Molecular and biological Study of Colibacillosis Susceptibility in broiler

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Abstract

This study was aimed to Detection of virulence genes(rfbE) It was carried in College of Veterinary Medicine at Baghdad university, During a time span of November (2021) to March (2022). (100) samples were collected from diarrheal chicken. 100 chicks were used in the present study, One day old chicks were weighed (Average of chicken weight was 160 gm±2) then transferred to the poultry house. Chickens in wards that were divided by wood barriers. Diagnosis of bacterial colonies were first done by the bacteriological methods. From (100) fecal samples of diarrheal cases of chicken, 90(90%) isolates were Escherichia coli. In the present study using vitek 2 compact system and Api 20 Escherichia coli for the identification isolated bacteria. PCR technique was used to Detection of virulence genes(rfbE). The result for rfbE gene in this study was showed that this gene was detected in(60) isolates of (90) samples of chicken observed carried the rfbE gene which represent (67%).

Keywords: Diarrhea, rfbE, chicken, PCR.

I. INTRODUCTION

Avian pathogenic Escherichia coli (APEC), an extra-intestinal pathogenic E. coli (ExPEC), causes diverse local and systemic infections in poultry, including chickens, turkeys, ducks, and many other avian species (Mellata.,2013). The most common infections caused by APEC in chickens are perihepatitis, airsacculitis, pericarditis, egg peritonitis, salpingitis, coligranuloma, omphalitis, cellulitis, and osteomyelitis/arthritis; these are commonly referred as avian colibacillosis (Bandyopadhyay et al., 2011). Colibacillosis in poultry includes systemic and localized infections. The localized infections were; omphalitis, swollen head syndrome, cellulitis, and diarrhea. Whereas systemic infection including; respiratory colisepticemia, enteric colisepticemia, and neonatal colisepticemia. (Ewers et al., 2003) reported that E. coli pathogenicity was generally enhanced or initiated by several influencing factors such as; environmental
factors, viral infections, mycoplasma infections, and immune-suppression. Susceptibility to APEC infection increased after exposure to many intrinsic and extrinsic factors. Extrinsic factors involve environment; exposure to other infections, virulence, duration and levels of exposure, whereas intrinsic factors involve age; route of exposure, passive and active immune status, in addition to strain and breed of chickens. Generally, young birds were more susceptible to severe infections than adult (Rodriguez et al., 2005). Escherichia coli O157:H7 has been associated with many outbreaks of diarrhea and haemolytic uraemic syndrome (HUS). The main pathways for the infection of this pathogen are contaminated food (including water), environmental, and person to person contact (Verma et al., 2007). Lipopolysaccharide (LPS), the characteristic of outer membrane of gram-negative bacteria, is an important virulence factor for E. coli O157:H7. It serves to protect the pathogen from the host’s defense system such as hydrophobic agents, serum-mediated killing, phagocytosis, and cationic peptides. LPS is composed of three domains: lipid A, core oligosaccharide and O-antigen. O-antigen is located at the outer layer of LPS and is almost specific to each gram-negative bacterium. The modification/alteration of LPS by various O-antigens likely aids the success of the pathogens during human infection. Some research suggested that the mutation on O-antigen synthesis could improve the adhesion of E. coli O157 to epithelial cells seven fold more than the wide-type bacteria. The rfb gene cluster has a lower GC content (30–40%) than that of E. coli chromosome (51%) with the exception of manB gene, which suggests that these genes are originated from a non- E. coli species. In the rfb gene cluster, per gene is predicted to encode GDP-perosamine synthetase. The gene is essential for the O-antigen synthesis. The mutation in per gene made bacteria deficient on the O-antigen, (Kalb., 2022)

II. MATERIALS AND METHODS

100 chicks were used in the present study, One day old chicks were weighed (Average of chick weight was 160 gm±2) then transferred to the poultry house. Chicks in wards that were divided by wood barriers, area of each barrier were 1×1.5 m. The ground bed was a sawdust and it's thickness was 7cm. Drinking water and food being freely accessed (ad libitum) made of plastic manhels and with using of continuous lighting system. The chicks were neither vaccinated nor medicated throughout the 35-day of study. The chicks were neither vaccinated nor medicated throughout the 35-day of study.

Polymerase Chain Reaction

PCR assay was performed for detection The Escherichia coli O157 IQ1 rfbE gene for GDP-perosamine synthetase in E. coli bacterium isolated from chicken

PCR master mix preparation

PCR master mix for each gene was prepared by using (Maxime PCR PreMix kit) and this master mix done according to company instructions as following table(3-12):
Table (1) PCR Master Mix preparation

<table>
<thead>
<tr>
<th>PCR Master mix</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA template 5-50ng</td>
<td>5µL</td>
</tr>
<tr>
<td>Forward primer (10pmol)</td>
<td>1µL</td>
</tr>
<tr>
<td>Reverse primer (10pmol)</td>
<td>1µL</td>
</tr>
<tr>
<td>PCR water</td>
<td>13µL</td>
</tr>
</tbody>
</table>

After that, these PCR master mix components that mentioned in table above placed in standard AccuPower PCR PreMix Kit that containing all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, MgCl₂, and loading dye). Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes, and then placed in PCR Thermocycler (BioRad USA).

PCR Thermocycler Conditions

PCR thermocycler conditions were done by using conventional PCR thermo cycler system is same for each gene as following table (2)

Table (2): PCR Thermocycler Conditions

<table>
<thead>
<tr>
<th>PCR step</th>
<th>Temp.</th>
<th>Time</th>
<th>Repeat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>94C</td>
<td>3min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94C</td>
<td>30sec</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>59C</td>
<td>60sec</td>
<td>30 cycle</td>
</tr>
<tr>
<td>Extension</td>
<td>72C</td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72C</td>
<td>7min</td>
<td>1</td>
</tr>
<tr>
<td>Hold</td>
<td>4C</td>
<td>∞</td>
<td>-</td>
</tr>
</tbody>
</table>
III. RESULTS AND DISCUSSION

1) Isolation of *Escherichia coli*:

Fecal samples were inoculated on various bacteriological media such as Blood agar, Nutrient agar, MacConkey agar, and EMB agar then incubated at (37°C) for (18-24) hours to allow the growth of bacteria. Diagnosis of bacterial colonies were first done by the bacteriological methods including colonial morphology, Gram’s staining, and cultural and biochemical characteristics as described in (Cheesbrough, 2007). From (100) fecal samples of diarrheal cases of chicken, 90(90%) isolates were *Escherichia coli*.

(Alonso et al., 2012); (Amadi et al.,2015), reported prevalence rate of *E. coli* associated with diarrhea in chicken which accounted for 67%. These results were shown in Figure (1).

![Figure (1): Percentage of Bacterial Isolates of diarrhea cases in chicken](image-url)
The results of this study are similar to the findings of (Abbassi et al., 2017) who found that prevalence of 67% and 100% was reported in rectal swabs of indigenous and caecal samples of broiler chicken respectively. (Adelaide et al., 2008) found that about 99% of diarrheal cases caused by E.coli, while other bacteria about for 1% of cases.

Mixed infection of samples with other microbes has been reported to affect prevalence of some bacteria such as E. coli and this could possibly be the cause of different prevalence from other studies. Environmental conditions, geographical location and other host factors affect prevalence of E. coli among related animals and could also account for the differences (Gambushe et al., 2022).

2) Detection and Characterization of (rfbE) Gene

To determine if E.coli isolates was E.coli 0157H7, we characterized the isolates on the basis of the presence of virulence genes (rfbE) by using real time PCR technique. Figure (3) shows Agarose gel electrophoresis image that showed the PCR product analysis of rfbE gene in Escherichia coli isolated from feces samples. Figure (4) shows Agarose gel electrophoresis image that showed the PCR product analysis of rfbE gene in Escherichia coli isolated from feces samples.
Figure (3): Agarose gel electrophoresis image that showed the PCR product analysis of rfbE gene in Escherichia coli isolated from feces samples. Where Marker ladder (1500-100bp), Lane (1-7) some positive rfbE gene in E. coli isolates at 292bp PCR product size.

Sixteen of the 90 (66.7%) samples of chicken observed carried the rfbE gene. Figure (4) illustrate this results.

Figure (4): rfbE gene in chicken

The result in this study was agreement with study of (Abdulmawjood et al., 2004) ; (Jeshveen et al., 2012), They focussed our method on the amplification of the rfbE gene to detect the presence of all E. coli O157 strains. They found that the O157 rfbE PCR is a selective and rapid method for confirmation of the O15 serogroup. The majority of E. coli strains are commensals inhabiting the intestinal tract of humans and warm-blooded animals.
and rarely cause diseases, unless they acquire VFs carried by mobile genetic elements such as bacteriophages, pathogenicity islands and plasmids. Additionally, E. coli can form a reservoir of AMR genes that may be transferred among different bacterial species, including pathogenic bacteria for both humans and animals (Bennameur et al., 2021). The O-antigen was the immunodominant agent for the gram-negative bacteria, which can disguise the pathogen to escape from the defense system of the host. Previous studies showed that the deficient on O-antigen could make the pathogens sensitive to the killing mechanism of host.

(Bakhshi et al., 2017) argued that the protein- encoding rfbE gene plays an essential role in the group of proteins involved in glucose-free biosynthesis and bacterial LPS synthesis. It is likely that the rfbE gene is involved in activating antibodies associated with E. coli antigen diseases and increases bacterial activity in the presence of sugar and LPS.

According to the knowledge obtained on the rfb gene function and its main activity as O antigens in E. coli through this study, creatinine, as a nutrient with a high level of nitrogen, can enhance the activities of O antigen and rfbE gene effectively.

The study by (Stevenson et al., 1994) showed that O antigen is removed during in vitro culture that effectively reduces the expression of rfb cluster genes. Still, in the present study, the expression of rfbE gene was enhanced as one of the 11 rfb genes, which indicates the importance of creatinine amplification compared to the genes encoding O polysaccharide in this bacterium. The temperature changes lead to the expression of the rfbE gene.

3) Genetic analysis of rfbE gene:

Sequence analysis of rfbE gene of local E. coli isolates were find to be identical to the rfbE gene in (NCBI) Blast. The results of all tested samples have high identity to another global sequence data when compared to NCBI- Blast. In the present study, the phylogenetic trees of the isolated rfbE genes from tested isolates were very close to each other with 100 percent compatibility, Phylogenetic analysis were done on 5 isolate in a local tree analysis.

The alignment study of the rfbE gene in revealed a genotype similar to other strains, from USA, deposited in the Gen Bank (accession numbers documented in (Fig.5). Sequencing of the gene demonstrated 99% compatibility with the global standard gene in Gen Bank. Global tree analysis were presented on figure (6) revealing that two cluster were shown one for our isolate and the other for the international gen bank isolate which revealing that the rfbE gene distantly a bit far from others isolates and to make it clear this might the effect of gene in producing EBLs effect. In addition, the gene sequencing and phylogenetic analysis from the E. coli isolates have reported 99 percent compatibility with the global standard genes of Gen Bank.
IV. REFERENCES


4. Rodriguez-Siek, K.E., Giddings, C.W., Doetkott, C., Johnson, T.J. and Nolan, L.K., 2005a. Characterizing the APEC pathotype. Veterinary Research 36, pp 241-256. doi.org/10.1099/mic.0.27499-0


