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Original Article

In vitro assessment of pathogenicity and virulence encoding gene profiles of avian pathogenic *Escherichia coli* strains associated with colibacillosis in chickens

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Abstract

Background: Avian pathogenic *Escherichia coli* (APEC) strains have been associated with various disease conditions in avian species due to virulence attributes associated with the organism. **Aims:** This study was carried out to determine the *in vitro* pathogenic characteristics and virulence encoding genes found in *E. coli* strains associated with colibacillosis in chickens. **Methods:** Fifty-two stock cultures of *E. coli* strains isolated from chickens diagnosed of colibacillosis were tested for their ability to produce haemolysis on blood agar and take up Congo red dye. Molecular characterization was carried out by polymerase chain reaction (PCR) amplification of virulence encoding genes associated with APEC. **Results:** Eleven (22%) and 41 (71%) were positive for haemolysis on 5% sheep red blood agar and Congo red agar, respectively. Nine virulence-associated genes were detected as follows: *FimH* (96%), *csgA* (52%), *iss* (48%), *iut* (33%), *tsh* (21%), *cva* (15%), *kpsII* (10%), *pap* (2%), and *felA* (2%). **Conclusion:** The APEC strains exhibited virulence properties and harbored virulence encoding genes which could be a threat to the poultry population and public health. The putative virulence genes were diverse and different in almost all isolate implying that pathogenesis was multi-factorial and the infection was multi-faceted which could be a source of concern in the detection and control of APEC infections.

Key words: APEC, Chicken, Colibacillosis, Virulence genes

Introduction

Avian pathogenic *Escherichia coli* (APEC) strains cause various diseases in chickens and are responsible for large economic losses in the poultry industry worldwide (Zhuang *et al*., 2014; Zeinab *et al*., 2018). Avian pathogenic *Escherichia coli* strains are associated with infection of extraintestinal tissues in chickens, turkeys, ducks, and other avian species (Barbieri *et al*., 2015). Thus, APEC strains have been implicated in a variety of disease conditions including: coligranuloma, air sac disease, perihepatitis, airsacculitis, pericarditis, egg peritonitis, salpingitis, omphlitis, cellulitis and osteomyelitis or arthritis (Nolan *et al*., 2013).

The most important disease syndrome associated with APEC begins as a respiratory tract infection and is often known as airsacculitis or the air sac disease, which in turn can evolve into severe sepsis or systemic infection ultimately leading to the death of the bird. Respiratory lesions observed include airsacculitis with a serous to fibrinous exudates, an initial infiltration with heterophils, and a subsequent predominance of mononuclear phagocytes (Mellata, 2013). Sites of entry into the bloodstream are presumed to be the gas exchange region of the lung and the air sacs, which are relatively vulnerable to colonization and invasion by bacteria due to lack of resident macrophages (Guabirabaand and Schouler, 2015).

Avian pathogenic *E. coli* isolates possess several potential virulence factors related to colonization, temperature-sensitive haemagglutinin, complement resistance and increased serum survival (Mainil, 2013). Knowledge of virulence factors associated with APEC is necessary in explaining the pathogenesis of these organisms which could be helpful in management and control of the disease. These virulence factors are usually encoded or mediated by genes which often are transferable from pathogenic to non-pathogenic strains and vice versa, in a multi-cultural environment like the gastrointestinal tract (GIT) (Ogura *et al*., 2009).

The presence of certain virulence-associated genes among APEC strains as well as similar disease patterns and phylogenetic background is an indication of a significant zoonotic risk of avian-derived *E. coli* infections (Bauchart *et al*., 2010). *Escherichia coli* isolated from healthy chickens have been reported to contain extraintestinal pathogenic *Escherichia coli* (ExPEC)-associated gene and can cause ExPEC-associated infections in animal models and thus may pose a health threat to the host, including humans

(Stromberg *et al*., 2017). Since chickens are usually in close contact with humans in the poultry industry value chain, APEC could also be of high public health risk.

Many studies across the globe including Nigeria have shown the prevalence of APEC strains among chickens diagnosed of colibacillosis (Olarinmoye *et al*., 2013; Barbieri *et al*., 2015; Ali *et al*., 2019). Different virulence-associated genes of APEC have also been documented worldwide (Wang *et al*., 2015; Sarowska *et al*., 2019). The purpose of this study was to determine the virulence-associated genes among *E. coli* strains isolated from chickens with colibacillosis in Enugu State, Nigeria.

Materials and Methods

Escherichia coli strains

Fifty-two stock cultures of *E. coli* strains isolated from the liver (20 strains), spleen (10 strains), heart blood (18 strains), and oviduct (4 strains) of broilers and layers diagnosed with colibacillosis in Nsukka, Nigeria were screened for the presence of virulence encoding genes. The stock cultures were inoculated onto nutrient broth and incubated for 24 h at 37°C. An aliquot of the broth culture was sub-cultured onto MacConkey agar (MCA) and confirmed by biochemical tests.

In vitro pathogenicity testing

Haemolytic activity

Escherichia coli isolates were streaked on blood agar plates. The inoculated blood agar plates were incubated at 37°C for 24 h and colonies producing clear zones of haemolysis were then recorded as hemolytic strains (Fakruddin *et al*., 2012). A known haemolytic *Staphylococcus* spp. and non-haemolytic *Klebsiella pneumoniae* isolates were used as positive and negative controls, respectively.

Congo red uptake

Each isolate was inoculated on a separate Congo red agar plate and incubated at 37°C for 24 h. After 24 h incubation, the cultures were left at room temperature for 48 h to facilitate annotation of results (Osman *et al*., 2012). Congo red uptake was indicated by the appearance of red colonies on the Congo red agar while colourless colonies indicated an inability to take up Congo red. A known Congo red positive *E. coli* and Congo red negative *Salmonella* isolates were used as positive and negative controls, respectively.

DNA extraction

DNA extraction was done following the standard phenol-chloroform method described by Sharpe (2005). Each *E. coli* strain was inoculated into 10 ml nutrient broth (HiMedia, India) and incubated at 37°C for 24 h. One ml of the culture was centrifuged at 12,000 g for 2 min. The cell pellet was then re-suspended in 200 µL of Tris-EDTA buffer (pH = 7.2) and 30 µL of lysozyme (2000U/µL). The mixture was incubated at 37°C for 1 h. It was then mixed with 33 µL of 10% sodium dodecyl sulphate (v/v) and incubated at 62°C for 30 min. Three-hundred microlitres of phenol: chloroform: isoamyl alcohol (25:24:1) were added to the mixture and vortex for 10 s followed by centrifugation at 12,000 g for 1 min. The top aqueous phase was collected into a new centrifuge tube and added to 1/10 volume of 3 M sodium acetate and mixed by inversion. It was then mixed with 2 volumes of 100% ethanol and incubated on ice for 5 min. The samples were then centrifuged at 12,000 g for 5 min and the supernatant removed. The DNA pellet was washed with 1 ml 70% ethanol and centrifuged at 12,000 g for 1 min and air-dried for 10 min. The DNA was re-suspended in 100 µL of Tris-EDTA buffer (pH = 8.0).

Detection of virulence-associated genes

The APEC strains were investigated for the presence of virulence-associated genes by polymerase chain reaction (PCR) following the procedure described by Rocha *et al*. (2008). The genes investigated were *FimH*, *pap*, *felA*, *sfa*, *fac*, *csgA*, *tsh*, *cvaC*, *kpsII*, *iss*, *iutA*, and *cnf*. The sequences of the primers used and PCR conditions are presented in Table 1.

The PCR assay was performed in a total volume of 25 µL of a mixture containing 3 µL of DNA template, 1 µL of each primer (IDT-Integrated DNA Technologies, Singapore), 1 × Taq buffer (10 mM Tris-HCl, pH = 8.8, 50 mM KCl), 1.0 mM $MgCl_2$, 0.2 mM of dNTPs and 1.25U Taq DNA polymerase (Promega, USA). The PCR condition was as follows: initial denaturation at 94°C for 5 min; followed by repeated cycle of denaturation at 94°C for 1 min, annealing for 30 s, and extension at 72°C for 30 s, and a final extension at 72°C for 7 min. Reaction products were separated by agarose gel electrophoresis by adding 1 µL of EZ-Vision DNA dye (Amresco, USA) to 5 µL of PCR product onto a 1% agarose gel (Vivantis, Malaysia). The buffer in the electrophoresis chamber and the agarose gel was 1 × Tris-acetate-EDTA (TAE) buffer. One-hundred volts and 400 mA were applied across the gel for 30 min. DNA in the gel was visualized under ultraviolet light (UV) using UVItec Gel Documentation System (USA). A 1 kb molecular weight marker (Promega, USA) was used.

Statistical analysis

Data were presented in the form of percentages, tables and images. Chi-square test was used to determine the association between virulence genes and chicken type and source of infection. Significance was accepted at 5% probability level.

Results

In vitro pathogenicity test

Out of fifty-two *E. coli* isolates used in this study, 11 (19%) of them were haemolytic on 5% sheep blood agar while 41 (71%) of the isolates were Congo red positive. Five of the haemolytic strains were isolated from broilers while six were gotten from layers. Out of the forty-one strains that bound to Congo red dye, 18 were from layers while 23 were from broilers chickens.

Table 1: Sequence of PCR primers, product size, annealing temperature and cycles (Rocha *et al*., 2008)

Gene	Primer sequence (5´-3´)	Product size (bp)	Annealing temp (°C)	cycles
fimH	CGA GTT ATT ACC CTG TTT GCT G (F) ACG CCA ATA ATC GAT TGC AC (R)	878	55	35
papC	GAC GGC TGT ACT GCA GGG TGT GGC G ATA TCC TTT CTG CAG GGA TGC AAT A	328	63	30
felA	GGC AGT GGT GTC TTT TGG TG GGC CCA GTA AAA GAT AAT TGA ACC	270	63	35
Sfa	CTC CGG AGA ACT GGG TGC ATC TTA C CGG AGG AGT AAT TAC AAA CCT GGC A	410	55	35
Fac	GGT GGA ACC GCA GAA AAT AC GAA CTG TTG GGG AAA GAG TG	388	58	35
csgA	ATC AGT ACG GTG GTG GTA ACT C CCA ACA TCT GCA CCG TTA CCA C	103	64	40
Tsh	GGT GGT GCA CTG GAG TGG AGT CCA GCG TGA TAG TGG	620	55	30
Cva	CAC ACA CAA ACG GGA GCT GTT CTT CCC GCA GCA TAG TTC CAT	680	63	30
Kpsll	GCG CAT TTG CTG ATA CTG TTG CAT CCA GAC GAT AAG CAT GAG CA	272	65	30
Iss	GTG GCG AAA ACT AGT AAA ACA GC CGC CTC GGG GTG GAT AA	760	61	30
iutA	GGC TGG ACA TCA TGG GAA CTG G CGT CGG GAA CGG GTA GAA TCG	300	63	35
cnf	CTG GAC TCG AGG TGG TGG GAA CTT ATT AAG GAT AGT	533	55	30

PCR: Polymerase chain reaction

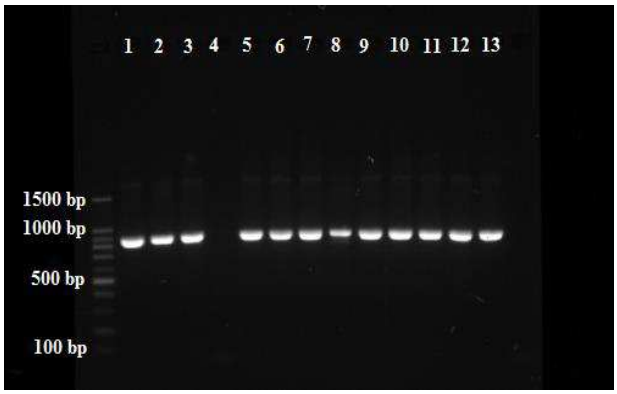

Fig. 1: Representative gel of *FimH* gene in APEC strains. Positive strains produced 878 bp band. First Lane on the right is 1.5 kb DNA ladder, Lanes 1-3, and 5-13: Positive, and Lane 4: Negative

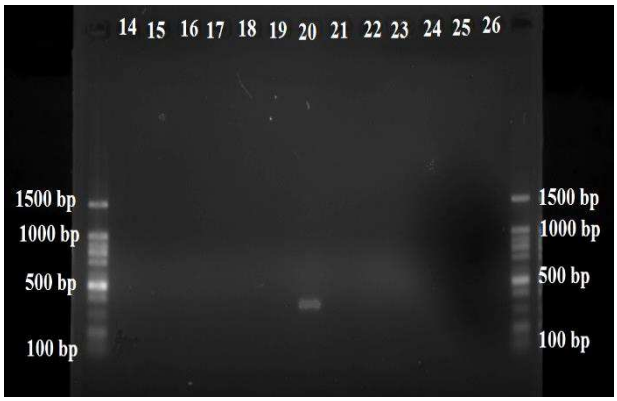

Fig. 2: Representative gel of *papC* gene detection in APEC strains. Positive strains produced 328 bp band. First and last Lanes are 1.5 kb DNA ladder, Lane 20: Positive and the other Lanes: Negative

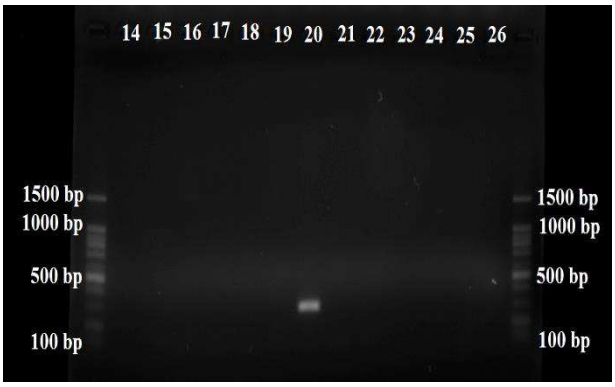

Fig. 3: Representative gel of *felA* gene detection in APEC strains. Positive strain produced 270 bp band. First and last Lanes are 1.5 kb DNA ladder, Lane 20: Positive and the other Lanes: Negative

Occurrence and distribution of virulence-associated genes of APEC from chickens

The APEC strains harbored 9 out of the 12 genes investigated. *FimH* (878 bp) gene (Fig. 1) has the highest occurrence (96.2%) while *pap* and *felA* genes (Figs. 2 and 3, respectively) had the least occurrence (2%). None of the strains was positive for *sfa*, *fac*, and *cnf* genes (Table 2). Out of the 50 *FimH* positive strains, 14 (27.5%) were from broilers while 37 (72.5%) were from layers (Table 3).

The patterns of occurrence of the virulence-associated gene detected from the APEC strains are presented in Table 4. A total of 22 patterns were observed, with *fimH-csgA-iss* being the predominant combination. The number of virulence genes per strain ranged from 1 to 8, with the majority (35.3%) of the strains harboring three virulence genes.

Table 2: Percentage distribution of the virulence associated genes detected in APEC strains in Enugu State

Genes	*fimH*	*pap*	*felA*	*sfa*	*fac*	*csgA*	*tsh*	*cvaC*	*kpsII*	*iss*	*iut*	*cnf*
No. of positive	50	1	1	0	0	27	11	8	5	25	17	0
(%)	(96.2)	(1.9)	(1.9)	(0.0)	(0.0)	(51.9)	(21.2)	(15.4)	(9.6)	(48.1)	(32.7)	(0.0)

Table 3: Distribution of *E. coli* strains positive for virulence gene from different tissues and bird type

Bird type	Tissue collected	No. of positive (%)
Laying bird (layers)	Heart	13 (35.1)
	Liver	12 (32.4)
	Spleen	8 (21.6)
	Oviduct	4 (10.8)
	Sub total	37 (72.5)
Broilers	Heart	4 (28.6)
	Liver	8 (57.1)
	Spleen	2 (25)
	Sub total	14 (27.5)
	Grand total	51 (100)

Table 4: Patterns of occurrence of virulence associated genes in APEC strains from chicken samples

S/No.	Virulence gene pattern	No. of positive (%)
1	*fimH*	11 (21.6)
2	*fimH-csgA*	5 (9.8)
3	*fimH-iss*	3 (5.9)
4	*fimH-tsh*	1 (2.0)
5	*fimH-iut*	1 (2.0)
6	*fimH-csgA-iss*	9 (17.6)
7	*fimH-tsh-kpsII*	1 (2.0)
8	*fimH-tsh-cvaC*	1 (2.0)
9	*fimH-csgA-iut*	2 (3.9)
10	*fimH-cvaC-iut*	3 (5.9)
11	*fimH-iss-iut*	1 (2.0)
12	*csgA-tsh-iss*	1 (2.0)
13	*fimH-csgA-tsh-iss*	1 (2.0)
14	*fimH-csgA-kpsII-iss*	2 (3.9)
15	*fimH-csgA-iss-iut*	2 (3.9)
16	*fimH-tsh-cvaC-iut*	1 (2.0)
17	*fimH-csgA-kpsII-iss-iut*	1 (2.0)
18	*fimH-csgA-tsh-iss-iut*	1 (2.0)
19	*fimH-tsh-cvaC-iss-iut*	1 (2.0)
20	*fimH-csgA-tsh-kpsII-iss-iut*	1 (2.0)
21	*fimH-csgA-tsh-cvaC-iss-iut*	1 (2.0)
22	*fimH-pap-felA-csgA-tsh-cvaC-iss-iut*	1 (2.0)
Total		51 (100)

S/No.: Serial number

The association between virulence gene and bird type and source of isolation is shown in Table 5. There was no significant association between virulence gene and bird type (P>0.05) but there was a significant association between the virulence genes and the tissue of isolation (P<0.05).

Discussion

Haemolysis is usually associated with pathogenicity of *E. coli*, especially the more severe forms of infection and is usually seen in *E. coli* strains isolated from blood (Daga *et al*., 2019). In this study, 11% of the *E. coli* strains were haemolytic which is higher than 1.5% haemolytic strains reported by Shankar *et al*. (2010) among APEC strains isolated from colisepticeamic chickens lower than 37.03% of APEC strains isolated from broiler chickens reported by AL-Saiedi and Al-Mayah (2014). Although it was reported that haemolytic activity is one of the important factors of pathogenicity in APEC strains, Sharada *et al*. (1999) stated that avian *E. coli* must not be haemolytic before they can be classified as pathogenic. Al-Arfaj *et al*. (2016) recognised haemolysis, Congo red uptake among others as phenotypic markers of virulence among *E. coli* strains associated with colibacillosis in chicken. Avian *E. coli* strains isolated from blood usually record high heamolytic activity but since these strains were isolated from other organs, it is only probable that haemolysis may not be the major pathway to their pathogenesis.

Congo red uptake by *E. coli* is a marker for differentiation of colisepticaemic (invasive) strains from non-coliseptecaemic *E. coli* in poultry (Al-Arfaj *et al*., 2016). An increase in virulence of bacteria strains has also been reported in bacteria that bind to Congo red dye (Ambalam *et al*., 2012). Among the 57 APEC strains isolated from chickens in this study, 41 (71%) bound to Congo red dye (positive), suggesting that most of the APEC strains studied were invasive and therefore pathogenic. This result was higher than the findings who reported that 40% of *E. coli* isolates from clinical cases of colibacillosis in the northern part of Nigeria were Congo red positive. This result was also higher than the 60% reported by AL-Saiedi and Al-Mayah (2014).

Ninety-eight percent of the 52 *E. coli* strains screened were positive for at least one of the 12 virulence genes studied which are usually implicated in the pathogenicity

Table 5: Association between virulence genes and type of bird and tissue samples

Variables		Virulence genes								
		fimH	*pap*	*felA*	*csgA*	*tsh*	*cvaC*	*kpsII*	*iss*	*iut*
Type of bird	Broilers	14	0	0	7	3	1	0	9	3
	Layers	37	1	1	20	9	7	5	15	12
Organ	Liver	20*	0	0	10	6	2	2	9	4
	Spleen	10*	0	0	5	1	3	1	4	6
	Heart	17*	1	1	10	5	3	2	10	4
	Oviduct	4*	0	0	2	0	0	0	1	1

* Significant association (P<0.05)

of APEC strains (Nakazato *et al*., 2009). This indicates that the *E. coli* strains are APEC strains since they were isolated from confirmed cases of colibacillosis in chicken. This finding is in agreement with the findings of Mbanga and Nyararai (2015) and Mohamed *et al*. (2018) who found 93% and 98% of *E. coli* strains from chicken in Zimbabwe and Algeria, respectively, haboring at least one virulence-associated gene.

The type I fimbrial adhesion gene (*FimH*) was the most prevalent gene detected in this study. *FimH* encodes for type 1 pili (Ionica *et al*., 2012) and plays a role in extraintestinal *E. coli* translocation through the intestinal epithelium and invasion (Poole *et al*., 2017). *FimH* is also the gene responsible for the mannose-specific or receptor-specific adhesin encoding the synthesis of type 1 fimbriae (Ionica *et al*., 2012). *FimH* in the form of *Fim DsG* complex is a relevant target for the development of anti-adhesive drugs (Sauer *et al*., 2016). The high binding ability of *FimH* could result in increased bacterial binding to target cells and increased pathogenicity of *E. coli*; thus, *FimH* could be used to design vaccine for the prevention of *E. coli* infections by blocking the bacterial attachment and colonization (Hojati *et al*., 2015). Previously, authors like Rodriguez-Siek *et al.* (2005) in USA and Wu *et al*. (2012) in the UK had reported similar occurrence of 98.1% and 100% of the *FimH* gene among APEC strains, respectively. However, a lower prevalence rate of 33.3% of *FimH* was recorded in APEC strains in Zimbabwe (Mbanga and Nyararai, 2015).

From the present study, 2% of the APEC strains harboured the *papC* gene. The *pap* operon which encodes for P fimbriae, is involved in bacterial colonization in respiratory epithelium which directly affects the intensity of infection (Melican *et al*., 2011). The *pap* gene has been reported to play a significant role during septicaemic infection as it was observed to be associated more with pathogenic *E. coli* isolates from septicaemic chickens than from healthy chickens (Subashchandrabose and Mobley, 2015). This gene is often present in urinary tract infections in humans and chickens (Rahdar *et al*., 2015), thereby making the strains a potential zoonotic danger. This finding is lower than that of Samah *et al*. (2015) and Hasani *et al*. (2017) who reported 8.3% and 20% prevalence, respectively of *papC* gene in *E. coli* strains tested. The *fel*A is the operon that codifies a serological variant of P fimbriae (F11) (Tseng *et al*., 2018). In this study, 2% of the strains were also positive for *fel*A gene. Different results have been obtained by researchers in different regions. For instances, Rocha *et al*. (2008) reported 38.8% in Brazil and Rodriguez-Siek *et al*. (2005) reported a higher prevalence rate of 78% in the United Kingdom. The variation in these reports could be attributed to regional differences and sample size investigated.

The *csgA* gene had a prevalence rate of 51.9% in the present study. Curli fibers encoded by *csgA* gene have been reported to be essential for the internalization of bacteria causing avian septicaemia *in vitro* (Van Gerven *et al*., 2018). This finding was lower than what Dho-Moulin and Fairbrother (1999) reported (99%) in *E. coli* from diseased chicken. *csgA* gene has been linked to biofilm formation in rats and increased invasion (Oppong *et al*., 2015) which could be a source of concern in antimicrobial resistance.

The *cvaC* gene encoding for Colicin V is involved in extra-intestinal infections affecting humans and animals by interfering with membrane formation and inhibiting bacterial growth (Gérard *et al*., 2005) thereby reducing bacterial population and competition. Expression of numerous virulence genes including *cvaC*, *iss*, and *iutA* were associated with the pathogenesis of colibacillosis in boiler chickens with gross and histopathological lesions (Sharif *et al*., 2018). In this study, 15.38% of the strains were positive for *cvaC* gene. This finding was similar to the findings of Ghafoor e*t al*. (2017) who reported 10.52% in their studies. Our finding was lower than the findings of Kumar *et al*. (2013) who reported a higher prevalence of 35%.

Twelve isolates (21.15%) haboured temperature-sensitive hemagglutinin (*tsh*) gene which encodes for autotransporter protein which is frequently found in APEC (Sarowska *et al*., 2019). Hasani *et al*. (2017) reported prevalence rates of 49.3% of the *tsh* gene in 71 APEC strains studied in Iran which is slightly higher than what was found in this study. Similarly, Won (2009) reported 55% of *tsh* gene among 118 APEC strains studied in Korea. The low prevalence rate may be connected with a relatively low prevalence of *cvaC* which has been reported to be associated with *tsh.* Paixao *et al*. (2016) have reported its role during pathogenesis of APEC infections in high-lethality *E. coli* isolates and its link to colicin V genes when they were present on the same plasmid. It contributes to the development of lesions in the air sac and is associated with high virulence among APEC strains.

The *iutA* gene is one of genes that encodes for siderophores (aerobactin operon). Aerobactin is produced more especially by invasive *E. coli*. The aerobactin system enables microorganisms to grow in iron-free media at low concentration (Garénaux *et al*., 2011). The aerobaction system plays a role in the persistence and generation of lesions in APEC infected chicken (Mbanga and Nyararai, 2015). The *iutA* prevalence of 32.7% recorded in this study is lower than that reported by Sharif *et al*. (2005) and Mbanga and Nyararai (2015) who found 96% and 80% prevalence of *iutA* gene, respectively. However, our finding was similar to Wu *et al*. (2012) who reported prevalence rate of 50% in their studies.

The *iss* gene was prevalent in 48.08% of the APEC strains. Increased serum survival (*iss*) gene is known to be associated with serum resistance (Barbieri *et al*., 2013). It is considered the most significantly associated gene with APEC strains (Dissanayake *et al*., 2014). The *iss* gene has been detected at a higher percentage in extraintestinal strains of the diseased birds that reached 72.2% when compared to no detection in the intestinal strains and this gives insight to the importance of its pathogenicity (Mohamed *et al*., 2014). This finding was

lower than Dissanayake *et al*. (2014) who reported that 80.5% of APEC isolates were positive for the *iss* gene in the United States of America. Samples analysed in this study showed 9.6% positive for *kpsII* gene. The K1 and K5 antigens are codified by *kps* genes (Wijetunge *et al*., 2015). The K1 antigen is thought to be an important virulence factor of *E. coli* while K5 antigen occurs frequently amongst *E. coli* strains isolated from extra-intestinal infections (Sarowska *et al*., 2019). This finding was lower than the 18% reported by Rocha *et al*. (2008) in Brazil.

The virulence genes investigated in this study, occurred in various combinations with *fimH-csgA-iss* being the most predominant. Avian pathogenic *E. coli* strains possess virulence traits that make them live extraintestinally and each strain has several virulence factors with several combinations of genes (Circella *et al*., 2012). In this study, 12 (23%) strains had 4 or more genes while 18 (35.3%) revealed 3 of the virulence-associated genes. Possessing *iutA*, *tsh* and *cva/cvi*, colicin V plasmids have been considered to be a defining feature of the APEC strains (Borzi *et al*., 2018). The diversity of genes associated with pathogenicity detected among the *E. coli* strains tested in this study and other studies especially among APEC strains may indicate an interaction among these virulence traits.

There was no significant association ($P>0.05$) between the virulence genes and the chicken type and source of isolation but there was a significant association ($P<0.05$) between *fimH* gene and the organ of isolation. This was in disagreement with the findings of Vandamaele *et al*. (2005) who did not find an association between the occurrence of *fimH* gene and chicken type and organ of isolation.

The *E. coli* strains showed multiple pathways to virulence which highlights the danger imposed by these organisms to their hosts. Because these genes may be carried by mobile genetic elements, the spread of virulence genes among *E. coli* strains could be a huge risk. This is more dangerous since some *E. coli* strains are usually commensal and could acquire virulence attributes thereby becoming pathogenic especially in immune-compromised hosts. The public health implication of this is also enormous as there is always a continual exposure of humans to chicken and poultry manure. *Escherichia coli* from chicken in the study area had been reported to be multi-drug resistant (Ugwu *et al*., 2017) and following the report of Stella *et al*. (2016) that virulence genes are common in *E. coli* strains resistant to one or more antimicrobials, then there is a possibility of animal and human health being hypothetically in danger. A difficult to treat, highly virulent *E. coli* strains could be a problem to the poultry industry and have the potential to be a major public health hazard.

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Conflict of interest

The authors declare that they do not have any conflict of interest.

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