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

M.H. Hasan¹, S.M. Abdulla² and A.H. Ulaiwi²

¹Department of Microbiology, College of Veterinary Medicine, University of Thi-Qar, Thi-Qar, ²Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

**Abstract**

There are 13 virulence-related genes in *E. coli* isolates. The 10 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.

**Keywords**: Avian Pathogenic *E. coli* Virulence Gene PCR

**Article information**

**Article history:**
Received December 17, 2021
Accepted July 04, 2022
Available online July 04, 2022

**Keywords:**
Avian Pathogenic *E. coli* Virulence Gene PCR

**Correspondence:**
A.H. Ulaiwi
amjed.h@covm.uobaghdad.edu.iq

**Introduction**

In chicken farms, *Escherichia coli* is a chief hazard (1). Many strains of *E. coli* can be classified into enterotoxigenic, 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, airsacculitis 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, IUC, 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, frz 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.

**Primer sequences used in PCR reaction used for amplification related six genes' fragments and predictable size**

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence (5'-3')</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>iroN</em></td>
<td>F - AAGTCAAA GCAGGGTTGCCC</td>
<td>667</td>
</tr>
<tr>
<td></td>
<td>R - GATCGCCGCACTTAAGACGCAG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F - CGCCGTGGCTGGGGTAAG</td>
<td></td>
</tr>
<tr>
<td><em>iucC</em></td>
<td>R - CAGCCGGGTTACCAATGATCCTG</td>
<td>541</td>
</tr>
<tr>
<td>*Frz operon</td>
<td>F - GAGTCCTGGCTGGCCGTT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R - CCGCTTCTCGCAGCCTGA</td>
<td>843</td>
</tr>
<tr>
<td><em>iucD</em></td>
<td>F - ACAAAGTITCATCGCTCC</td>
<td>714</td>
</tr>
<tr>
<td><em>papC</em></td>
<td>R - CCTGATCCAGATGATGCTC</td>
<td></td>
</tr>
<tr>
<td><em>R4</em></td>
<td>F - TGATATCAGCAGTCAGTACGC</td>
<td>501</td>
</tr>
<tr>
<td></td>
<td>R - CCG GCCATATTTACAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F - TGCCATACTTTATTACATCA</td>
<td>699</td>
</tr>
<tr>
<td></td>
<td>R - TGGATGATGTCGCCGTAT</td>
<td></td>
</tr>
</tbody>
</table>

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, *iroNN* was found to be 100% positive, *IUC* 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**

<table>
<thead>
<tr>
<th>Detected genes</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
<th>S8</th>
<th>S9</th>
<th>S10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  IUC C (641 bp)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2  IUC D (714 bp)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3  IRO N (667 bp)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4  FRZ OPERON (843 bp)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
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 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.
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 environment, 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.

Acknowledgments

This study supported by Authors, University of Baghdad and Thi-Qar.

Conflict of interest

No conflict.

References

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## عزل وتوصيف جزيئي لبعض جينات الضراوة المرتبطة بجرثومة الأشريكية الضارية الطيور

**ماجد حمد حسن**

*، سمير ماهر عبد الله* و *أحمد حسين عليوي?

أفرع الأحياء المجهرية، كلية الطب البيطري، جامعة ذي قار، ذي قار، العراق

أفرع الأمراض وأمراض الدواجن، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

**الخليفة**

هناك 13 جينًا مرتبطة بالضراوة في عزلات جرثومة الأشريكية الطيور، حيث تمَّ تأكيد قدراتها المرضية بشكل منفصل في جميع العزلات ولكن تم العثور على جينات الضراوة الستة بنسب مختلفة في العزلات وتم الكشف عن هذه جينات الضراوة في العزلات بواسطة تفاعل البوليمير المتسلسل على أنها تحتوي على هذه العزلات بواسطة 80٪ من العزلات تحتوي على جينات الضراوة المرتبطة بالضراوة و100٪ إيجابية للجينات *iucD* و *iucC* معاً، مما يجعلها كلاً مسببة للأمراض. تم تأكيد نتائج هذه العزلات بواسطة تفاعل البوليمير المتسلسل على أنها تحتوي على iucD و *iucC* في جميع العزلات. بالإضافة إلى ذلك، تتوفر جينات *iucD* و *iucC*ól في جميع العزلات. الهدف من هذه الدراسة هو أن تكون هذه العزلات مسببة للأمراض.

* DOI: 10.1186/s13099-019-0290-0