 O-antigen–based serogroups (out of about 180 detected in *E. coli*) to human extraintestinal diseases. The growth of K and H antigen composition resulted in more precise targets (16). Antibiotics agents have been used more frequently in

the poultry and domesticated animal production industries for treating and avoiding contagious bacterial diseases, as well as development advertisers at sub-remedial levels in feeds, resulting in bacterial protection from antibiotics agents in recent years (17). Treatment of APEC contamination mostly depends on antibiotics;however, the expanding rise of medication obstruction makes treatment less effective (8)

O2, O1, and O78 are the most common serogroups linked to colibacillosis, and their prevalence varies by country and farm (18). Nonetheless, sero-groups don't represent virulence of strains, and so serotyping can't be used as beneficial analytic tool (19). The link between virulence and serotyping is also unclear, raising questionof whether *E. coli* contaminations in poultry should be viewed purely opportunistic. (20) The pathogenicity regarding *E. coli* strains is determined by many virulence-associated genes or pathogenicity. Recent research has focused on sharing and type of virulence-related genes, in addition to the link between virulence-related gene sharing and O serotypes (6). The key link between pathogenicity and serum obstruction was Iss protein. The membrane attack complex (MAC) statement is suppressed by the (Iss) gene (21). Interest in a variety of grouping arrangements and phylogenetic analysis of (ISS) gene revealed that this trait was extremely well conserved amongst most serogroups andstrains of APEC. Iss gene has been discovered in human septicemic *E. coli* isolate and has been found to be more prevalent in the isolates of theAPEC compared to it in isolates of *E. coli* from healthy birds, leading to the suggestion that (Iss) gene and its outer membrane protein item may be utilized in order to identify and regulate APEC. As a result, it could be a competitive gene for delivering a poultry colibacillosis immunization (22). The Iss gene has long been known for its role in the virulence of ExPEC. Iss was recognized as distinguishing feature of avian ExPEC, yet not human ExPEC. (23). The most widely accepted methodology for investigating ExPEC is genetic finding of virulence genes via PCR (2). Those genes are usually found on plasmids and could be transferred to other types of the bacteria through the conjugation. Col V plasmids, in particular, produce virulence genes like the ompT, hlyF, cvaC and ISS (24).

## EXPERIMENTAL PART

A total of 120 sterile cotton swabs have been taken from lesions (hock joint, pericardium, liver, crop, air sac, cellulitis infection, ceca, and intestines) dead and diseased broiler birds and inoculated in the Brain Heart Infusion (BHI) broth and oppressed for bacteriological examination on the MacConkey agar and incubated during the night at a temperature of 37°C for verifying lactose fermentation. In addition, lactose fermentation was thought to be positive in isolates (25). API-20E framework (Bio-MEIREUX) was used for performingbiochemical detection (26). Congo red restricting examination and hemolytic activity serotyped and established its virulence (27). On a separate plate, each one of the isolates was cultured and incubated at a temperature of 37°C, after incubation period. Congo Red (CR+) colonies that appeared red were regarded positive, while Congo Red (CR -) colonies which didn't tie the color and remained dark or white have been termed negative.

Motility test medium has been prepared as directed by Himedia manual instructions through the addition of5 grams of sodium chloride, 10 grams of the tryptose, and 5 grams of agar to 1L of distal water and heating till boiling. The pH has beenaltered to 7.20, the medium was loaded into screw-top jugs, autoclaved, then used right away. A bacterial culture was added to the motility test medium. Following an incubation period at a temperature of 37°C, the motility was measured. The occurrence related to diffuse development far from the inoculation line and appeared to extend from the place of inoculation into agar like painting brush revealed motility (28). The isolates of the *E. coli* have been propagated on the blood agar with 5% wahsed human blood erythrocytes for the hemolysis test. Colonies delivering obvious haemolysis zones have been registered as hemolysin positive after being cultured at a temperature of 37°C for 24 hours (29). The hemagglutination test was carried out as shown in (30). The presence of type 1, P fimbriae has been quickly identified by existence or lack of hemagglutination in absence or presence of mannose. Hemagglutination was identified by the clustering of erythrocytes through microbe fimbriae in the presence of d-mannose. The direct bacterial hemagglutination test-slide approach was used, as well as mannose-resistant (MRHA) and mannose-sensitive (MSHA) hemagglutination. For full fimbriation, *E. coli* strains have been inoculated into 1% supplement broth and incubated at a temperature of 37°C for 48 hours. Human blood group "O" red blood cells have been washed for 3 times in the normal saline and suspended in fresh saline at a concentration of 3%. They were all used at the same time. The testing has been conducted on concavity slide with multiple concavities. A drop of red platelets (RBCs) suspension has been added to stock culturedrop, and the slide has been shaken back and forth for 5 minutes at room temperature. The presence of clumping was considered a sign of hemagglutination. The MSHA has been identified by hemagglutination absence in parallel set where red cells were treated with drop of 2% w/v d-mannose (S.d.fine-Chem tech, Mumbai) and a drop of broth culture. The existence of hemagglutination of 3% "O" group human Red Blood Cells in presence of 2% mannose was used to detect MRHA.

Serological test was utilized for serotyping of *E.coli* O2 by utilizing Monovalent *E. coli* antisera (S.S.I,Denmark) to distinguish the somatic antigen and performed by the bearings of the maker: The *E.coli* isolate was grown on EMB agar medium not inhibit motility and approximately20 µl of antiserum was connected on a glass slide, at that point the culturewas moved to each drop of antiserum and mixed well. The measure of culture ought to be adequate to give an unmistakable smooth turbidity.The slide was tilted for (5-10) seconds. The response was perused with the naked eye through holding a slide in front of a source oflight against a dark foundation (indirect illumination). A positive response was viewed as an obvious agglutination. A negative response was industriousness of the homogenous milkyturbidity. A later or frail agglutination was considered as negative (29).

Antimicrobial susceptibility testing has been performed in accordance with the procedure for (31). Chloramphenicol, Ampicillin, Penicillin, Gentamicin, Streptomycin, and Vancomycin discs were used for disc diffusion tests. A pure culture has been sub-cultured on a nutrient agar in order to activate Bacteria and incubated at a temperature of 37°C for a

period between 18 and 24 hrs, after which 3-5 colonies have been diluted in 0.85% of the normal saline, and turbidity regarding activated growing culture has been changed to get turbidity nearly identical to 0.5 McFarland measures. To remove overload inoculums from the swab, sterile cotton swab has been submerged into balanced suspension and after that rotated a few times solidly within tube wall over liquid level. The plunging swab streaked the whole surface of a Mueller-Hinton agar plate. The anti-microbial discs have been dispensed on the outside of inoculated agar plate for the use of thedisc. Overnight, the plates were turned over and placed in a 37°C incubator. The inhibitory zone has been computed by measuring dishunderside. Results have been compared to Bioanalys Co.'s base hindrance measurement (Turkey). The CLSI principles were used to determine antimicrobial breakpoints and interpretation(32).

PCR Amplification of Virulence-Associated Genes Iss. Primer 5.0 programming was used to create the primers for PCR intensification (Table 1), which were based on recently distributed sequences (33). Nanjing GenScript Bio-innovation Co. (China) produced the primers. All of thestrains of E. coli and reference strains have been cultivated overnight on LB agar plates at a temperature of 37°C. colonies of *E. coli* have been suspended in 500µL of the deionized water then boiled for 10mins, then chilled on ice for 5 mins and centrifuged at 10000xg for 5mins, with supernatant utilized as a DNA template for amplification of thePCR. 10µL of 2x PCR Master blend (which includes2x Taq DNA polymerase, 2x dNTP blend and2x PCR Buffer) (TaKaRa), 1µL of the primer pair, 4µL of the template of the DNA, and 25 µL of deionized water Initial heat activation of five mins at a temperature of 95°C was followed by 30 cycles of 30secs at 94°C, 30secs at Tm, and 45 secs at 72°C, followed by 10 min extension at 72°C. PCR products have been sorted by size on 1% agarose gel electrophoresis with DL2000 DNA markers and seen on a UV transilluminator after staining with ethidium bromide (6).

Table1: primer sequences that have been utilized for the PCR amplification.

| Target genes | Sequences | size pb | |
|---|---|---|---|
| Iss | F:ATCACATAGGATTCTGCCG<br>R: CAGCGGAGTATAGATGCCA | 266 | (*Ewers et al., 2005*) |

**Results and Discussion**:

APEC strains result in a wide range of the illnesses in the birds and are responsible for massive financial losses in avian industry. Until yet, various studies were conducted to better understand the pathogenesis of APEC in order to perhaps design equipment that may prevent economic losses that are caused by such strains (34).*E. coli* was divided into over 50000 different serotypes, only some have a potential to cause disease due to their pathogenicity (29). *E. coli's* ability to result in extraintestinal illnesses is mostly dependent on some virulence factors that help to make due under unfavorable environments. Many methods for recognizing proof and detecting virulence to distinguish between non-pathogenic and pathogenic *E. coli*, serovars that include old-style phenotypic cultural confirming strategies and molecular approaches (14) that could also supply in as a new target in medication and vaccine development (35).

According to standard isolation and detection methodology, the general detect *E. coli* isolate was 45 (36.7%). This result has been consistent with those of both (36) who isolated isolates of *E. coli* from dead chickens with colisepticemia (28.3%) and (14) who used phenotypic virulence markers to determine that 100% of the isolates have been motile and 97% were hemolytic. In contrast to (20), who demonstrated that (63.60%) *E. coli* isolates have been recovered from 110 of the collected samples ofbroiler, 95% were Congo red positive with a mean of (36%). The CR binding activity test was used to distinguish between non-pathogenic and pathogenic *E. coli* recovered in this research, and results have revealed that 23 out of 45 (51.1%) isolates have been CR+. This result is consistent with (37) which isolates (44.29%) for Congo red binding activities of researched serotypes. On the other side, this resulted in disagreement with (38) people who were Congo red positive (95%).

The end result of the motility test revealed that 10/23 (43.4%) of the *E.coli* isolates have been motile. This assay has been used by *Zinnah et. al.,* (39) to classify *E. coli* isolates from various natural and ecological sources. In addition, all 448 *E. coli* isolates have been shown to be motile, mediated by flagella, which is one of the virulence determinants for *E. coli* pathogenicity. In contrast to (41) who found that (97%) of *E. coli* have beencapable of creating hemolysis, and (20) who found that (92.9%) Enterotoxigenic *E. coli* (ETEC) have been detected, the present work found that 8/23 (34.7%) of *E. coli* were able to produce hemolysis. In keeping with our findings (42) that 44.60% of clinical *E. coli* isolates have been hemolytic. Furthermore, hemolysin is frequently produced for more serious infections(43).

For hemagglutination test, the present investigation showed that only 3/23(13.04 %) of test was sure. This outcome was in agreement with Maurer *et.al.*,(44) and Knobl*etal*,(45) revealed that (26%)has demonstrated adhesion capability.And disagreementwith (46) who expressed that (73%) for adhesion.

The O2 monovalent antisera that were utilized in this researchexposed that 10 out of 23 tested isolates (43.47%) were distinguished as *E. coli* serotype O2,whereas the other 13(56.52%) isolate were uncertain and we considered them as untypable. This result similar

to investigation of Knobl *et.al.*, (2004),who revealed that the most common, which represent(62%) of strains. Serogroups O2, O21, and O78, regularly found in poultry influenced by the colibacillosis and disagreement what was recently distributed by (26) that Serotype O2 with(4.55%)many examinations proved that serotyping can't be utilized as a particular effectiveindicativetool, since it doesn't reproducestrains'virulence. It might be utilized distinctly to describe strains. APEC O78, O2 and O1 are the most common serogroups in numerous countries (11).

Exact pathogenesis of APEC isn't obviously characterized, and choice specific virulence factors are additionally not known for APEC control and, recognition is difficult (22).

Antimicrobial sensitivity testing of the three (mid, highly, and less) virulent *E. coli* isolates revealed that the slightly andhighly virulent isolates have been susceptible to Gentamicin, Chloramphenicol, Streptomycin andPenicillin, while the mid virulent isolate has been susceptible to the Gentamicin, Chloramphenicol,Penicillin, and Streptomycin. Table 2 shows the results.

Table (2):The results of antimicrobial sensitivity test of the three isolates.

| Antimicrobial agent | References | Highly virulent isolate | Mid virulent isolate | Slightly virulent isolate |
|---|---|---|---|---|
| Chloramphenicol | ≥ 18 | 29 | 30 | 30 |
| Ampicillin | ≥ 14 | 0 | 0 | 0 |
| Penicillin | ≥ 13 | 19 | 0 | 16 |
| Vancomycin | ≥1 | 0 | 0 | 0 |
| Streptomycin | ≥ 15 | 17 | 0 | 18 |
| Gentamicin | ≥ 15 | 20 | 20 | 20 |

This result was in agreement with that of (47) who expressed that antibiotics example different according various isolate, time and improvement of multiple drug resistance amongst various bacteria samples. In this manner, this can bring about the disappointment of the possibility of antibiotictreatment because of the occurrence of various isolate in the mean time. The best avian colibacillosis treatment that has been brought about by APEC strains for the most part relies upon the utilization of antimicrobials. In any case, increasing protection from seriously significant antimicrobials, for example, third-generation fluoroquinolones and cephalosporins is presently a day normal in *E. coli* from the source of thepoultry.These protections can be transmitted to people by means of the food supply. A few investigations have shown that the *E. coli* populace of broilersmay be transferred for antimicrobial resistance genesby mobile genetic elementto the people by food(1).

The present work used molecular apparatuses for detecting the (Iss) gene, whose role is to mediate the resistance of thebacteriato serum's complement. The results indicated that 15

(65.2%) of pathogenic *E. coli* have been positive for the studying gene. The frequency of the Iss that had been detected in this study has been in agreement with that found by Pfaff-McDonough et. al, (48) with (77%) and (20) (73.8%), respectively. The high frequency reveals the function of Iss, which allows *E. coli* to avoid host defense, increase, and distribute, thus advancing disease improvement(46).

*Clermont et al.* (49) discuss the possibility of commensal bacteria obtaining virulence genes. Dziva and Stevens virulence genes for Iss allow intestinal commensal *E. coli* to evolve into APEC, capable of contaminating extra-intestinal sites (36). The ISS quality has not been only more common in APEC than in AFEC, but it has been observed as well in different serotypes from a variety of the geographic areas (50).

This investigation also revealed presence of the virulence genes that have been linked with the APEC strains in the *E. coli*, which indicate the presence of potential reservoir of virulence-related genes in this population. Virulence-associated genes or pathogenicity islands have recently been discovered in a number of pathogenic bacteria, and this could be linked to development of the bacterial virulence.

**Conclusion:**

● In poultry, *E. coli* is a typical part of the microbiota, and pathogenic *E. coli* strains colonizing respiratory tract are linked to extraintestinal illness.

● *E. coli's* capability to produce extraintestinal infections is mostly dependent on various virulence factors that allow the bacteria survive in harsh environments.

● O2, O1, and O78 are the most common serogroups linked to colibacillosis.

● The first link between pathogenicity and serum resistance was the Iss protein.

● Using antimicrobials is crucial in treating avian colibacillosisthat results from the strains ofAPEC.

**REFERENCES**

[1] Jiddu Joseph, Li Zhang , Pratima Adhikari , Jeffrey D. Evans, Reshma Ramachandran . Avian Pathogenic Escherichia coli (APEC) in Broiler Breeders:An Overview2023, Pathogens 2023, 12, 1280. https://doi.org/10.3390/pathogens12111280.

[2] Hejair A, Zhu Y, Ma J, ZhangY, Pan Z, Zhang W, Yao H. Functional role of ompF and ompC porins in pathogenesis of avian pathogenic *Escherichia coli*. Microbial Pathogenesis.2017,107:29–37.

[3] LeStrange K, Markland M, Hoover G, Sharma M, KnielE. An evaluation of the virulence and adherence properties of avian pathogenic Escherichia coli. One Health2017,4: 22–26.

[4] Kathayat, D., D. Lokesh, S. Ranjit, and G. Rajashekara. 2021. Avian Pathogenic Escherichia coli(APEC): An Overview of Virulence and Pathogenesis Factors, Zoonotic Potential, andControl Strategies. Pathogens. 10:467

[5] Min Wang, Min Jiang, Zhongxing Wang, Rui Chen, Xiangkai Zhuge, , Jianjun Dai. Characterization of antimicrobial resistance in chicken-source phylogroup F Escherichia coli: similar populations and resistance spectrums between E. coli recovered from chicken colibacillosis tissues and retail raw meats in Eastern China. Poult Sci. 2021 Sep; 100(9): 101370..

[6] Sofie Kromann & Henrik Elvang Jensen. In vivo models of Escherichia coli infection in poultry Acta Veterinaria Scandinavica .2022. 64:33

[7] https://doi.org/10.1186/s13028-022-00652-z

[8] Lianci P, Mieke M, Henk H, Edwin V.Avian pathogenic Escherichia coli-induced activation of chicken macrophage HD11cells.Developmental and Comparative Immunology.2018, 87: 75–83.

[9] Priscila G, Ana HF, Susanne P, Shahana A, Dan W, da T, Fabio A, John O.The membrane transporter PotE is required for virulence in avian pathogenic Escherichia coli (APEC).Vet. Micro.2018, 216: 38–44.

[10] Fernanda M. Coura , Soraia A. Diniz , Marcos X. Silva , Thiago L. M. Arcebismo , Silvia Minharro , Adriana C. F. Feitosa , Andrey P. Lage , Terezinha Knöbl , Jamili Maria Suhet Mussi , Marcos B. Heinemann . Phylogenetic Group of Escherichia coli Isolates from Broilers in Brazilian Poultry Slaughterhouse. The Scientific World Journal; Cairo Vol. 2017, (2017). DOI:10.1155/2017/5898701

[11] Dalia M. Sedeek, Mohamed M. Rady, Hanaa S. Fedawy, Nagwa S. Rabie. Molecular Epidemiology and Sequencing of Avian Pathogenic Escherichia coli APEC in Egypt. Adv. Anim.2020. Vet. Sci. 8(5): 499-505

[12] deCampos A, LagoC, Nakazato G, Stehling EG, Brocchi M, deCastro FP. Occurrence of virulence-related sequences and phylogenetic analysis of commensal and pathogenic avian Escherichia coli strain (APEC). Pesq Vet Bras.2008,28: 533–40.

[13] Saiful Islam, Mehedi Hasan Nayeem , Abdus Sobur, Samina Ievy, Amirul Islam,Saifur Rahman , Abdul Kafi, Hossam M. Ashour, Tanvir Rahman. Virulence Determinants and Multidrug Resistance of Escherichia coli Isolated from Migratory Birds Antibiotics 2021, 10(2), 190; https://doi.org/10.3390/antibiotics10020190

[14] OsmanM, HessainM, Abo-shama Usama H, GirhM,KabliA, Hemeg A, MoussaM. An alternative approach for evaluating the phenotypic virulence factors of pathogenic Escherichia coli. Saudi Journal of Biological Sciences.2018, 25(2): 195–197.

[15] Joseph J., M. Jennings, N. Barbieri, L. Zhang, P. Adhikari, and R. Ramachandran..Characterization of Avian Pathogenic Escherichia coli Isolated from Broiler Breederswith Colibacillosis in Mississippi. Poultry.2023. 2:24-39.

[16] Michael Kowarik, Michael Wetter, Micha A. Haeuptle, Martin Braun, Michael Steffen, Stefan Kemmler, Neil Ravenscroft, Gianluigi De Benedetto, Matthias Zuppiger, Dominique Sirena, Paola Cescutti, Michael Wacker. The development and characterization of an E. coli O25B bioconjugate vaccine. Glycoconj J. 2021; 38(4): 421–435.

[17] Awad, A. M., N. A. El-shall, D. S. Khalil, M. E. A. El-hack, A. A. Swelum, A. H. Mahmoud, H.Ebaid, A. Komany, R. H. Sammour, and M. E. Sedeik. 2020. Incidence, pathotyping, andantibiotic susceptibility of Avian Pathogenic Escherichia coli among diseased broilerchicks. Pathogens. 9: 114.

[18] Jiangang Hu, Dossêh Jean Apôtre Afayibo, Beibei Zhang, Hong Zhu, Lan Yao, Weiqi Guo, Xinyu Wang, Zhiyang Wang, Di Wang, Haoheng Peng, Mingxing Tian, Jingjing Qi, Shaohui Wang.Characteristics, pathogenic mechanism, zoonotic potential, drug resistance, and prevention of avian pathogenic Escherichia coli (APEC) Front Microbiol. 2022; 13: 1049391.

[19] Eman Jassim Mohammed,Mohammed Allami, Mohammad Reza Sharifmoghadam ,Masoumeh Bahrei. Relationship Between Antibiotic Resistance Patterns and O-Serogroups in Uropathogenic Escherichia coli Strains Isolated from Iraqi Patients. 2021. Jundishapur Journal of Microbiology: Vol.14, issue 8; e118833

[20] Al-ArfajA, Ali S, HessainM, Zakri M, Dawoud M, Al-MaaryS,MoussaM.Phenotypic and genotypic analysis of pathogenic Escherichia coli virulence genes recovered from Riyadh, Saudi Arabia. Saudi Journal of Biological Sciences.2016, 23(6): 713–717.

[21] Dvora Biran, Thomas Sura, Andreas Otto, Yael Yair, Dörte Becher, Eliora Z. Surviving Serum: the Escherichia coli iss Gene of Extraintestinal Pathogenic E. coli Is Required for the Synthesis of Group 4 Capsule. Infect Immun. 2021 Oct; 89(10): e00316-21.

[22] Zahraei Salehi T, DerakhshandehA, Tadjbakhsh H, Karimi V.Comparison and phylogenetic analysis of the ISS gene in two predominant avian pathogenic E. coli serogroups isolated from avian colibacillosis in Iran. Research in Veterinary Science.2013, 94(1): 5–8.

[23] Timothy J, Yvonne M, Lisa N.Evolution of the iss Gene in Escherichia coli.Applied and environmentalmicrobiology. 2008,74: 2360–2369.

[24] Johnson J, Nolan K. Pathogenomics of the virulence plasmids of Escherichia coli. Microbiology and molecularbiology reviews. 2009, 73:750–74.

[25] Tana, K, Elena C, Pranvera A,Sonila O,Antonio C. Serotyping and Biotype Prevalence of Avian Pathogenic Escherichia coli in Albanian Poultry Industry. IV Int.Sym.2013, (10): 1098-1104.

[26] Ismail Raheel,Ahmed Orabi, Ahmed Erfan,Mohamed Ashery Raslan,Shaimaa Hassan Abd ElWahab,and Eman A. A. Mohamed. Intestinal Tract of Broiler Chickens as a Reservoir of Potentially Pathogenic Curli Producing ESβL Escherichia coli. Int.J. Microbiol.Res. 2022. International Journal of Veterinary Science 11(4): 498-503.

[27] Azza A, AL Hussien D, Abd El-Mawgoudc A.Pathogenicity of Escherichia coli O157 in commercial broiler chickens.Beni-Suef University Journal of Basic and Applied Sciences. 2018,7:620–625.

[28] Abdullahi M. Incidence and antimicrobial susceptibility pattern of Salmonella species in children attending some hospitals in kano metropolis, Kano state –Nigeria.Bay. J. Pu.App. Sci. 2010,(1): 202 – 206.

[29] Kathayat, D., D. Lokesh, S. Ranjit,G. R ajashekara. 2021. Avian Pathogenic Escherichia coli(APEC): An Overview of Virulence and Pathogenesis Factors, Zoonotic Potential, and Control Strategies. Pathogens. 10:467

[30] Awad, A. M., N. A. El-shall, D. S. Khalil, M. E. A. El-hack, A. A. Swelum, A. H. Mahmoud, H. Ebaid, A. Komany, R. H. Sammour, and M. E. Sedeik. 2020. Incidence, pathotyping, and antibiotic susceptibility of Avian Pathogenic Escherichia coli among diseased broiler chicks. Pathogens. 9: 114

[31] Victoria O, Ismail O. Antibiogram profiles of Escherichia coli, Salmonella and Listeria Species along the processing line of sale frozen poultry foods. Res.J. Microbiol. 2012,7 (4):235-241.

[32] CLSI: Clinical and Laboratory Standards Institute. Performance Standards for antimicrobial susceptibility testing, twentieth informational supplement,2012, CLSI document M100-S20, Wayne, PA.

[33] JanbenT, Schwarz C, Preikschat P, Voss M, Philipp C, Wieler H. Virulence-Associated Genes in Avian Pathogenic Escherichia coli (APEC) Isolated from Internal Organs of Poultry Having Died from Colibacillo- sis. International Journal of Medical Microbiology.2001, 291: 371-378.

[34] CDC, Centers for Disease Control and Prevention Escherichia coli (E. coli) 1600 Clifton Road Atlanta, GA 30329-4027, USA, 800-CDC-INFO (800–232-4636), TTY: 888-232-6348 Email CDC-INFO April 16, 2015.

[35] Valerio M. Sora, Gabriele Meroni, Piera A. Martino, Alessio Soggiu, Luigi Bonizzi, and Alfonso Zecconi. Extraintestinal Pathogenic Escherichia coli: Virulence Factors and Antibiotic Resistance . Pathogens. 2021 Nov; 10(11): 1355.

[36] MacielF, MatterB, Trindade M, Camillo G, LovatoM, de Ávila BottonS, Castagna de Vargas A. Virulence factors and antimicrobial susceptibility profile of extraintestinal Escherichia coli isolated from an avian colisepticemia outbreak. Microbial Pathogenesis.2017, 103: 119–122.

[37] EmeryA, NagarajaV, ShawP, NewmanA, WhiteG ز Virulence factors of Escherichia coli associated with colisepticemia in chickens and turkeys. Avian Dis.1992 و36: 504– 511.

[38] AliZ.Isolation andIdentification of Avian Pathogenic E. coli O78:K80 Serotype from broilers and study of its experimental infection. Department of Pathology and Poultry Diseases,College of Veterinary Medicine, Basra University, Basra, Iraq. (2013).

[39] ZinnahA,Bari R,Islam T,Hussain T,Rahman T,Haque H, Babu M, Ruma P,Islam A. Characterization of Escherichia coli isolated from samples of different biological and environmental sources. Bangl.J.Vet.Med.2007, 5(1 and 2):25-32.

[40] Kao Y, Lin H, Tseng C, Wu.B, Wang C, Wu J. The complex interplay among bacterial motility and virulence factors in different Escherichia coli infections. Eur. J. Clin. Microbiol. Infect. Dis.2014, 33:2157–2162.

[41] Fatima N, AgrawalM, ShuklaI, KhanP.A. Characterization of uropathogenic E. coli in relation to virulence factors. Sci. Rep2012, 1, 342. doi: 10.4172.

[42] Fakruddin D, Reaz M,Abhijit C,Khanjada S. Bin Mannan.A preliminary study on virulence factors and antimicrobial resistance in extra-intestinal pathogenic Escherichia coli (ExPEC) in Bangladesh.Indian. J. Med.2013,137: 988-990.

[43] Johnson R. J. Virulence factors in Escherichia coli urinary tract infection. Clin. Microbiol. Rev.1991, (4): 80-128.

[44] Maurer JJ, Brown TP, Steffens WL, Trayer SG. The occurrence of ambient temperature-regulated adhesions, curli and the temperature-sensitive hemagglutinin tsh among avian Escherichia coli. Avian Dis.1998,42: 106-18.

[45] Knöbl T, Moreno AM, Paixao R, Tardelli T, Midolli M. (2011) Prevalence of avian pathogenic Escherichia coli (APEC) clone harboring sfa gene in Brazil. Sci World J.

[46] López M, Serrano Q, DelgadoM, RodríguezV, Olague-Marchán M, Rodríguez S, Luna L, de la Torre F, Santoyo R. Genes of Virulence and Phylogenetic Group in Isolates of Avian Pathogenic Escherichia coli. Arch Med.2017, 9:6:5.

[47] ShardaS, Wilfred R,ThiyageeswaranM.Isolation,Characterization and Antibiotic Resistance Pattern of Escherichia coli Isolated from poultry. Am.-Eu. J.Sci. Res.2010, 5 (1):18-22.

[48] Pfaff-McdonoughJ, Horne M, GiddingsW, EbertO, Doetkott C, SmithH, Nolan K. Complement resistance-related traits among Escherichia coli isolates from apparently healthy birds and birds with colibacillosis. Avian Dis.2000, 44:23-33.

[49] Clermont O,Bonacorsi S,BingenE.Rapid and Simple Determination of the Escherichia coli phylogenetic group, Appl. Environ. Microbiol. 2000,66: 4555-4558.

[50] RodriguezSiekE, GiddingsW, DoetkottC, JohnsonJ, NolanK. Characterizing the APEC pathotype. Veterinary Research.2005,36:241–256.