University of Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 12, Issue 2 (2023) PP 185-194

https://jam.utq.edu.iq/index.php/main ***https://doi.org/10.54174/utjagr.v12i2.282***

Molecular and biological Study of Colibacillosis Susceptibility in broiler

[1]Hassan Zaher, [2]Balqees Hassan Ali

[1]College of veterinary medicine, University of Baghdad.

[2]Dept. of poultry Diseases , College of veterinary medicine, University of Baghdad.

E-mail: hassanza944@gmail.com

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, salphingitis, 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

University of Thi-Qar Journal of agricultural research
ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 12, Issue 2 (2023) PP 185-194
https://jam.utq.edu.iq/index.php/main ***https://doi.org/10.54174/utjagr.v12i2.282***

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 sever 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-peroamine 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 synthase 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):

University of Thi-Qar Journal of agricultural research
ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 12, Issue 2 (2023) PP 185-194
https://jam.utq.edu.iq/index.php/main ***https://doi.org/10.54174/utjagr.v12i2.282***

Table (1) PCR Master Mix preparation

PCR Master mix	Volume
DNA template 5-50ng	5µL
Forward primer (10pmol)	1µL
Reveres primer (10pmol)	1µL
PCR water	13µL

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, $MgCl_2$,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 convential PCR thermo cycler system is same for each gene as following table(2)

Table (2):PCR Thermocycler Conditions

PCR step	Temp.	Time	Repeat
Initial Denaturation	94C	3min	1
Denaturation	94C	30sec.	30 cycle
Annealing	59C	60sec	
Extension	72C	1 min	
Final extension	72C	7min	1
Hold	4C	∞	-

University of Thi-Qar Journal of agricultural research
ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 12, Issue 2 (2023) PP 185-194
https://jam.utq.edu.iq/index.php/main ***https://doi.org/10.54174/utjagr.v12i2.282***

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).

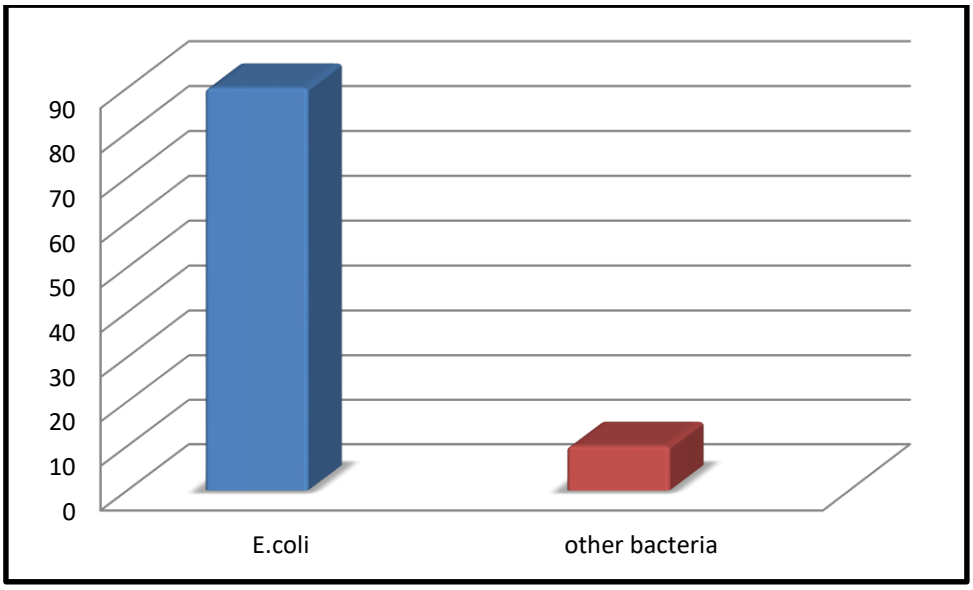

Figure (1): Percentage of Bacterial Isolates of diarrhea cases in chicken

University of Thi-Qar Journal of agricultural research
ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 12, Issue 2 (2023) PP 185-194
https://jam.utq.edu.iq/index.php/main ***https://doi.org/10.54174/utjagr.v12i2.282***

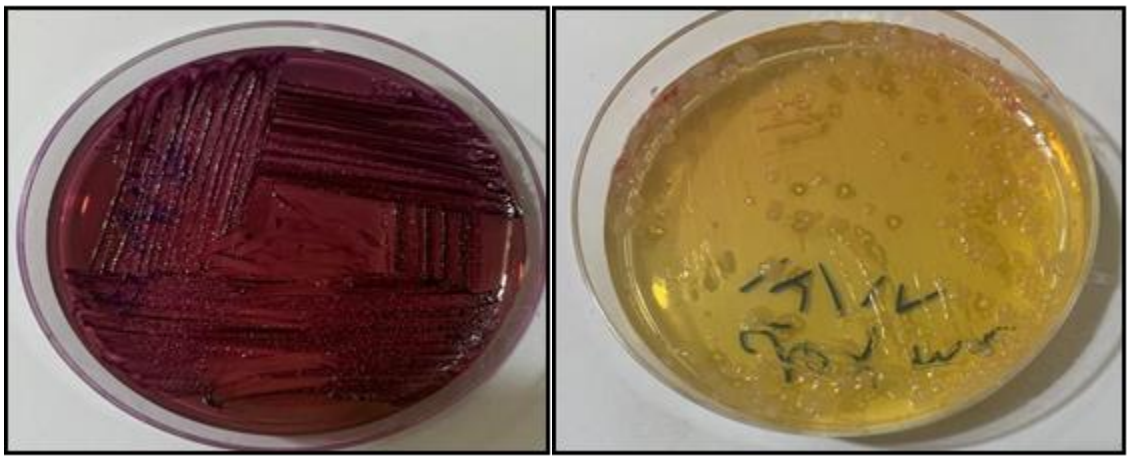

Figure (٢): Bacterial Isolates of diarrhea cases in chicken

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 for1% 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 o157H7 ,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

University of Thi-Qar Journal of agricultural research
ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 12, Issue 2 (2023) PP 185-194
https://jam.utq.edu.iq/index.php/main ***https://doi.org/10.54174/utjagr.v12i2.282***

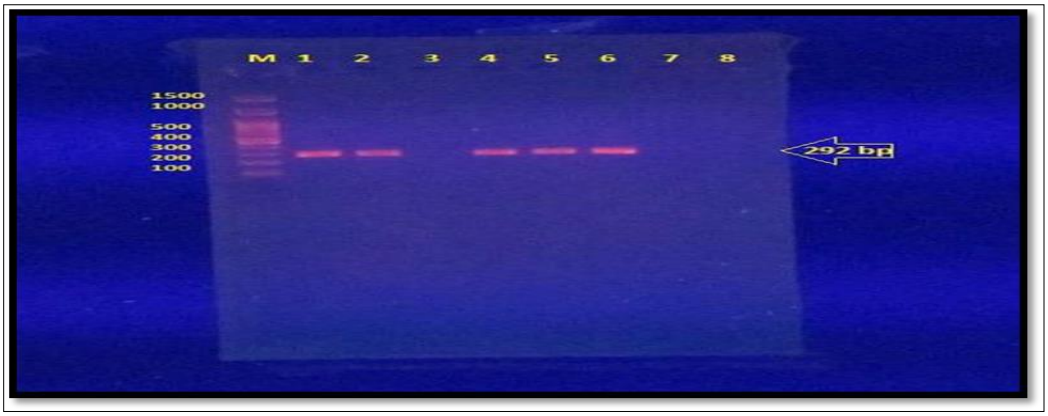

Figure (٣): Agarose gel electrophoresis image that showed the PCR product analysis of *rfbE* gene in *Escherichia coli* isolated from feces samples . Where Marker ladder (1500-100bp), Lane (1-7) some positive *rfbE* gene in *E. coli* isolates at 292bp PCR product size.

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

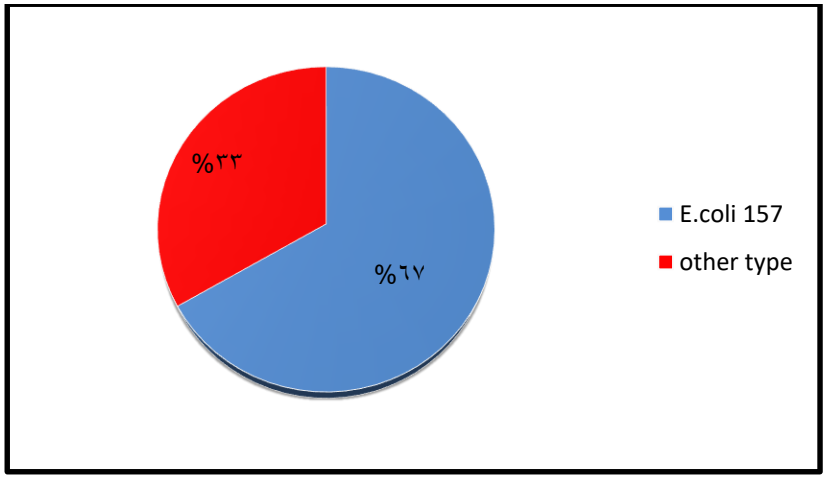

Figure (4): *rfbE* gene in chicken

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 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

University of Thi-Qar Journal of agricultural research
ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 12, Issue 2 (2023) PP 185-194
https://jam.utq.edu.iq/index.php/main ***https://doi.org/10.54174/utjagr.v12i2.282***

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(Benameur *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 GenBank.

University of Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 12, Issue 2 (2023) PP 185-194

https://jam.utq.edu.iq/index.php/main ***https://doi.org/10.54174/utjagr.v12i2.282***

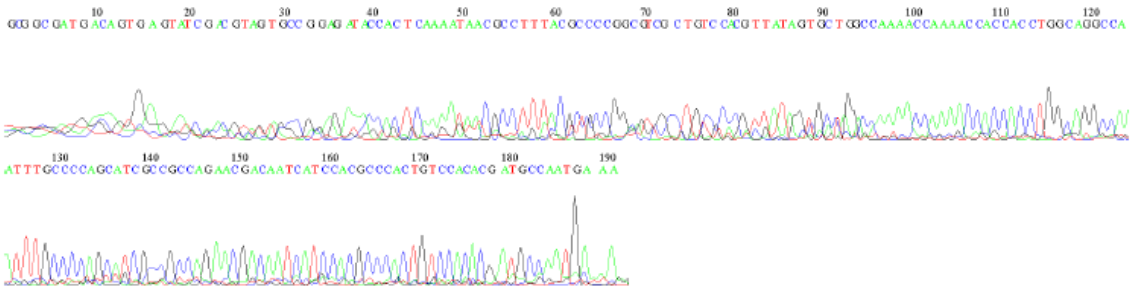

Figure (5): genetic analysis based on the *rfbE* gene for (5) isolates of E. coli

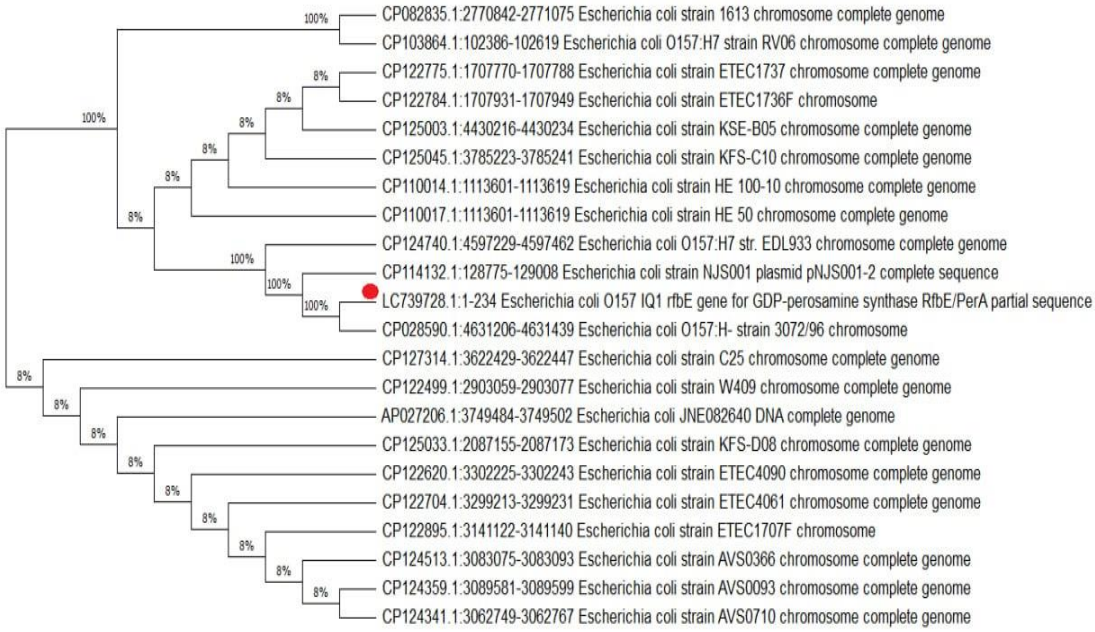

Figure (6): Phylogenetic tree based on the *rfbE* gene for (5) isolates of E. coli

IV. REFERENCES

1. Mellata, M., 2013. Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks and antibiotic resistance trends. Foodborne Pathogens and Disease 10, pp 916-932. doi.org/10.1089/fpd.2013.1533

2. Bandyopadhyay S., Mahanti A., Samanta I., Dutta T.K., Ghosh M.K., Bandyopadhyay S.& Bhattacharya D.(2011). Virulence repertoire of Shiga toxin-producing *Escherichia coli* (STEC) and enterotoxigenic *Escherichia coli* (ETEC) from diarrhoeic lambs of Arunachal Pradesh, India.*Trop. anim. Hlth Prod*..43 (3):705–710. doi.org/10.1007/s11250-010-9757-1

3. Ewers, C., Janssen, T. and Wieler, L.H., 2003. Avian pathogenic *Escherichia coli* (APEC). Berliner und Münchener Tierärztliche Wochenschrift 116, pp 381-395.

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

5. Verma, F.J. Bolton, D. Fiefield, P. Lamb, E. Woloschin, N. Smith, R. McCann, An outbreak of E. coli O157 associated with a swimming pool: an unusual vehicle of transmission, Epidemiol. Infect. (2007)1–4. doi.org/10.1017/S095026880700794

6. Kalb, M. J. (2022). *Development of genetic tools in environmental Pseudomonas species* (Doctoral dissertation, University of Minnesota).

7. Cheesbrough M. (2006). Biochemical tests to identify bacteria. In: Cheesbrough M (ed.). District laboratory practice in tropical countries, Part 2.2nd Edition. Cambridge University Press, UK. 7(11): 62-70.

8. Alonso, M. Z., Lucchesi, P. M. A., Rodríguez, E. M., Parma, A. E., & Padola, N. L. (2012). Enteropathogenic (EPEC) and Shigatoxigenic Escherichia coli (STEC) in broiler chickens and derived products at different retail stores. *Food Control*, *23*(2), 351-355. doi.org/10.1016/j.foodcont.2011.07.030

9. Amadi, V. A., Matthew-Belmar, V., Tiwari, K., Brathwaite, E., Sharma, R., & Hariharan, H. (2015). Antimicrobial susceptibility profiles of Escherichia coli recovered from feces of young healthy domestic pigs in Grenada, West Indies. *British Microbiology Research Journal*, *5*(3), 300. doi.org/10.9734/BMRJ/2015/14071

10. Abbassi, M. S., Kilani, H., Zouari, M., Mansouri, R., Oussama, E. F., Hammami, S., & Chehida, N. B. (2017). Antimicrobial resistance in Escherichia coli isolates from healthy poultry, bovine and ovine in Tunisia: a real animal and human health threat. *J Clin Microbiol Biochem Technol*, *3*(2), 019-123.

11. Adelaide, O. A., Bii, C., & Okemo, P. (2008). Antibiotic resistance and virulence factors in Escherichia coli from broiler chicken slaughtered at Tigoni processing plant in Limuru, Kenya. *East Afr Med J*, *85*(12), 597-606.

12. Gambushe, S. M., Zishiri, O. T., & El Zowalaty, M. E. (2022). Review of Escherichia Coli O157: H7 prevalence, pathogenicity, heavy metal and antimicrobial resistance, African perspective. *Infection and Drug Resistance*, 4645-4673.

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 12, Issue 2 (2023) PP 185-194
https://jam.utq.edu.iq/index.php/main ***https://doi.org/10.54174/utjagr.v12i2.282***

13. Abdulmawjood, A., Bülte, M., Roth, S., Schönenbrücher, H., Cook, N., Heuvelink, A. E., & Hoorfar, J. (2004). Development, validation, and standardization of polymerase chain reaction-based detection of E. coli O157. *Journal of AOAC International*, *87*(3), 596-603.

14. Jeshveen, S. S., Chai, L. C., Pui, C. F., & Son, R. (2012). Optimization of multiplex PCR conditions for rapid detection of Escherichia coli O157: H7 virulence genes. *International Food Research Journal*, *19*(2).

15. Benameur, Q., Gervasi, T., Giarratana, F., Vitale, M., Anzà, D., La Camera, E., ... & Marino, A. (2021). Virulence, antimicrobial resistance and biofilm production of Escherichia coli isolates from healthy broiler chickens in western Algeria. *Antibiotics*, *10*(10), 1157.

16. Bakhshi M, Ebrahimi F, Nazarian S, Tarverdizade Y, Sadeghi D. Identifi cation of E. coli O157: H7 by Using Specific Primers for rfb E and stx2b Genes. ISMJ. 2017;20(3):267—77.

17. Stevenson G, Neal B, Liu DAN, Hobbs M, Packer NH, Batley M, et al. Structure of the O antigen of Escherichia coli K-12 and the sequence of its rfb gene cluster. Journal of bacteriology. 1994; 176(13):4144—56.