Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Research article

Molecular and phylogenetic study of avian pathogenic *Escherichia coli*(APEC) isolated from broilers in Al-Diwaniyah Province, Iraq

Nafea Sabeeh Jaseim
Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Al-Qadisiyah, , Iraq
Corresponding Author Email: Nafea.Jasim@qu.edu.iq

(Received 08/11/2018, Accepted 28/01/2019)

Abstract

The current work was performed to study the infections induced by avian pathogenic Escherichia coli (APEC) in broiler farms in Al-Diwaniyah province, Iraq, using molecular and phylogenetic-based methods. For these purposes, 200 omphalitis, air sacculitis, kidney, liver, and gastrointestinal (GIT)-based samples were collected using cloacal and oropharyngeal swabs. These samples were exposed to traditional microbiological processing methods including cultivation and biochemical methods. Polymerase chain reaction-based identification was induced targeting the 16S rRNA gene. The results showed the amplification of this gene in all samples. The phylogenetic study detected 5 local isolates that were close in their identities to isolates from Egypt, Ecuador, and Nigeria. The current study provides important information about the status of E. coli infection in poultry in addition to the evolutional status of the local isolates of this microbe.

Keywords: *E. coli*, PCR, phylogeny, poultry**.**

Introduction

The infection induced by *E. coli*, under the category of avian pathogenic *E. coli* (APEC), still represents a major health and economic problem affecting large population of chickens around the world leaving humongous production losses(1, 2, 3). The disease is called Colibacillosis that affects large numbers of chickens especially in bad rearing conditions inducing high percentages of mortalities in all ages of chickens. Although *E. coli* is considered as one of the normal gut microbiota of humans and animals, some pathogenic or opportunistic strains affect chickens generating a fatal disease that is initiated in the intestine mucosa and disseminated to different body organs such as air sacs. Colibacillosis via *E. coli* infection can affect different organs of a chicken body including liver, air sacs, heart, GIT, and navel part in chicks(4, 5, 6, 7). The disease is endemic in different parts of the world. Globally, the disease control is a big challenge due to the presence of infection with high resistant strains of *E. coli* to wide range of antibacterial agents. Understanding the current situation of bacterial evolution leads to better tracking of the genetic modifications occurred via bacterial resistance to these antibacterial agents(8, 9, 10, 11). The current work was performed to study the infections induced by *Escherichia coli* in poultry farms in Al-Diwaniyah province, Iraq using molecular and phylogenetic-based methods.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study.

Sampling

In this study, 200 samples of yolk sac, air sacculitis, kidney, liver, and gastrointestinal (GIT) samples from infected broilers were

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

collected using cloacal and oropharyngeal swabs. The samples were provided by Al-Diwaniyah Veterinary Medicine Hospital, Al-Diwaniyah, Iraq, and was approved by the appropriate institutional evaluation committee. The sampling from Al-Diwaniyah Province, Iraq, was lasted throughout a period from July-November, 2015. The study involved collecting 60, 40, 55, and 45 samples, and they were from Al-Diwaniyah, Afak, Hamza, and Saniyah cities respectively. Immediately, the samples were inoculated in 2ml of transport media (nutrient broth or brain-heart infusion broth), transported to a Lab, and incubated at 37^0C for 24hrs.

Sample processing

The growth on the nutrient broth was examined under the microscope. Then, the samples from that growth were cultivated and sub-cultivated on MacConkey, EMB, and TBXagar plates (Difico Labs) at 37^0C for 24hrs until obtaining single pure colonies. These bacteria were studied for their Gram staining and morphological properties. Hanging drop test was done to test the motility of these bacteria. Biochemical tests and API 20E kit (Biomerieux, France) were performed. The processes of the cultivation were performed according to(12).

Molecular analyses

PCR technique

The 16S rRNA gene and primers were used for confirming the identification of *E. coli* isolates as shown in table 2. These primers were designed according to NCBI-Gen Bank and provided by (IDT, Canada). These primers are F: 5'-ATGCTTAGTGCTGGTTTAGG-3' and R: R-5'-GCCTTCATCATTTCGCTTTC-3' targeting a piece at 1500bp of length. The thermocycler conditions used for the current work were 1 cycle for initial denaturation at 95^0C for 5min, 30 cycles of (denaturation at 95^0C for 30s, annealing at 55-60^0C for 30s, and extension at 72^0C for 1min), and 1 cycle for final extension at 72^0C for 5min.

Phylogeny study

The tree was built up using Mega v6 software depending on the Neighbor-Joining method. The distances were calculated by the Maximum Composite Likelihood method (13, 14).

Results

Phenotype-based findings

The biological examination consisted of cultivation, identification of the colonies, and the biochemical tests were interpreted using Bergys Manual of Determinative Bacteriology. The results demonstrated the presence of *E. coli* in the studied samples.

Genotype-based findings

The results of the PCR confirmed the presence of the microorganism in the tested samples as shown in figure (1). The results revealed the amplification of this gene in all samples at 1500bp of target.

Phylogeny-based findings

Five positive samples were sent for sequencing analysis where they were submitted to GeneBank under the accession numbers of KY681420, KY681421, KY681422, KY681423, KY681424, and KY681425. KY681420 and KY681425 were close in their identities to an isolate from Egypt, MF197876. KY681421 was close in its identity to LC317300, an isolate from mastitis in dairy cattle from Ecuador. However, KY681422 and KY681423 aligned together in a distinct branch of the phylogenetic tree. Moreover, KY681424 was close in its identity to MH396737, an isolate from pharmaceutical wastewater from Nigeria. These results are shown in figure (2).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

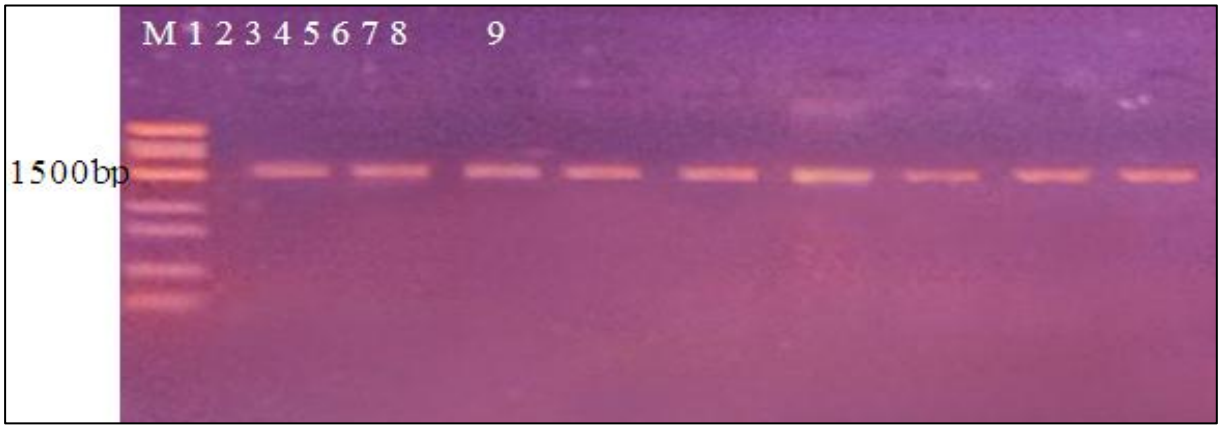

Figure (1): Electrophoresis image of DNA amplification of a1500bp region detecting 16srRNA gene using PCR. Lane1 to lane9 are positive results. Lane M is the ladder.

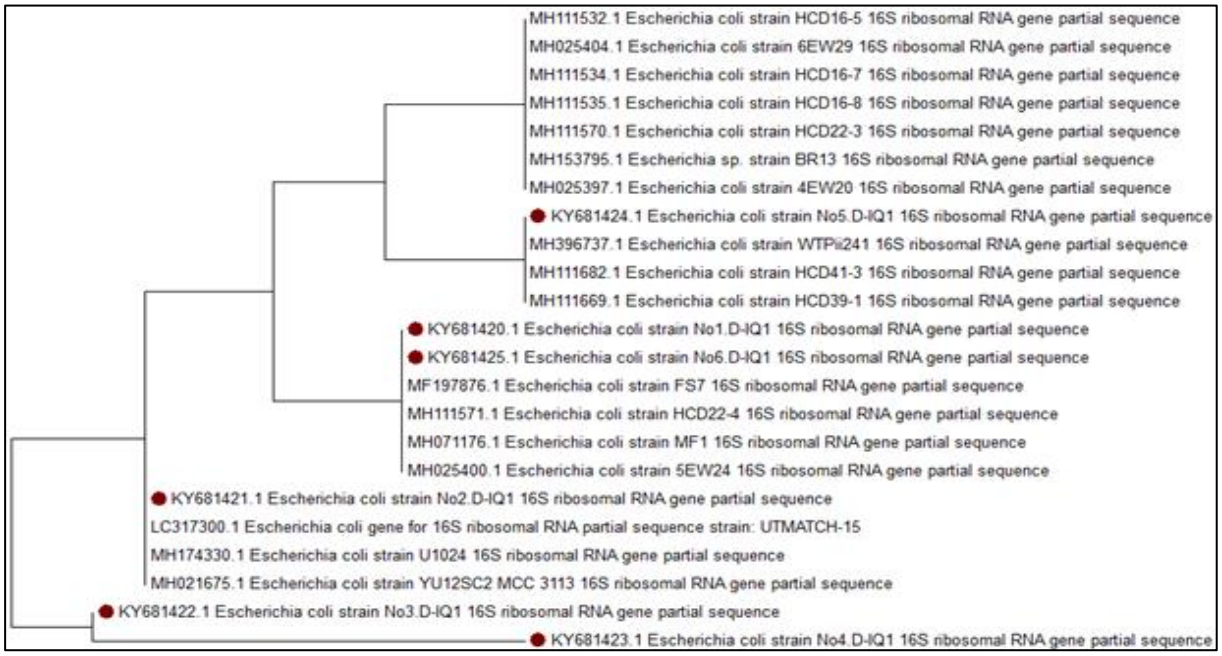

Figure (2): Phylogenetic tree. The phylogenetic study detected 5 local isolates with accession numbers of KY681420, KY681421, KY681422, KY681423, KY681424, and KY681425. KY681420 and KY681425 were close in their identities to an isolate from Egypt, MF197876. KY681421 was close in its identity to LC317300, an isolate from mastitis in dairy cattle from Ecuador. However, KY681422 and KY681423 aligned together in a distinct branch of the phylogenetic tree. Moreover, KY681424 was close in its identity to MH396737, an isolate from pharmaceutical wastewater from Nigeria. The phylogenetic tree done using Mega v6 software depending on the Neighbor-Joining method. The distances were calculated by the Maximum Composite Likelihood method.

Discussion

The infection induced by *E. coli*, under the category of APEC, still represents a major health and economic problem affecting large population of chickens around the world leaving humongous production losses (1, 2, 3). Colibacillosis control is considered as a major obstacle facing the poultry production in the world, and this may be due to different factors especially that *E. coli* comes from different sources such as human and animal wastes generating humongous sources for infection (1, 15, 16, 17). The results identified the presence of *E. coli* in the tested samples using the traditional lab techniques. This indicates the importance of using such tools in primary detection of the infections induced by this microorganism, and these results agree with (18, 19) who identified the presence of *E. coli* in samples collected of different types especially cloacal swabs of poultry using standard methods revealing the importance of these techniques

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

in initiating vital studies of the same concept. The results using PCR were shown to be interesting as they confirmed the results of the standard methods used in the current work. The amplification of the PCR products at 1500bp of size indicating the successful efforts in detecting the current microorganism in the tested samples. The PCR technique is an important method in detecting the presence of infections by different microorganisms such as *E. coli* in the current study. The current PCR findings reveal agreement with (20, 21, 22, 23, 24) who detected the presence of *E. coli* in their samples using PCR-based techniques identifying the importance of this method in supporting the traditional techniques in diagnosing such infection. The phylogenetic study results revealed matching of the current local isolates with high identity similarity with global isolates detected in different parts of the world. This technique is an important method in understanding the evolution history of the current microorganism, *E. coli*, and knowing its matched strains from the neighbor or the world countries. The current isolates and the matching global isolates refer that they might come from similar source of rearing or reproduction(25, 26). This gives important information in controlling the disease in poultry. The current study provides important information about the status of *E. coli* infection in poultry in addition to the evolutional status of the local isolates of this microbe.

References

1-Stromberg ZR, Johnson JR, Fairbrother JM, Kilbourne J, Van Goor A, Curtiss R, et al. Evaluation of Escherichia coli isolates from healthy chickens to determine their potential risk to poultry and human health. PLoS One [Internet]. 2017 [cited 2018 Dec 21];12 (7): e0180599. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28671990

2-Ballou AL, Ali RA, Mendoza MA, Ellis JC, Hassan HM, Croom WJ, et al. Development of the Chick Microbiome: How Early Exposure Influences Future Microbial Diversity. Front Vet Sci [Internet]. 2016 Jan 20 [cited 2018 Dec 21];3:2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26835461

3-Mellata M. Human and Avian Extraintestinal Pathogenic *Escherichia coli* : Infections, Zoonotic Risks, and Antibiotic Resistance Trends. Foodborne Pathog Dis [Internet]. 2013 Nov [cited 2018 Dec 21];10(11):916-32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23962019

4-Dziva F, Stevens MP. Colibacillosis in poultry: unravelling the molecular basis of virulence of avian pathogenic *Escherichia coli* in their natural hosts. Avian Pathol [Internet]. 2008 Aug 14 [cited 2018 Dec 21];37(4):355-66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18622850

5-Ozaki H, Matsuoka Y, Nakagawa E, Murase T. Characteristics of Escherichia coli isolated from broiler chickens with colibacillosis in commercial farms from a common hatchery. Poult Sci [Internet]. 2017 Oct 1 [cited 2018 Dec 21]; 96(10): 3717–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28637225

6-Matin MA, Islam MA, Khatun MM. Prevalence of colibacillosis in chickens in greater Mymensingh district of Bangladesh. Vet World [Internet]. 2017 Jan [cited 2018 Dec 21];10(1):29–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28246445

7-Uotani Y, Kitahara R, Imai T, Tsutsumi N, Sasakawa C, Nagai S, et al. Efficacy of an avian colibacillosis live vaccine for layer breeder in Japan. J Vet Med Sci [Internet]. 2017 Jul 19 [cited 2018 Dec 21]; 79(7):1215–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28603216

8-Collingwood C, Kemmett K, Williams N, Wigley P. Is the Concept of Avian Pathogenic Escherichia coli as a Single Pathotype Fundamentally Flawed? Front Vet Sci [Internet]. 2014 [cited 2018 Dec 21];1:5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26664913

9-Mellata M. Human and avian extraintestinal pathogenic Escherichia coli: infections, zoonotic risks, and antibiotic resistance trends. Foodborne Pathog Dis [Internet]. 2013 Nov [cited 2018 Dec 21]; 10 (11): 916–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23962019

10-Salehi TZ, Tabatabaei S, Karimi V, Fasaei BN, Derakhshandeh A, Jahromi AON. Assessment of immunity against avian colibacillosis induced by an aroA mutant containing increased serum survival gene in broilers. Braz J Microbiol [Internet]. 2012 Jan [cited 2018 Dec 21]; 43(1): 363–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24031840

11-Subedi M, Luitel H, Devkota B, Bhattarai RK, Phuyal S, Panthi P. Antibiotic resistance pattern

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

and virulence genes content in avian pathogenic Escherichia coli (APEC) from broiler chickens in Chitwan, Nepal. BMC Vet Res [Internet]. 2018 Mar 27 [cited 2018 Dec 21]; 14(1):113. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29587768

12-Sanderson MW, Gay JM, Hancock DD, Gay CC, Fox LK BT. Sensitivity of bacteriologic culture for detection of Escherichia coli O157:H7 in bovine feces. J Clin Microbiol Microbiol. (1995); 33(10):2616-9.

13-Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol [Internet]. 1987 Jul [cited 2017 Dec 16];4(4):406–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3447015

14-Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol [Internet]. 2013 Dec [cited 2017 Dec 16]; 30 (12): 2725–9. Available from: https://academic.oup.com/mbe/article-lookup/ doi/ 10.1093/ molbev/mst197

15-Dho-Moulin M, Fairbrother JM. Avian pathogenic Escherichia coli (APEC). Vet Res [Internet]. 1999 [cited 2018 Dec 21];30(2–3):299–316. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10367360

16-Tuntufye HN, Lebeer S, Gwakisa PS, Goddeeris BM. Identification of Avian pathogenic Escherichia coli genes that are induced in vivo during infection in chickens. Appl Environ Microbiol [Internet]. 2012 May [cited 2018 Dec 21]; 78(9): 3343–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22344666

17-Tivendale KA, Logue CM, Kariyawasam S, Jordan D, Hussein A, Li G, et al. Avian-pathogenic Escherichia coli strains are similar to neonatal meningitis E. coli strains and are able to cause meningitis in the rat model of human disease. Infect Immun [Internet]. 2010 Aug [cited 2018 Dec 21]; 78 (8): 3412–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20515929

18-Hricová K, Röderová M, Pudová V, Hanulík V, Halová D, Julínková P, et al. Quinolone-resistant Escherichia coli in Poultry Farming. Cent Eur J Public Health [Internet]. 2017 Jun 1 [cited 2018 Dec 21]; 25(2): 163–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28662329

19-Hanson R, Kaneene JB, Padungtod P, Hirokawa K, Zeno C. Prevalence of Salmonella and E. coli, and their resistance to antimicrobial agents, in farming communities in northern Thailand. Southeast Asian J Trop Med Public Health [Internet]. 2002 [cited 2018 Dec 21];33 Suppl 3:120–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12971491

20-Zhu S-Y, Lu H, Wang J. [Isolation of avian Escherichia coli and PCR detection of their F1 and HPI genes]. Wei Sheng Wu Xue Bao [Internet]. 2007 Oct [cited 2018 Dec 21];47(5):795–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18062251

21-Sekhar MS, Sharif NM, Rao TS, Metta M. Genotyping of virulent Escherichia coli obtained from poultry and poultry farm workers using enterobacterial repetitive intergenic consensus-polymerase chain reaction. Vet world [Internet]. 2017 Nov [cited 2018 Dec 21];10(11):1292–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29263587

22-Younis G, Awad A, Mohamed N. Phenotypic and genotypic characterization of antimicrobial susceptibility of avian pathogenic Escherichia coli isolated from broiler chickens. Vet world [Internet]. 2017 Oct [cited 2018 Dec 21]; 10(10):1167-72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29184361

23-Mohapatra BR, Broersma K, Mazumder A. Differentiation of fecal Escherichia coli from poultry and free-living birds by (GTG)5-PCR genomic fingerprinting. Int J Med Microbiol [Internet]. 2008 Apr [cited 2018 Dec 21]; 298 (3–4):245–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17572150

24-Zhu XY, Zhong T, Pandya Y, Joerger RD. 16S rRNA-based analysis of microbiota from the cecum of broiler chickens. Appl Environ Microbiol [Internet]. 2002 Jan [cited 2018 Dec 21]; 68(1):124–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11772618

25-Shamsi H, Mardani K, Ownagh A. Phylogenetic analysis of Escherichia coli isolated from broilers with colibacillosis based on gyrA gene sequences. Can J Vet Res [Internet]. 2017 Jan [cited 2018 Dec 21]; 81(1): 28–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28154459

26-Coura FM, Diniz SA, Silva MX, Arcebismo TLM, Minharro S, Feitosa ACF, et al. Phylogenetic Group of Escherichia coli Isolates from Broilers in Brazilian Poultry Slaughterhouse. Scientific WorldJournal [Internet]. 2017 [cited 2018 Dec 21]; 2017:5898701. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29130064

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm