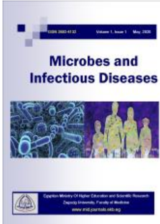
Microbes and Infectious Diseases 2023; Article-In-Press, **DOI:** 10.21608/mid.2023.218901.1548



Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Molecular determination of virulent genes from avian pathogenic *Escherichia coli* isolated from poultry farm droppings in Bayelsa State, Nigeria

***Pere-ere S. Tobia** [*1] **, Elijah I. Ohimain** [2]*

1- Department of Science Laboratory Technology, Federal Polytechnic Ekowe, Bayelsa State, Nigeria.
2- Department of Microbiology, Niger Delta University, Wilberforce Island Bayelsa State, Nigeria .

ARTICLE INFO

Article history:
Received 20 June 2023
Received in revised form 10 July 2023
Accepted 14 July 2023

Keywords:
Colibacillosis
ExPEC
Multiplex PCR
Pathogenesis
VAG

ABSTRACT

Background: Colibacillosis caused by avian pathogenic *Escherichia coli* (APEC), which is an extraintestinal pathogenic *E. coli* (ExPEC), is the commonest bacterial disease of poultry, which has led to severe economic losses. **Methods:** In this study, we isolated ten suspected strains of *Escherichia coli* (*E. coli)* from the droppings of birds suffering from colibacillosis at the teaching and research farm of the Niger Delta University, Wilberforce Island, Nigeria. Eight virulence associated genes (VAGs) were analysed for, using multiplex PCR techniques. **Results:** Only four (Isolates 1, 5, 6 and 8) were positive for the tested virulent genes. The iron uptake gene iutA, was predominant, being present in all the four isolates (1, 5, 6 and 8). Another iron uptake gene iucD, was detected only in isolates 1 and 5, while outer membrane protein gene ompT, was detected in isolates 1 and 8 only. The toxin gene hlyF, was only detected in isolate 8. In all, one VAG was detected in isolate 6 (iutA), two VAGs in isolate 5 (iutA, iucD) and three each in isolate 1 (iutA, iucD, ompT) and isolate 8 (iutA,ompT, hlyF). When isolate 1 was inoculated in a healthy chicken, it exhibited symptoms of colibacillosis with visible lesions. **Conclusions:** The results confirm the importance of VAGs in the pathogenesis of APEC.

Introduction

Avian pathogenic *Escherichia coli* (APEC) is a strain of *E. coli* that causes diseases primarily in poultry leading to economic losses [1]. The pathogen has been implicated in several infectious diseases of birds, of which colibacillosis is by far the most common bacterial disease [2, 3]. **Nakazato et al.** [1] reviewed several diseases associated with APEC including colisepticaemia (colibacillosis), inflammation of the oviduct, salpingitis, peritonitis, cellulitis and swollen head syndrome. Colisepticemia caused by APEC often facilitates other infections such as viral (bronchitis virus, Newcastle virus) and other bacterial (*Mycoplasma*) infection of the upper respiratory system [4]. The infectious often results in yield loss and ultimately death of chickens. **Bhattarai et al.** [3] estimated economic losses linked to colibacillosis including causing up to 20% mortality, reduction in meat production by about 2% live weight loss and 2.7% reduction in feed conversion ratio, up to 20%

DOI:10. 21608/MID.2023.218901.1548
* *Corresponding author:* Pere-ere S. Tobia
E-mail address: pereogoun77@gmail.com
© 2020 The author (s). Published by Zagazig University. This is an open access article under the CC BY 4.0 license ***https://creativecommons.org/licenses/by/4.0/.***

reduction in egg production and up to 43% carcass rejection by customers.

Janßen et al. [5] analysed for 17 virulence-associated genes (VAG) in APEC isolated from the internal organs of chickens that died from colibacillosis, among which *iucD, fimC*, *tsh, stx2f* and *hlyE* were used to identify APEC. They found that a combination of *tsh*, *iucD*, *fimC*, *fyuA* and *irp2* genes rendered the strains predominantly virulent. **Bhattarai et al.** [3] reported that the major VAGs predominant among APEC involved in colibacillosis were *traT*, *iutA*, *iss*, *hlyF*, *iroN*, *fimH*, *ompT*, *iucD*, *cvi/cva*, *sit chro.*, *tsh*, *kpsMTII* and *aerJ*.

Pathogenesis are somewhat related to virulence. Although, *E. coli* is a normal flora in the guts of humans, other mammals and birds, extraintestinal pathogenic *E. coli* (ExPEC) like APEC exhibit enhanced ability to cause infectious diseases even beyond the gut of chickens [6], hence they are referred to as facultative pathogens [7]. The survival of *E. coli* beyond the gut of birds is facilitated by the presence of several virulence factors (VF) which are coded by virulence genes [8]. **Dissanayake et al.** [9] reported that ExPEC enters through diverse routes particularly respiratory and genital tracts causing various extra intestinal illnesses collectively known as colibacillosis [8]. APEC have been reported to be related to extraintestinal human strains such as uropathogenic *E. coli* (UPEC), sepsis-associated *E. coli* (SEPEC) and neonatal meningitis-causing *E. coli* (NMEC) [1, 7, 10, 11]. **Bhattarai et al.** [3] reported that human ExPECs share major 10 virulence genes with APEC including *iucC*, *iucD*, *cvaA*, *cvaB*, *cvaC*, *iutA*, *etsA*, *ompT*, *hlyF* and *cvi*) suggesting a probable zoonotic transmission of APEC from birds to humans. Several other authors have mentioned the zoonotic potential of APEC [1, 9, 11-16]. **Olsen et al.** [12] detected virulence genes in APEC including *iss*, *ompT*, *hlyF*, *iutA traT*, *eitABC*, *sitABCD*, *iroBCDEN*, and *lucABCD*, several of which are found in with human ExPEC.

APEC virulence gene contribute to pathogenicity of the strain through production of toxins, iron uptake mechanisms, adhesion, invasion, immune suppression, resistance to host's serum and bacterial survival [1, 13], which are typically encoded on plasmids, pathogenicity islands (PAIs), transposons, bacteriophage and other mobile genetic elements [10, 7, 17]. For instance, **Singh et al.** [18] detected the 'tsh' gene in *E. coli* isolates that correlated positively with pathogenicity *in- vivo*. The *iss* gene increases resistance to serum [19]. **Hussain et al.** [13] reported the most prevalent virulence genes associated with multidrug resistant APEC to be *iroN, papC, iucD, fimC, fimH, traT, irp2,* and *iutA*. **Ievy et al.** [20] reported multidrug resistance of APEC possessing three virulence genes (*papC*, *iucD* and *fimC*) for the first time in Bangladesh. Other authors have also reported multidrug resistance (MDR) linked to the virulent and drug resistance genes of APEC [17]. **Lee et al.** [21] reported that tetracycline resistance in *E. coli* is associated with the presence of virulent genes particularly increased serum survival (iss), iron uptake (iroN) and temperature-sensitive haemagglutinin (Tsh) genes.

Based on detection frequency, subcellular localization and protein uniformity, some of the VAGs including *iroN*, *iss*, *ompT*, *iutA*, *traT* and *etsC* has been suggested as suitable vaccine candidates [12]. **Kathayat et al.** [11] suggests that anti-virulence inhibitors can be explored as alternative measure for the control of APEC in poultry especially because the strains are unlikely to develop resistance to these inhibitors. Also, some virulence genes that do not play a role in pathogenesis of APEC and its drug resistance patterns can be explored for the development of countermeasures such as vaccines and inhibitors.

Authors have used a combination of three or more VAGs viz; *iroN, ompT, iucD, iss, ompT, hlyF* and *iutA* to classify strains of E. coli as APEC [8, 22]. **Aslam et al.** [14] defined ExPEC with the presence of at least two of the following VAGs; papA/*papC*, *iutA*, *sfa* and *kpsMT II*. However, **Johnson et al.** [23] observed that the APEC plasmid used as defining trait of APEC are prevalent both in diseased birds and in the caecum of healthy birds. They suggested sequence types (STs) especially ST23, ST131, ST117, ST355 and ST428 which dominated infected birds but rare in the caecum of healthy birds. **Dissanayake et al.** [9] found that a combination of four genes (*ompT*, *iroN*, *hlyF* and *papC*) adequately predicted APEC and also reported four STs (ST10, ST117, ST48 and ST2016) contained APEC isolates. Hence, the aim of this study was to characterize A*PEC* strains isolated from the droppings of poultry suspected to be suffering from colibacillosis for the presence of VAGs.

Material and methods

Source of isolate

The isolate used for the study was obtained from the droppings of sick birds suspected to be suffering from colibacillosis. *Escherichia coli* was isolated from the droppings using MacConkey agar and eosin–methylene blue agar at the Microbiology Laboratory of the Niger Delta University. Ten pure cultures of the isolates were prepared on Nutrient broth and stored in sterile McCartney bottle and incubated for 18 hours at 35°C before transporting them to the Nigerian Institute for Medical Research (NIMR) Yaba, Lagos for molecular analysis.

DNA extraction and detection of virulence genes

DNA was extracted on overnight culture of the bacteria using the Jena Bioscience DNA purification kit according to the manufacturer's instructions (Jena Bioscience GmbH. Germany). Virulent genes of the isolates were identified using a multiplex PCR technique. The ten isolates were screened for eight virulent genes which include *iutA, iss, ompT, iroN, hlyF, vat, papC* and *iucD* using their respective primers **(Table 1).** In order to test for the virulent genes, two multiplex PCR cycles were carried out as described below. The first multiplex PCR involved identifying the presence of APEC virulent genes; iroN, ompT, hlyF, iutA and iucD. The multiplex PCR was done in a 20µl reaction mixture containing 1X Blend Master mix buffer (Solis Biodyne), 1.5 mM $MgCl_2$, 200µM of each deoxynucleotide triphosphates (dNTP) (Solis Biodyne), 2 units of hot FIREPol DNA polymerase (Solis Biodyne), 20 pMol of each primer (Stab Vida, Portugal), proofreading Enzyme, 2µl of the extracted DNA, while sterile distilled water was used to make up the reaction mixture.

The second multiplex PCR involved identifying the presence of the APEC virulent genes iss, vat, and papC. The multiplex PCR was done in a 20µl reaction mixture comprising of 1X Blend Master mix buffer (Solis Biodyne), 200µM of each dNTP (Solis Biodyne), 1.5mM $MgCl_2$, 20 pMol of each primer (Stab Vida, Portugal), 2 unit of hot FIREPol DNA polymerase (Solis Biodyne), proofreading Enzyme, 2µl of the extracted DNA, while sterile distilled water was added to make up the reaction mixture.

Thermal cycling was conducted using the MJ Research Peltier Thermal Cycler, PTC 200 (GMI, Inc) for an initial denaturation of 95°C for 15 minutes followed by 30 amplification cycles of 30 seconds at 95°C, 30 seconds at 56°C and 1 minute at 72°C. This step was followed by a final extension step of 10 minutes at 72°C. The amplification product was separated on a 1.5% agarose gel electrophoresis that was carried out at 100V for 1 hour 30 minutes, which was followed by DNA bands visualization using ethidium bromide staining. A 100bp DNA ladder (Solis Biodyne) was used as DNA molecular weight marker.

Table 1. Virulence gene primers for APEC.

Gene	Description	Primer sequence	Size (bp)	Reference
iutA	Iron uptake	F:GGCTGGACATCATGGGAACTGG R: CGTCGGGAACGGGTAGAATCG	302	[24]
iucD	Iron uptake	F:ACAAAAAGTTCTATCGCTTCC R: CCTGATCCAGCTGATGCTC	714	[21, 25]
iroN	iron uptake	F: AATCCGGCAAAGAGACGAACCGCCT R: GTTCGGGCAACCCCTGCTTTGACTTT	553	[24]
Iss	serum survival	F: ATCACATAGGATTCTGCCG R: CAGCGGAGTATAGATGCCA	309	21, 25]
ompT	Outer membrane protein	F:TCATCCCGGAAGCCTCCCTCACTACTAT R: TAGCGTTTGCTGCACTGGCTTCTGATAC	496	[23, 24]
hlyF	Toxin	F:GGCCACAGTCGTTTAGGGTGCTTACC R: GGCGGTTTAGGCATTCCGATACTCAG	450	[23, 24]
vat	Toxin	F: TCCTGGGACATAATGGTCAG R: GTGTCAGAACGGAATTGT	981	[25]
papC	Adhesins for attachment	F: TGATATCACGCAGTCAGTAGC R: CCGGCCATATTCACATAA	501	[21, 25]

F=forward primer, R=Reverse primer

Results and discussion

Of the ten isolates (isolates 1-10), only four (Isolates 1, 5, 6 and 8) were positive for the tested virulent genes, hence, 40% of the isolates tested positive for APEC genes. Among the eight VAG, tested, only 4 (iutA, iucD, ompT, hlyF) was detected in all the isolates **(Figure 1).** The genes that were not detected in any of the isolates were *iss, vat, iroN* and *papC* **(Figure 2).** The iron uptake gene iutA, was present in all the four isolates (1, 5, 6 and 8), i.e. 100% presence in all the positive isolates. Another iron uptake gene iucD, was detected only in isolates 1 and 5, thus representing 50%. The outer membrane protein gene ompT, was detected in isolates 1 and 8 only, which therefore represented 50% among the positive isolates. The toxin gene hlyF, was only detected in isolate 8, thus representing 25% of the positive isolates. In all, one VAG was detected in isolate 6 (iutA), two VAGs in Isolate 5 (iutA, iucD) and three each in isolate 1 (iutA, iucD, ompT) and isolate 8 (iutA, ompT, hlyF). When isolate 1 was inoculated in a healthy chicken, it exhibited symptoms of colibacillosis with visible lesions. The results show that iron uptake gene (iutA) was predominant among the isolates.

Using multiplex PCR technique, **Abu El Hammed et al.** [26] determined the prevalence of APEC in *E. coli* isolates based on the detection of 8 VAGs viz; iss, astA, irp2, iucD, papC tsh, cva/cvi and vat. **Bhattarai et al.** [3] analysed for 57 VAGs from four types of chickens infected by colibacillosis in Nepal, and found that the top 5 VAGs are *fimH*, *iss*, *traT*, *sit chro* and *ironEC*. Using multiplex PCR techniques, **Johnson et al.** [24] screened 124 avian *E. coli* isolates of known pathogenicity for 44 VAGs and demonstrated that five genes (*iutA*, *iss*, *hlyF*, *ompT* and *iroN)* carried by plasmids were the most significantly associated with highly pathogenic APEC strains. **Hossain et al.** [27] used four virulence genes, iutA, hlyF, iroN, and iss detected by PCR analysis to characterize APEC in Bangladesh. Other authors have also used the presence of virulent genes to identify APEC linked to colibacillosis in poultry [28 - 31].

In our study, isolate 1, in which three VAGs (iutA, iucD, ompT) were detected, was able to induce colibacillosis in inoculated healthy chicken. This could either mean that these three genes were enough to induce the disease or more VAGs were probably present, which were not analyzed for. The relatively small number of isolates studied is a limitation of this work. Notwithstanding, our study, confirms the detection of markers of APEC in the birds suffering from colibacillosis and shows that VAGs play prominent roles in their pathogenicity. Similarly, other authors have reported VAGs contributing to invasiveness and pathogenicity of other bacteria. For instance, **Oladapo et al.** [32] detected hil A, iro B and stn genes of *Salmonella enterica* serovar typhimurium from non-ripened cheese, which are associated with pathogenicity of the organism. Likewise, Olusola-**Makinde et al.** [33] demonstrated the presence of virulence factors in *Proteus penneri* and *Bacillus cereus* responsible for haemolytic activities of the bacteria. These studies linked the contribution of genetic elements to bacterial pathogenicity.

Figure 1. Result for the multiplex PCR identification of the virulent genes ompT, hlyF, iutA and iucD.

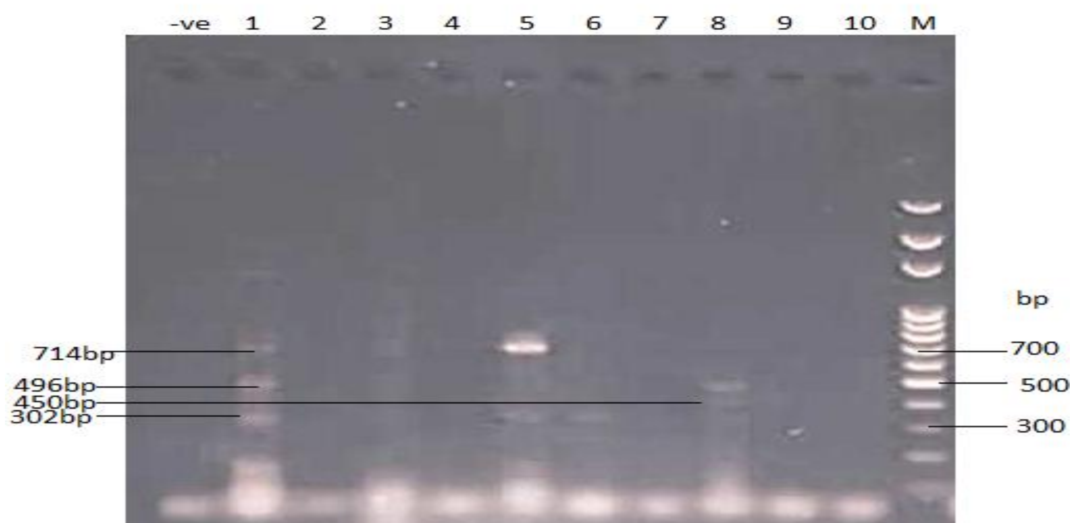
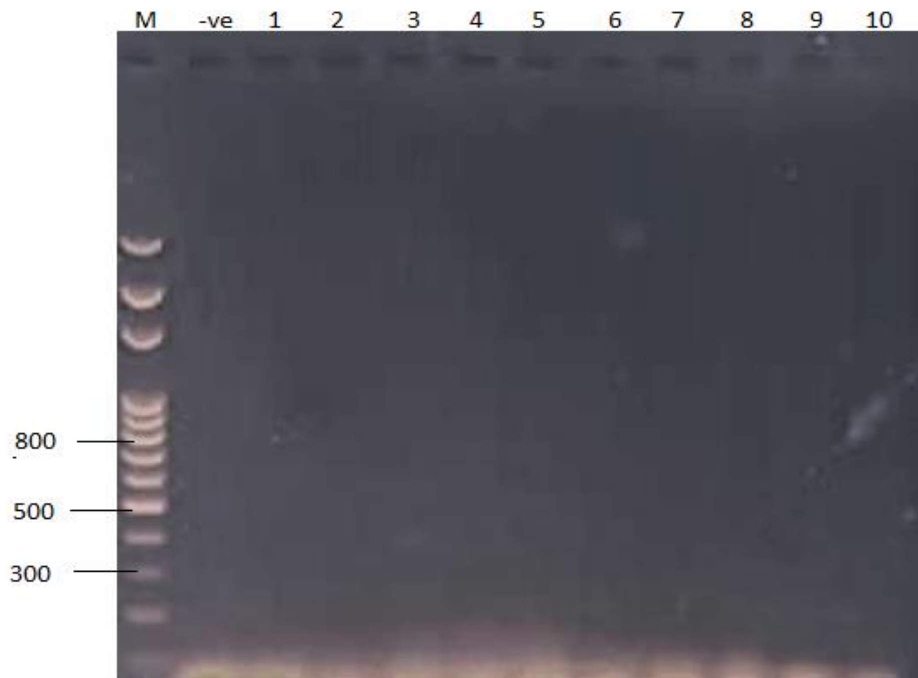


Figure 2. Result for the multiplex PCR identification of the virulent genes iss, vat, and papC.



Conclusion

Ten strains of *E. coli* that was isolated from the droppings of chickens suffering from colibacillosis was analysed for the presence of 8 VAGs using multiplex PCR techniques. The study detected the presence of four VAGs (iutA, iucD, ompT, hlyF) that characterized APEC. Isolate 1, which expressed three of the genes (iutA, iucD, ompT), induced colibacillosis in healthy birds when inoculated. The iron uptake gene, iutA was detected in all the positive isolates. The study therefore confirms the importance of VAGs in the pathogenesis of APEC and their use as markers for its determination.

Acknowledgement

This publication is based on the Ph.D. research work of PST supervised by EIO and RTSO. The study was carried out at the Department of Biological Sciences and Department of Microbiology both of the Faculty of Science and Teaching and Research Farm of the Faculty of Agriculture, Niger Delta University, Wilberforce Island, Nigeria. Molecular analysis was carried out at Nigerian Institute for Medical Research (NIMR), Yaba, Lagos.

Conflicts of interest

Not declared.

Funding

None.

References

1- **Nakazato G, Campos TAD, Stehling EG, Brocchi M, Silveira WDD.** Virulence factors of avian pathogenic *Escherichia coli* (APEC). Pesquisa Veterinária Brasileira 2009; 29: 479-486.

2- **Delannoy S, Schouler C, Souillard R, Yousfi L, Le Devendec L, Lucas C, et al.** Diversity of *Escherichia coli* strains isolated from day-old broiler chicks, their environment and colibacillosis lesions in 80 flocks in France. Veterinary Microbiology 2021; 252: 108923.

3- **Bhattarai RK, Basnet HB, Dhakal IP, Alocilja EC.** Virulence genes of avian pathogenic *Escherichia coli* isolated from commercial chicken in Nepal. *Comparative* Immunology, Microbiology and Infectious Diseases 2023; 95: 101961.

4- **Giovanardi D, Lupini C, Pesente P, Rossi G, Ortali G, Catelli E.** Characterization and

antimicrobial resistance analysis of avian pathogenic *Escherichia coli* isolated from Italian turkey flocks. Poultry Science 2013; 92(10): 2661-2667.

5- **Janßen T, Schwarz C, Preikschat P, Voss M, Philipp HC, Wieler LH.** Virulence-associated genes in avian pathogenic *Escherichia coli* (APEC) isolated from internal organs of poultry having died from colibacillosis. International Journal of Medical Microbiology 2001; 291(5): 371-378.

6- **Mu X, Gao R, Xiao W, Gao Q, Cao C. Xu H, et al.** EntE, EntS and TolC synergistically contributed to the pathogenesis of APEC strain E058. Microbial Pathogenesis 2020; 141: 103990.

7- **Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G, Choroszy-Krol I.** Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. Gut Pathogens 2019; 11: 1-16.

8- **Subedi M, Luitel H, Devkota B, Bhattarai RK, Phuyal S. Panthi P, et al.** Antibiotic resistance pattern and virulence genes content in avian pathogenic *Escherichia coli* (APEC) from broiler chickens in Chitwan, Nepal. BMC Veterinary Research 2018; 14(1): 1-6

9- **Dissanayake DRA, Octavia S, Lan R.** Population structure and virulence content of avian pathogenic *Escherichia coli* isolated from outbreaks in Sri Lanka. Veterinary Microbiology 2014; 168(2-4): 403-412.

10- **Kaper JB, Nataro JP, Mobley HL.** Pathogenic *Escherichia coli*. Nature Reviews Microbiology 2004; 2(2): 123-140.

11- **Kathayat D, Lokesh D, Ranjit S, Rajashekara G.** Avian pathogenic *Escherichia coli* (APEC): an overview of virulence and pathogenesis factors, zoonotic potential, and control strategies. Pathogens 2021; 10(4): 467.

12- **Olsen RH, Christensen H, Bisgaard M.** Comparative genomics of multiple plasmids from APEC associated with clonal outbreaks demonstrates major similarities and identifies several potential vaccine-targets. Veterinary Microbiology 2012; 158(3-4): 384-393.

13- **Hussain HI, Iqbal Z, Iqbal M, Kuang X, Wang Y, Yang L, et al.** Coexistence of virulence and β-lactamase genes in avian pathogenic *Escherichia coli*. Microbial Pathogenesis 2022; 163: 105389.

14- **Aslam M, Toufeer M, Bravo CN, Lai V, Rempel H, Manges A, et al.** Characterization of extraintestinal pathogenic *Escherichia coli* isolated from retail poultry meats from Alberta, Canada. International Journal of Food Microbiology 2014;177: 49-56.

15- **LeStrange K, Markland SM, Hoover DG, Sharma M, Kniel KE.** An evaluation of the virulence and adherence properties of avian pathogenic *Escherichia coli*. One Health 2017; 4: 22-26.

16- **Jørgensen SL, Stegger M, Kudirkiene E, Lilje B, Poulsen LL, Ronco T, et al.** Diversity and population overlap between avian and human *Escherichia coli* belonging to sequence type 95. MSphere 2019; 4(1): e00333-18.

17- **Kim YB, Yoon MY, Ha JS, Seo KW, Noh EB, Son SH, et al.** Molecular characterization of avian pathogenic *Escherichia coli* from broiler chickens with colibacillosis. Poultry Science 2020; 99(2): 1088-1095.

18-Singh SD, Mahender M, Dhama K. Studies on virulence factors associated with pathogenicity in Avian *Escherichia coli*. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases 2009; 30(2): 95-100.

19-Mellata M, Dho-Moulin M, Dozois CM, Curtiss III R, Brown PK, Arné P, et al. Role of virulence factors in resistance of avian pathogenic *Escherichia coli* to serum and in pathogenicity. Infection and Immunity 2003; 71(1): 536-540.

20-Ievy S, Islam MS, Sobur MA, Talukder M, Rahman MB, Khan MFR, et al. Molecular detection of avian pathogenic *Escherichia coli* (APEC) for the first time in layer farms in Bangladesh and their antibiotic resistance patterns. Microorganisms 2020; 8(7): 1021.

21-Lee A, Aldeieg M, Woodward MJ, Juniper DT, Rymer C. The effect of *Candida famata* and *Lactobacillus plantarum* on the number of coliforms and the antibiotic resistance and virulence of *Escherichia coli* in the gut of broilers. Animal 2021; 15(8): 100310.

22-Barbieri NL, Pimenta RL, De Melo DA, Nolan LK, De Souza MMS, Logue, CM. Mcr-1 identified in fecal *Escherichia coli* and avian pathogenic *E. coli* (APEC) from Brazil. Frontiers in Microbiology 2021; 12: 659613.

23-Johnson TJ, Miller EA, Flores-Figueroa C, Munoz-Aguayo J, Cardona C, Fransen K, et al. Refining the definition of the avian pathogenic *Escherichia coli* (APEC) pathotype through inclusion of high-risk clonal groups. Poultry Science 2022; 101(10): 102009

24-Johnson TJ, Wannemuehler Y, Doetkott C, Johnson SJ, Rosenberger SC, Nolan LK. Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. Journal of Clinical Microbiology 2008; 46(12): 3987-3996.

25-Paixao AC, Ferreira AC, Fontes M, Themudo P, Albuquerque T, Soares MC, et al. Detection of virulence-associated genes in pathogenic and commensal avian Escherichia coli isolates. Poultry Science 2016; 95(7): 1646-1652.

26-Abu El Hammed W, Soufy H, EL-Shemy A, Nasr S, Dessouky MI. Isolation and Molecular Identification of Avian Pathogenic *Escherichia coli* in Broiler Chickens Suffering from Colibacillosis in some Governorates in Egypt. Egyptian Journal of Veterinary Sciences 2022; 53(1): 31-41.

27-Hossain FE, Islam S, Islam MA, Ahmed F. Detection of virulence genes of APEC (avian pathogenic *Escherichia coli*) isolated from poultry in Noakhali, Bangladesh. Bioresearch Communications 2021; 7(1): 967-972.

28-Schouler C, Schaeffer B, Brée A, Mora A, Dahbi G, Biet F, et al. Diagnostic strategy for identifying avian pathogenic *Escherichia coli* based on four patterns of virulence genes. Journal of Clinical Microbiology 2012; 50(5): 1673-1678.

29-Rao M, Devi V, Sreedevi C, Rao T, Chowdary C, Annapurna P. Molecular Detection of Certain Virulence Genes of Avian Pathogenic *Escherichia coli* (APEC) in Chicken. International Journal of Livestock Research 2022; 12(5): 15-18. Doi:10.5455/ijlr.20220601053400

30-Mehat, JW, van Vliet AH, La Ragione RM. The Avian Pathogenic *Escherichia coli* (APEC) pathotype is comprised of multiple distinct, independent genotypes. Avian Pathology 2021; 50(5): 402-416.

31-Kravik IH, Kaspersen H, Sjurseth SK, Dean KR, David B, Aspholm M, et al. A molecular epidemiological study on *Escherichia coli* in young chicks with colibacillosis identified two possible outbreaks across farms. Veterinary Research 2023; 54(1): 10.

32-Oladapo OD, Onifade AK, Bayode MT. Direct detection of iro B, stn and hil A virulence genes in Salmonella enterica serovar typhimurium from non-ripened cheese. Bulletin of the National Research Centre 2022; 46(1): 175.

33-Olusola-Makinde O, Bayode M, Somefun A. Upshot of virulence markers and effects of temperature and pH on haemolytic bacteria in South-west Nigeria. Microbes and Infectious Diseases 2022; 3(2), 472-483

Tobia PS and Ohimain EI. Molecular determination of virulent genes from avian pathogenic *Escherichia coli* isolated from poultry farm droppings in Bayelsa State, Nigeria. Microbes Infect Dis 2023; Article-In-Press, **DOI:** 10.21608/mid.2023.218901.1548.