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# Isolation and molecular detection of some virulence associated genes in avian pathogenic *E. coli*

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**Abstract**

There are 13 virulence-related genes in *E. coli* isolates. The10 genes of these isolates were selected from avian pathogenic *E. coli* in some Iraqi broiler farms. Six of these virulence-related genes (*iroN*, *iucC*, *frz operon*, *iucD*, *papC*, and *R4*) were investigated in these isolates by PCR. Eighty percent of the isolates had one or more virulence-associated genes. Two APEC separates carried just one gene, *iroN* or *iucC*. According to preliminary evidence, the *iroN* and *iucC* genes may express their pathogenicity independently. All of the strains had the same *iroN* gene, making them all pathogenic. The results of these isolates were confirmed by PCR to have the six pathogenic genes: 80% positive for *iucC*, 50% positive for *iucD*, 100% positive for *iroN*, 10% positive for *frz operon*, 10% positive for *papC*, and 0% positive for *R4* respectively. These six virulence genes were detected with different percentages in isolates; the *iroN* gene was found in all isolates but the other virulence genes were found with different percentages in *E. coli* isolates. According to, detection the *iroN* and such genes are displaying their pathogenicity separately from each other.

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## Introduction

In chicken farms, *Escherichia coli* is a chief hazard (1). Many strains of *E. coli* can be classified into entero-toxigenic, entero-pathogenic, and entero-hemorrhagic types, according to (2,3). The *E. coli* infections have widespread in Iraqi farms and caused many diseases by *E. coli* only or other associated poultry diseases and were detected *E. coli* through several molecular methods 16Sr RNA and VITEK (4). Also, some avian pathogenic *E. coli* were classified according to Individual Pathogenicity Index (IPI), bacteremia death time (BTd), lesion as (pericarditis, per hepatitis, peritonitis, airsaculitis and cellulitis (5,6). Poultry and other bird species were infected by the most virulent strain of (APECs) *E. coli* infections, particularly in young broilers, and continue to be major sources of financial loss in poultry farms due to decreased egg production and a high mortality rate (2,7,8). *E. coli* can cause septicemia, liver and spleen enlargement, and necrosis of the intestines obviously it depends on the chicken's age (9). Acute airsacculitis, pericarditis, pneumonia, and arthritis are also symptoms of the subacute phase (4,6). The virulence factors of *E. coli* Multiple genes from the nucleus and plasmids are required (6). There are at least 13 genes related with virulence in the most isolated members of APEC (10,11). According to a molecular study, APEC isolates' virulence factors may be polygenic or individual, depending on how common they are (12,13). For one thing, no one knows exactly how many separate genes work together to cause disease in APECs, and the relationships among them are rarely predictable (14,15). According to the investigations, APEC is a diverse strain in all groups, since it is uncommon for the virulence genes to

be found in all isolates at once. *Iss*, *tsh*, *IUCC*, *CVI*, *IUTA*, *HLYF*, and *OmpT* can be detected utilizing PCR methods for APEC pathogenicity genes (10,16).

This study aimed to used PCR to identify six APEC virulence genes, including *iucC*, *froz operon*, *iucD*, *papC*, and *R4*, to identify multiple Iraqi strains of the avian pathogenic *E. coli* that were found in the current investigation. According to research data, the genes that are frequently found in other countries have been selected (17,18).

## Materials and methods

The six virulence genes, *iroN*, *iucC*, *frz operon*, *iucD*, *papC*, and *R4*, was examined in 10 strains of *Escherichia coli*. All of the samples tested were from Baghdad province/ Iraq. Avian colibacillosis cases in which the disease was identified in broiler of different ages have yielded several of these strains (19,20). Samples of broiler and layer birds were collected from a variety of holding facilities. As a precaution, the *Escherichia coli* strains were cultivated on Eosin-Methylene Blue (EMB) agar, MacConkey agar, and nutritional broth for 24 hours. The *Escherichia coli* strains were cultivated in brain heart broth (BHI). The culture was transferred to MacConkey agar plates after a day of incubation at 37°C. After that, the colonies were harvested and kept at a temperature of -20°C. As instructed by the manufacturer, the QIAamp Cador Mini Kit (Qiagen, Dusseldorf, Germany) the primers were used to amplify the DNA and to identify *E. coli* virulence genes isolated by the manufacturer (21,22) (Table 1).

### Ethical approval

Write the name of the scientific or institutional board that give the ethical approval to conduct this scientific work and give the approval issue number and date.

Table 1: Primers sequences used in PCR reaction used for amplification related six genes' fragments and predictable size

| Primer name | Primer sequence (5′-3′) | Product size (bp) |
|---|---|---|
| *iroN* | F -AAGTCAAAGCAGGGGTTGCCCG<br>R - GATCGCCGACATTAAGACGCAG | 667 |
| *iucC* | F - CGCCGTGGCTGGGGTAAG<br>R - CAGCCGGTTCACCAAGTATCACTG | 541 |
| *Frz operon* | F - GAGTCCTGGCTTGCGCCGTT<br>R - CCGCTCCATCGCAGCCTGAA | 843 |
| *IucD* | F - ACAAAAAGTTCTATCGCTCC<br>R - CCTGATCCAGAT GATGCT C | 714 |
| *papC* | F - TGATATCACGCAGTCAGTAGC<br>R - CCG GCCATATTCACATAA | 501 |
| *R4* | F - TGCCATACTTTATTCATCA<br>R - TGGAATGATGTGGCGTTAT | 699 |

bp = base pair, F=forward primer, R= reverse primer (19,20).

## Results

Six virulence genes were found in the PCR analysis of ten *E. coli* isolates, and these genes were then assigned to specific pathotypes based on the results (*iroN*, *iucC*, frzOperon, *iucD*, *papC*, and *R4*). In the ten strains of ill birds studied, *iroN*N was found to be 100% positive, *IUCC* was found to be 80% positive, *iucD* was found to be 50% positive, *frz operon* was found to be 10% positive, and *R4* was found to be 0% positive. The virulence genes are critical to a bacterium's autonomy and pathogenicity towards chickens, and each gene confers a specific trait and virulence on the bacteria it is present in. When the *IroN* gene has been activated, it promotes *iroN* chelation in the host (Table 2).

Table 2: Six genes for *Escherichia coli* were found in positive and negative strains for ten samples

| Detected genes | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 IUC C (641 bp) | + | + | - | + | + | + | + | - | + | + |
| 2 IUC D (714 bp) | - | + | - | - | + | + | - | + | - | + |
| 3 *IRON* N (667 bp) | + | + | + | + | + | + | + | + | + | + |
| 4 *FRZ OPERON* (843 bp) | - | + | - | - | - | - | - | - | - | - |
| 5 pap C (501 bp) | - | - | - | - | - | - | - | - | - | + |
| 6 *R4* (699 bp) | - | - | - | - | - | - | - | - | - | - |

The electrophoretic patterns of PCR products for each gene tested positive in nine strains were consistent with the expected size of the associated base pairs (bp), but the bacteria did not have the all tenth virulence genes since no amplicon was detected only sixth virulence gene (Figures 1-6).

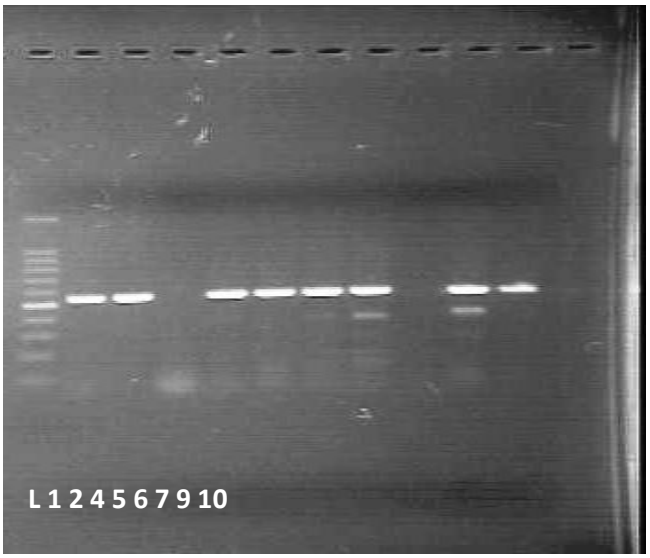


Figure 1: Detection of *iucC* gene in samples (1-10). Positive samples produce band (641 bp); lane M: 1Kb DNA Ladder; lanes 1, 2, 4, 5, 6, 7, 9 &10 were positive samples.

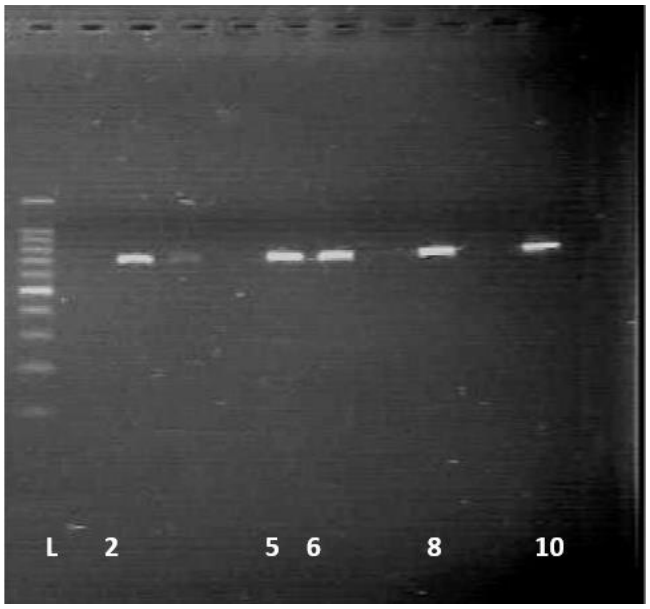


Figure 2: Detection of iuc D gene in samples (1-10). Positive samples produce band (714 bp); lane M: 1Kb DNA Ladder; lanes 2, 5 ,6, 8 & 10 were positive samples.

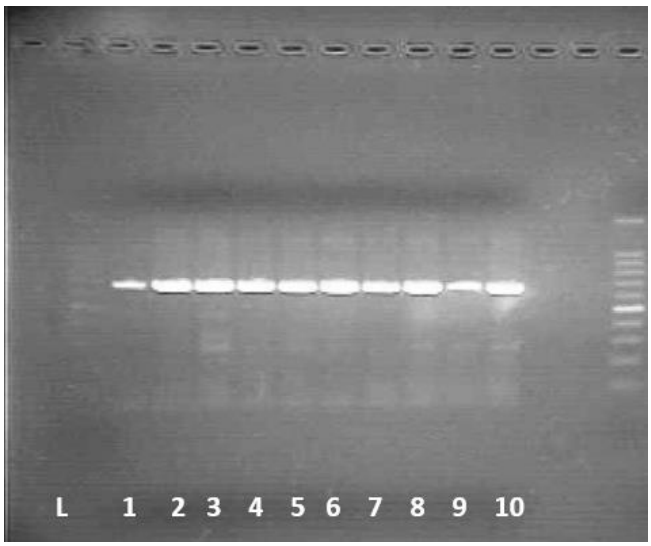


Figure 3: Detection of *iroN* gene in samples (1-10). Positive samples produce band (667 bp); lane M: 1 Kb DNA Ladder; all lanes were positive samples.

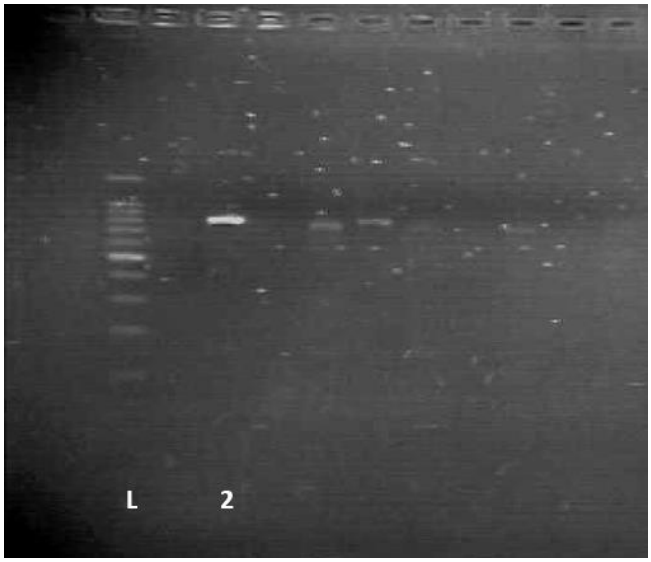


Figure 4: Detection of *frz operon* gene in samples (1-10). Positive samples produce band (843 bp); lane M: 1 Kb DNA Ladder; only lane 2 was positive sample.

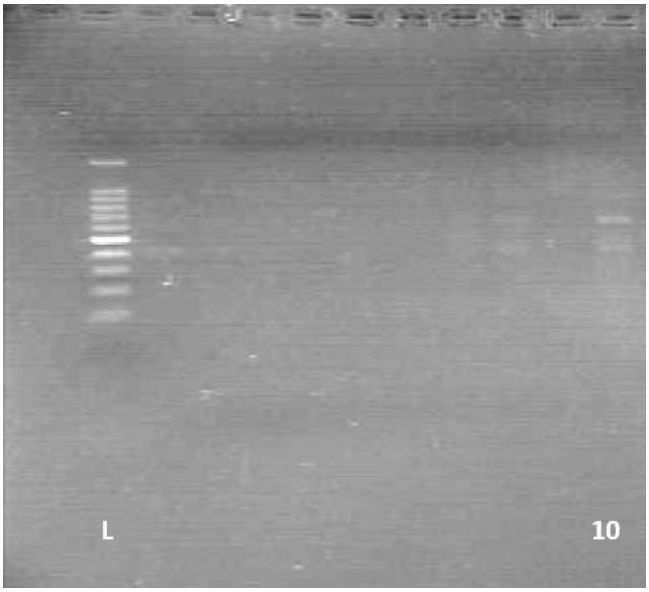


Figure 5: Detection of *papC* gene in samples (1-10). Positive samples produce band (501 bp); lane M: 1 Kb DNA Ladder; only lane 10 was positive sample.

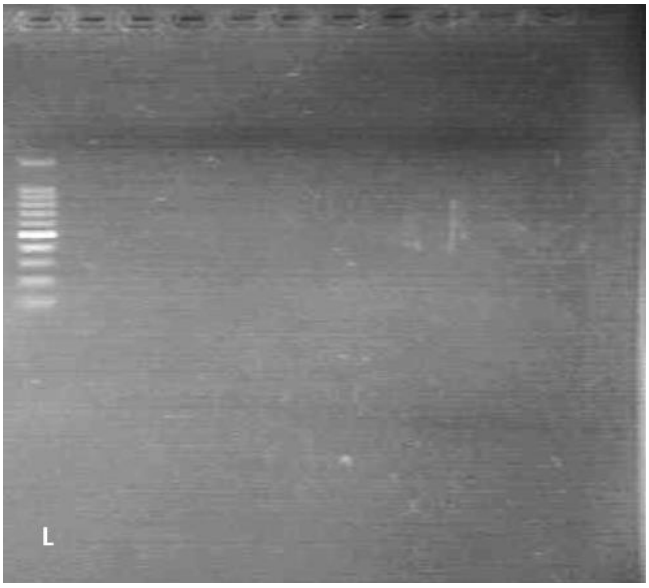


Figure 6: Detection of *R4* gene in samples (1-10). Positive samples produce band (699 bp); lane M: 1 Kb DNA Ladder; lanes were negative samples

**Discussion**

According to the result of PCR detection with different genes showed each gene responsible on target to the pathogenicity of *E. coli* pathways like, the *iucC* gene had the *iroN* acquisition, as well as had two sites (sitA and FeoB) which all contribute to *iroN* acquisition for bacteria (23-25). While it has been demonstrated that the frz gene increases productivity in serum under oxygen-restricted conditions (26). Also, the ColV-*iucD* K30 gene act as an enzyme that generates N6-hydroxylysine is encoded, which encodes a membrane-bound enzyme in *Escherichia coli* (27). In order to aid in the formation of pili, the *PapC* gene produces a channel in the outer membrane of the bacteria (28,29). R1-*R4* and K-12 are the five identified as outer core oligosaccharide (core OS) structures in *E. coli*, and the *R4* gene playing the most important function in the outer membrane integrity of the organisms (20).

Two APEC isolates of the same breed and age were obtained from outbreaks, but neither virulence gene was present (30). Our findings support the idea that not all pathogenic genes are present in all *Escherichia coli* strains, in accordance with previous studies that indicated that varied *E. coli* strains included distinct virulent genes (31,32).

current research showed more than 80% of strains have both the *IroN* and *iucC* pathogenicity genes. Research backs up this claim. in eastern China, some provinces were the sources of 71% of the APEC isolates (33). The percentages of *iucC* and *iroN* genes found in ten of the thirty-five strains examined were 28.57% and 54.28%, respectively (31). Also, ten strain were detected both *iucC* and *iroN* genes in Eastern Europe (34). Because the amount of APEC strains with highly pathogenic *iroN* and *iucC* genes varies by region, not all APEC strains have the same level of pathogenicity. When the chicken was exposed to stress factors such as other diseases, the env*iroN*ment, or age-related variables, the pathogenicity of APEC will variable, the low pathogenic isolate change to high pathogenic (35,36).

It was discovered that avian pathogenic *Escherichia coli* in the intestinal tract and serum may be reduced by 10% thanks to the *frz operon*'s ability to promote bacterial competence under stressful conditions such as oxygen restriction, which was demonstrated in a study of the *frz operon* (37).

The *papC* gene percentage was 10% (1/10), which indicates that a low pathogenic strain of APEC was responsible for the septicemia that infected many organs later on in the infectious process (38). In, contrast prior research showed that 33.33 % of the *papC* gene was found in *E. coli* isolates, which was higher than the average (39).

The *Escherichia coli iucD*, the gene encodes a membrane-bound enzyme capable of producing N6-hydroxylysine enzyme. Also, according to Paixao's findings in 2016, other genes like *iucD*, chuA, and fyuA assemblies were detected in APEC isolates, In addition, *E. coli iucD* gene isolates with a high percentage (40). The *E. coli R4* virulence gene was lipopolysaccharides that include five distinct oligosaccharide cores (core OS), Also the *R4* virulence genes were not found in isolates; the results showed differ from prior studies about the *R4* gene was

detected with the variable percentage in different isolates especially, in *Escherichia coli* O157:H7 (41,42).

## Conclusions

All isolates have 5 Virulence genes, as well as, all isolates lack *R4* gene, so all 10 isolates possess 5 V. genes but not an *R4* gene. Only one gene, *iroN*, was identified in one of the APEC isolates, these bacteria are less dangerous than those with many genes (10 percent). Not every virulence gene was present in all *Escherichia coli* strains, and not every pathogenic gene was present in all *Escherichia coli* strains. According to preliminary findings, the *iroN* and *iucC* genes display their pathogenicity in separate ways.

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## Conflict of interest

No conflict.

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# عزل وتوصيف جزيئي لبعض جينات الضراوة المرتبطة بجرثومة الاشريشكيا الضارية للطيور


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## الخلاصة

هناك ١٣ جينا مرتبطا بالضراوة في عزلات جرثومة الاشريكية القولونية اختيرت ١٠ عزلات لجرثومة الاشريكية القولونية المرضية الطيرية التي تم جمعها من حالات الاصابة بالاشريكية القولونية في حقول فروج اللحم. اجري التحري عن ٦ جينات مرتبطة بالضراوة (*iroN*، *iucC*، *frz operon*، *iucD*، *papC* و *R4* ) في هذه العزلات بواسطة اختبار تفاعل البوليمير المتسلسل. ثمانون في المائة من العزلات تحتوي على جينات مرتبطة بالضراوة وان جميع عزلات جرثومة الاشريكية القولونية تحمل جينًا واحدًا فقط من، *iroN* أو *iucC*. لذلك، قد تعبر جينات *iroN* و / أو *iucC* عن قدرتها المرضية بشكل مستقل. كل السلالات لديها نفس الجين *iroN*، مما يجعلها كلها مسببة للأمراض. تم تأكيد نتائج هذه العزلات بواسطة تفاعل البوليميراز المتسلسل على أنها تحتوي على الجينات الستة المسببة للأمراض وكانت كالاتي: ٨٠٪ إيجابية لـ *iucC*، و٥٠٪ إيجابية لـ *iucD*، و١٠٠٪ إيجابية للحديد N، و١٠٪ موجبة لـ *frz operon*، و١٠٪ إيجابية لـ *papC*، و٠٪ إيجابية لـ *R4* على التوالي. تم الكشف عن هذه جينات الضراوة الستة بنسب مختلفة في العزلات وتم العثور على جين *iroN* في جميع العزلات ولكن تم العثور على جينات الضراوة الأخرى بنسب مختلفة في عزلات الإشريكية القولونية. ووفقا لذلك يبدو أن جينات *iroN* و*iucC* تُظهِر قدرتها المرضية بشكل منفصل عن بعضها البعض.