Molecular Screening Of Virulence Genes In Avian Pathogenic *Esherichia Coli*

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ABSTRACT

Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis, which is one of the main causes of economic losses in the poultry industry worldwide. This disease occurs only when the *E. coli* infecting strain presents virulence factors (encoded by specific genes) that enable the adhesion and proliferation in the host organism. Herin, 15 *E. coli* strains of different serogroups isolated from birds with colibacillosis were assigned to their phylogenetic groups and analyzed for the occurrence of 11 virulence associated genes. phylogenetic typing showed that group B2 was the largest (33.3%, 5/15) followed by group B1 (26.7%, 4/15), groups A and D were similar in size (22.1%, 19/86). The virulence profiles showed that *omp*A was found in most isolates 14/15 (93.3%). The *iss* gene was found in13/15 (86.6%). Followed by *tra*T and *iut*A genes which were found in12/15 (80%). *cva*C, *stx*2 and *tsh* genes were present in 9/15(60%), 7/15(46%) and of the isolates, respectively. Only one isolate gave positive amplification for *stx*1 and *ibe*A genes each. The *hly* gene was not encountered in any of the tested isolates.

keywords: Avian pathogenic *Escherichia coli* (APEC), phylogenetic analysis, virulence genes.

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1. INTRODUCTION

Colibacillosis is one of the most important bacterial disease of poultry, the economic impact of this disease is difficult to assess, and it is widely assumed substantial based on losses due to mortalities, condemnations and lost productivity (Barnes et al., 2008). Avian pathogenic *Escherichia coli* (APEC), the etiologic agent of this disease, belonged to the broad *E. coli* pathotype known as extraintestinal pathogenic *E. coli* (ExPEC) (Russo and Johnson, 2000; Johnson et al., 2002; Smith et al., 2007). ExPEC are characterized by their ability to cause disease outside the intestinal tract **(**Russo and Johnson, 2000; Johnson et al., 2002; Kaper, 2005). Colonization of the trachea and air sacs considered the first step of a systemic infection by APEC, followed by the colonization of the liver and the pericardium, with subsequent bacteremia (Dziva and Stevens, 2008). To adhere, colonize, invade, and cause infection, APEC strains possess diverse set of virulence factors that enable their extraintestinal lifestyle and make them distinct from commensal and diarrheagenic *E. coli* strains (Russo and Johnson, 2000). These virulence factors, which involved in colonization, adhesion, invasion and survival the host defenses are adhesins, invasins, toxins, iron acquisition system (siderophores) and protictins (Ewers et al., 2004; Ewers et al., 2007; Rodriguez-Siek, et al., 2005 and Johnson et al., 2008**).**These virulence genes rarely found in the same isolate and that they can present either individually or polygenically with varying

frequencies (Delicato et al., 2003 and Vandekerchove et al., 2005). Phylogenetic analysis of *E.coli* shows that there are four main phylogenetic groups of *E.coli,* designated A, B1, B2 and D (Clermont et al*.,* 2000).Worldwide phylogenetic analyses have demonstrated that virulent extraintestinal *E. coli* strains belong mainly to group B2 and, to a lesser extent, to group D. In contrast, most of the commensal strains are associated with group A or group B1**(**Takahashi et al., 2006; Moreno et al., 2008 and Basu et al., 2013). Recent studies have shown that some APEC clones are very similar to extraintestinal pathotypes that affect humans (UPEC: uropathogenic *Escherichia coli* and NMEC: neonatal meningitis-causing *Escherichia coli*). These APEC strains are indistinguishable from human ExPEC by the possession of certain virulence factors and phylogenetic groups (Rodriguez-Siek et al., 2005; Ewers et al.,2007; Johnson et al., 2008; Zhao et al., 2009; Tivendale et al., 2010; Vincent et al., 2010; Bergeron et al., 2012 and Jakobsen et al., 2012). For this reason, some authors pointed to poultry as a reservoir for human ExPEC, and the possibilities exist for the transfer of APEC to human, which suggests a public health hazard (Vincent et al., 2010; Bergeron et al., 2012 and Jakobsen et al., 2012). In view of these considerations, this study was aimed to characterize *E.coli* isolates referring to their phylogenetic groups, presence of some virulence associated genes which are frequently found in APEC including *eae*A, *stx1*, *stx2*, *hly*, *iss*, *tsh*, *omp*A, *tra*T, *ibe*A, *cva*C and *iut*A as well as DNA sequencing for 2 of the isolated genes (*cva*C and *iss*).

2. MATERIAL AND METHODS

2.1. Sampling, bacteriological examination and serotyping:

This study investigated 451 samples from freshly slaughtered broilers showed different disease manifestations clinically diagnosed as colibacllosis obtained from farms of different localities in Sharkia and Dakahlia. Samples were cultured on MacConkey agar and (EMB) agar (Calnek et al., 1997). Then they were further identified using conventional biochemical tests in addition to confirmation by RapID ONE system (Remel), 25 isolates that were preliminary identified biochemically as *E. coli* were subjected to serological identification using slide agglutination test according to (Edward and Ewing, 1972).

2.2. Phylogenetic analysis of E. coli isolates:

E. coli phylogenetic grouping was accomplished by PCR as previously described by(Clermont et al., 2000**).** DNA of 15 isolates were extracted by QIAamp DNA mini kit instructions according to manufacture instructions, then placed in a PTC-100 programmable thermalcycler in a final volume of 25 µl consisting of 12.5 µl of Dream*Taq* TM Green Master Mix (2X) (Fermentas, USA), 1µl of each primer (Sigma, USA), 7 µl of template DNA and nuclease-free water up to 25 µl. Amplified PCR products was electrophoresed on 1.5% agrose gel in tris acetate EDTA and visualized by UV transilluminator. This method designates strains to one of four phylogenetic groups (A, B1, B2, and D) based on the presence of two genes (*chuA* and *yjaA*) and a specific DNA fragment(TSPE4.C2). Both *chu*A and TspE4.C2 negative and positive *E. coli* strains were grouped into group A and B2, respectively, and the *chu*A-negative and TspE4.C2-positive, and the *chu*A positive and *yja*A-negative *E. coli* strains were grouped into B1 and D, respectively. The used primers in this analysis are listed in Table (1).

2.3. Detection of Virulence Genes:

DNA of 15 *E. coli* isolates were extracted as previously mentioned in addition to plasmid extraction according to QIAprepSpin Miniprep Kits catalogue No. 27104. PCR was used to amplify some virulence genes of interest in APEC. Multiplex PCR was used for the detection of seven virulence genes (*eae, tra*T, *ibe*A,

*iut*A, *cva*C, *Stx*1*and Stx*2) in 3 different cycling protocols and 4 uniplex PCR systems were used for detection of other four virulence genes (*tsh, hly , omp*A and *iss*) in the same isolates. The primer sets, sequences, amplification product size and the positive control strain for each gene used in PCR programs were listed in Table (2).

2.4. DNA sequencing:

iss and *cva*C genes were sequenced each in 2 *E. coli* isolates representing serogroups O78 and O157 using Big dye Terminator V3.1 cycle sequencing kit.(Perkin-Elmer, Foster city, CA**)** then the samples were read using Applied Biosystems 3130 automated DNA Sequencer ***(****ABI, 3130, USA)*. The sequence analysis were performed with BIO EDIT and the basic local alignment search tool software (BLAST) by comparing genes under study with corresponding genes on the gene bank then deposited on the gene bank.

3. RESULTS

The obtained results revealed that, 236 *E. coli* isolates were successfully recovered with a total percent 52.3% (representing 64, 51, 48 and 52 out of 100 examined lung, liver, heart blood and trachea respectively, and 21 out of 51examined spleen). Serogrouping of 25 *E. coli* isolates revealed that O78 was the most predominant serogroup with a percentage of (20%) followed by O157 and O27 (16%) each, other serotypes as O168, O125 were also recorded with percentage of (12%) and O115 with percentage of (8%) while 4 isolates were untypable.

3.1. Phylogenetic typing:

Fifteen APEC strains were assigned to four different phylogenetic groups. the majority of examined strains fell into Group B2 5/15 (33.3%,) followed by group B1 (26.7%), groups A and D were detected with the same percentage 19 out of 86 (22.1%). the results were presented in Fig. (1) and Table (3).

3.2. Virulence genotyping:

The fifteen *E. coli* isolates were examined for the presence of 11 virulence associated genes. The virulence profiles showed that *omp*A was found in most isolates 14 out of 15 with percent 93.3% and gave an amplification product sizes of 919 bp (Fig.5). The *iss* and *iut*A genes gave positive amplification at 266 and 300 bp respectively. iss was found in 13 isolates (86.6%)and iutA 12 isolates (80%) as shown in (Fig. 7 and 3), *tra*T gene was detected in 12 out of 15 examined isolates (80%) with amplification product size of 307 bp (Fig. 2), in addition to *cva*C, *stx*2 and *tsh* genes were present in 9/15 (60%), 7/15 (46%) and 5/15 (33.3%) of the examined isolates, respectively with an amplicon product sizes of 760 bp, 779 bp and 620 bp, respectively as presented in (Fig.3, 4 and 6). Only one isolate harbored *eae*A, *ibe*A and *stx*1 genes each. They gave positive amplification at 248bp, 342bp and 614 bp, respectively (Fig. 2 and 4). Whereas, the *hly* gene hasn't been found in any of the isolates. Distribution of virulence genes in fifteen *E. coli* isolates and the relationship between APEC isolates phylogenetic group and gene prevalence shown in Table (3) and thier relation with isolates serogroups represented in Table (4)

3.3. DNA sequencing:

iss and *cvaC*/*cvi* genes of 4 APEC isolates representing serogroups O157 and O78 were subjected to DNA sequencing followed by analysis of the genes through their alignment with previously deposited genes on the gene bank showing high nucleic acid homogeneity between the two genes under study and the same genes on the gene bank harbored by different *E. coli* isolates published on the gene bank. The nucleotide sequences of *iss genes* of *E. coli* under study were deposited into gene bank under the following accession numbers KU904253, KU904254 while the nucleotide sequences of *cvaC* /*cvi genes* of

Table (1): The oligonucleotide primers used for phylogenetic grouping of *E. coli* isolates:

Gene	Primer sequence (5'-3')	Amplified product	Reference
*Chu*A	GAC GAA CCA ACG GTC AGG AT	279 bp	
	TGC CGC CAG TAC CAA AGA CA		
*Yja*A	TGA AGT GTC AGG AGA YGC TG	211 bp	Jeong et al., (2012)
	ATG RAG AAT GCG TTC CTC AAC		
*tsp*E4C2	GAG TAA TGT CGG GGC ATT CA	152 bp	
	CGC GYC AAC AAA GTA TTR CG		

Table (2): Oligonucleotide primers used for genotypic identification of *E. coli* isolates:

Gene	Primer Sequence (5'-3')	Amplified product	Reference	Positive control Reference strain
*Eae*A	ATGCTTAGTGCTGGTTTAGG	248 bp	Bisi-Johnson et al., 2011	M58154
	GCCTTCATCATTTCGCTTTC			
traT	GATGGCTGAACCGTGGTTATG	307 bp	Kaipainenet al., 2002	NC-005327.1
	GATGGCTGAACCGTGGTTATG			
*ibe*A	TGGAACCCGCTCGTAATATAC	342 bp	Ewers et al., 2007	AY248744.1
	CTGCCTGTTCAAGCATTGCA			
iutA	GGCTGGACATGGGAACTGG	300 bp	Yaguchi*etal*., 2007	JX446685.1
	CGTCGGGAACGGGTAGAATCG			
cvaC	CACACACAAACGGGAGCTGTT	760 bp	Dipineto et al., 2006	CP01031601
	CTTCCCGCAGCATAGTTCCAT			
stx1	ACACTGGATGATCTCAGTGG	614 bp	Dipineto et al., 2006	AJ413986
	CCATGACAACGGACAGCAGTT			
stx2	CCTGTCAACTGAGCAGCACTTTG	779 bp	Dipineto et al., 2006	FN252457
	AGCTATCGCGATTGCAGTG			
ompA	GGTGTTGCCAGTAACCGG	919 bp	Ewers et al., 2007	AF23428.1
	GGTGGTGCACTGGAG TGG			
tsh	AGT CCA GCG TGA TAG TGG	620 bp	Delicato et al., 2003	JX46685.1
	AACAAGGATAAGCACTGTTCTGGCT			
hly	ACCATATAAGCGGTCATTCCCGTCA	1177 bp	Piva et al., 2003	NG036728.1
	ATGTTATTTTCTGCCGCTCTG	260 bp		
iss	CTATTGTGAGCAATATACCC		Yaguchi et al., 2007	DQ309287.1
	CTATTGTGAGCAATATACCC			

Table (3): Relationship between APEC isolates phylogenetic group and gene prevalence

Isolate No.	*Eae*A	*traT*	*ibe*A	*iutA*	*cvaC*	*stx1*	*stx2*	*tsh*	*hly*	*ompA*	*iss*	No.ofvirul.genes	Phylo. Group
1	-	-	-	+	-	-	-	-	-	-	+	3	A
2	-	+	+	+	+	-	+	+	-	+	-	7	B1
3	-	-	-	-	+	-	-	-	-	+	+	3	A
4	-	-	-	-	-	-	-	-	-	+	+	2	A
5	+	+	-	+	-	-	-	+	-	+	+	6	B1
6	-	+	-	+	+	-	-	-	-	+	+	5	D
7	-	+	-	+	+	-	-	+	-	+	+	6	B2
8	-	+	-	-	+	+	-	-	-	+	+	5	B1
9	-	+	-	+	+	-	+	-	-	+	+	6	B1
10	-	+	-	+	+	-	+	-	-	+	+	6	D
11	-	+	-	+	-	-	+	+	-	+	-	5	B2
12	-	+	-	+	-	-	+	-	-	+	+	5	B2
13	-	+	-	+	-	-	+	-	-	+	+	5	B2
14	-	+	-	+	+	-	+	+	-	+	+	7	B2
15	-	+	-	+	+		-	-	-	+	+	5	D

Table (4):Relationship between *E. coli* serogroups, phylogenetic group and virulence genes pattern

Strain no.	Virulence genes	Serogroup	Phylo.group
1 (84lu)	*iutA* and *iss*	O168	A
2 (28lu)	*tra*T,*ibe*A, *iut*A, *cva*C, *stx*2, *tsh* and *omp*A	O125	B1
3 (66sp)	*cva*C, *omp*A and *iss*	O168	A
4 (52h)	*omp*Aand *iss*	O27	A
5 (72li)	*eae*A, *tra*T, *iut*A, *omp*A, *tsh* and *iss*	O27	B1
6 (14li)	*tra*T, *iut*A,*cva*C,*omp*Aand *iss*	O78	D
7 (70lu)	*tra*T, *iut*A, *cva*C, *omp*A, *tsh* and *iss*	O78	B2
8 (87h)	*tra*T, *iutA*, *cva*C, *stx*1, *omp*A, and *iss*	O27	B1
9 (81sp)	*tra*T, *iut*A, *cva*C, *stx*2, *omp*A and *iss*	O115	B1
10 (4h)	*tra*T, *iut*A, *cva*C, *stx*2, *omp*A, and *iss*	O157	D
11 (54h)	*tra*T, *iut*A, *stx*2, *omp*A and *tsh*	O157	B2
12 (12sp)	*tra*T, *iut*A, *stx*2, *omp*Aand *iss*	O157	B2
13 (2t)	*tra*T, *iut*A, *stx*2, *omp*Aand *iss*	O78	B2
14 (100sp)	*tra*T, *iut*A, *cva*C, *stx2*, *omp*A, *tsh* and *iss*	O78	B2
15 (68t)	*tra*T, *iut*A, *cva*C, *omp*Aand *iss*	O157	D

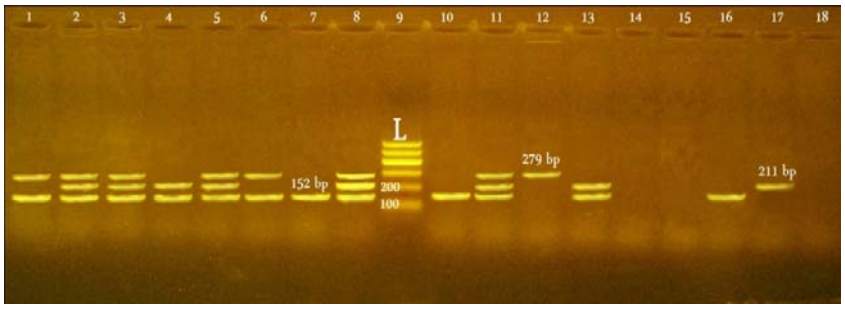

Fig. (1): Agarose gel electrophoresis of TspE4c2, chuA and yjaA genes among DNA products of of 15 E.coli isolates in a multiplex PCR Lane 9: 100-bp ladder, Lane 18: control negative, Lane 8: control positive. Lanes 1-7, 10,11,13 and 16: *E.coli* isolates had *TspE4c2* virulence gene at 152 bp Lane 2- 5, 11, 13 and17: *E.coli* isolate had *yja*A virulence gene at 211 bp. Lane 1-6, 11 and 12: *E.coli* isolates had *chuA* virulence gene at 279 bp.

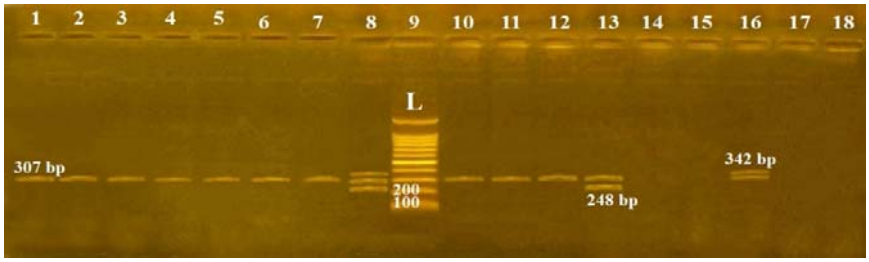

Fig. (2): Agarose gel electrophoresis of *eae*A, *tra*T *and ibe*A genes in *E.coli* isolates in multiplex PCR Lane 9: 100-bp ladder, Lane 18: control negative, Lane 8: control positive. Lane 13 *E.coli* isolate had *eae*A virulence gene at 248 bp Lanes 1-7 and 10-13 and 16 : *E.coli* isolate had *tra*T virulence gene at 307 bp. Lane 16: *E.coli* isolates had *ibe*A virulence gene at 342 bp.

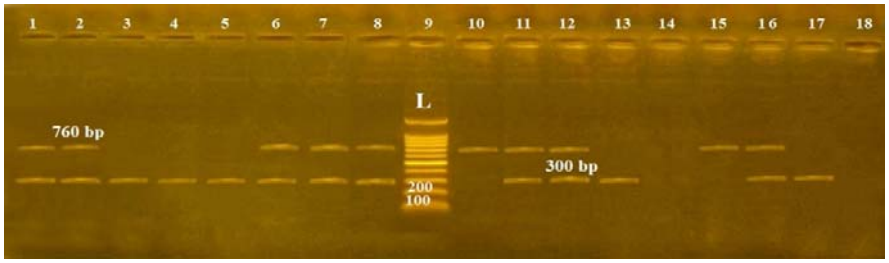

Fig. (3): Agarose gel electrophoresis of *iut*A *and cva*C genes Lane 9: 100-bp ladder, Lane 18: control negative, Lane 8: control positive. Lane 1-7 and 11,12,13,16 and 17 *E.coli* isolates had *iut*A virulence gene at 300 bp Lane 1,2, 6, 7, 10, 11, 12, 15 and 16: *E.coli* isolate had *cva*C virulence gene at 760 bp**.**

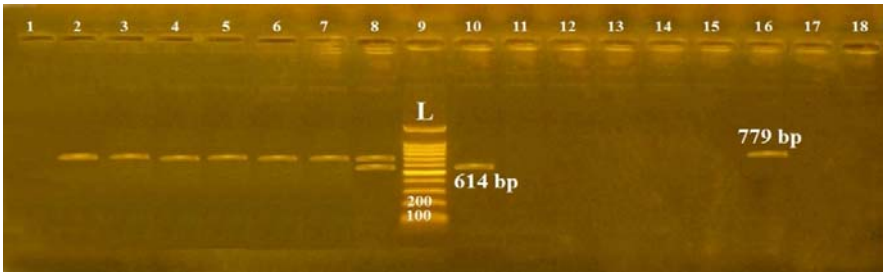

Fig. (4): Agarose gel electrophoresis of stx1and *stx*2 virulence genes among DNA products of of 15 *E.coli* isolates in a multiplex PCR, Lane 9: 100-bp ladder, Lane 18: control negative, Lane 8: control positive. Lane10: *E.coli* isolate had stx1 virulence gene at 614bp Lane 2 -7and 16: *E.coli* isolates had *stx*2 virulence gene at 779 bp.

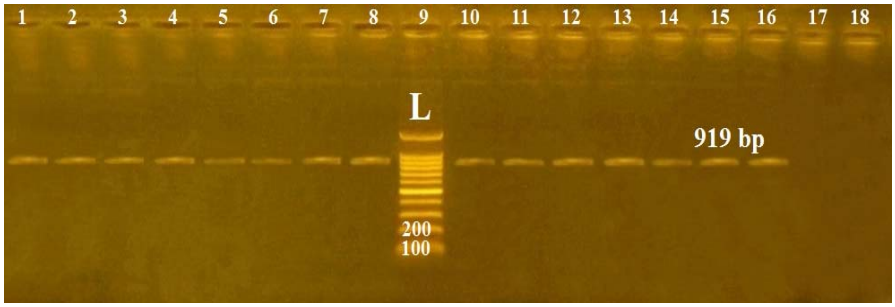

Fig. (5): Agarose gel electrophoresis of PCR- for amplification products of *ompA* gene of 15 *E.coli* isolates Lane 9: 100-bp ladder, Lane 18: control negative (reference strain*)*, Lane 8: control positive (reference strain*)*, Lanes 1-7and 10-16: *E.coli* isolates had *omp*A virulence gene at 919 bp.

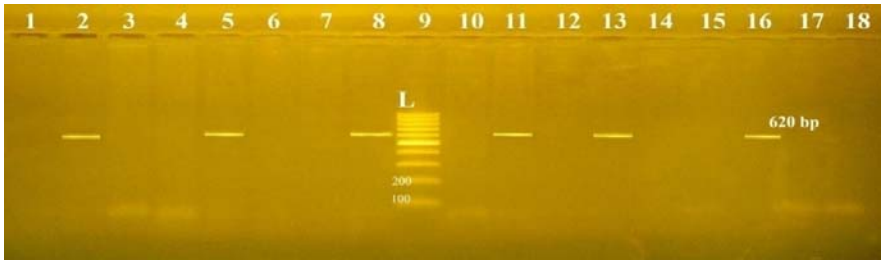

Fig. (6): Agarose gel electrophoresis of PCR- for amplification products of *tsh* gene of 17 *E.coli* isolates (Lane 9: 100-bp ladder, Lane 18: control negative (reference strain), Lane 8: control positive, Lanes 2, 5, 11, 13 and 16: *E.coli* isolates had *tsh* virulence gene at 620 bp.

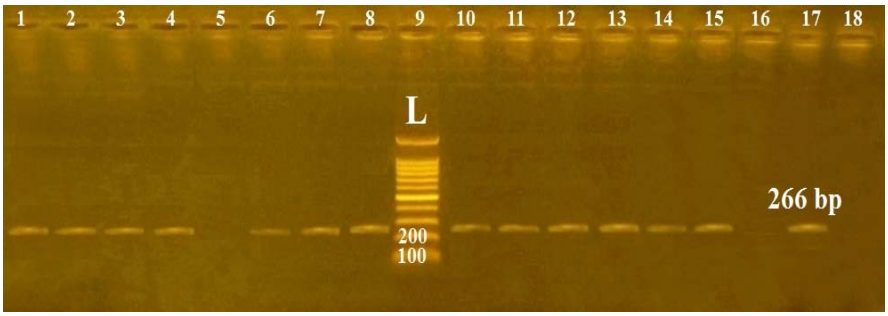

Fig. (7): Agarose gel electrophoresis of PCR- for amplification products of *iss* gene of 15 *E.coli* isolates (Lane 9: 100-bp ladder, Lane 18: control negative (reference strain), Lane 8: control positive, Lanes 1-4,6,7, 10- 15 and 17: *E.coli* isolates had *iss* virulence gene at 266 bp.

E. coli under study were deposited into gene bank under the following accession numbers 954329 and 954330.

4. DISCUSSION

APEC has been widely studied for its rule in extra intestinal infections which causes high economic losses in poultry industry (Barnes and Gross, 1997; Minarro et al., 2001; Assis et al., 2003;). The pathogenicity of an *E.coli* strain is based on the presence and expression of some potential virulence factors (Won et al., 2009). In spite of frequency variations, the following factors are significant including adhesion, invasion, colicin production, aerobactin presence, serum resistance and temperature sensitive haemagglutination (Dho and Lafont, 1982; Naveh et al., 1984; Rocha et al., 2002; Ngeleka et al., 2002; Brito et al., 2003; Delicato et al., 2003 and Mcpeake et al., 2005). In this study, 15 *E.coli* isolates were obtained from chickens with confirmed cases of colibacillosis and were screened for 11 virulence genes commonly associated with APEC and we discussed the association between these virulence genes and phylogenetic groups in which *E.coli* belonged to(A, B1, B2 and D). The PCR virulence gene detection revealed that the majority of pathogenic avian *E.coli* strains have 4 or more virulence related genes(Kwon et al., 2008)**.**The genetic determiners and the proteins that accompanied colicin production are mainly located in plasmids, which are called Col factors (Luria and Suit 1987). Colicin V is found mainly in virulent bacteria involved in extra-intestinal infections affecting humans and animals (Gilson et al. 1987, Lior 1994) and it inhibits the bacterial growth, interfering with the potential of membrane formation (Yang and Konsky 1984).Colicin V provides a competitive advantage in colonization of the intestinal tract (Wooley et al., 1994),which indicates that the presence of either colV plasmid or

its related sequences is associated with APEC virulence (Johnson et al., 2006 and 2008). This study showed high prevalence of genes harbored by this plasmid and aerobactin operon as (*iss, cva*C*, iut*A and *tra*T genes). The gene for colV synthesis, *cva*C was successfully amplified in 60% of tested *E. coli* isolates at 760bp. Constituent with previous studies, *cva*C gene was found with the same percentage (Rodriguez- seik et al., 2005), other investigations found different results either higher 99.1% (McPeake et al., 2005) or lower 22% (Rocha et al., 2002). *iss* gene has been detected with high frequency 13/15 (86.7%). *iss* gene is an important gene responsible for serum resistance and was identified in pColV-I-K94 plasmids, the protein of which is related to cytotoxic complex inhibition (Binns et al. 1982).

This gene was detected in previous studies with levels ranging from 80 to 100 % (Ewers et al., 2004; Zaho et al., 2005; Someya et al., 2007 and Kwon et al., 2008). The property of invading and multiplying presented by pathogens is influenced by iron availability, which is essential for growth in living cells (Neilands et al. 1985). The aerobactin system enables microorganisms to grow in iron free media at low concentration. *E. coli* especially uses this way of capture and transport (Rohrbach et al. 1995, Braun, 2003).The presence of operon aerobactin is in general related to ColV plasmids, although it can be chromosomal (Linggood et al. 1987and Johnson 1991). *iut*A gene was found in 12 of the examined isolates (80%). Rodriguez-seik et al., (2005), Johnson et al., (2008) and schouler et al., 2012) detected the outer membrane protein aerobactin receptor *iut*A gene with similar percentages 80.2%, 80.8% and 82.7% respectively. While, other authors detected lower levels 46.8% (Gomis et al., 2001), 63% (Delicato et al., 2003) and 23% (vandekerchove et al., 2005). Results showed that only 5 isolates (33.3%) harbored the *tsh* gene (temperature sensitive haemagglutinine gene),Whereas it was found in 46.3% of the *E. coli* strains analyzed by zhao et al., (2005) and won et al., (2009). High levels of The plasmid related outer membrane protein gene *tra*T (80%)were reported in the tested *E. coli* isolates, the same percent were recorded by others researchers in different countries (Binns et al.,1982; Pfaff-McDonough et al., 2000 and Rodriguez- seik et al., 2005). The *omp*A gene encodes the episomal outer membrane protease that cleaves colicins (Cavard and Lazdunski 1990) was found in most of the tested isolates (93.3%) some studies showed the same high detection rates (Rodriguez-Siek et al. 2005 and Kafshdouzan et al. 2013). Collectively, most of the plasmid related genes under study were found with high percent as previously mentioned in other studies and suggested that plasmids are widely distributed among APEC and that they might act as a reservoir of plasmid-mediated virulence genes transmissible to other bacteria (Rodriguez- seik et al., 2005). All isolates in this study lacked *hly*F gene although this gene has been well documented in chickens suffering from colibacillosis(Van der Westhuizen and Bragg, 2012) and found with very high prevalence rates in different studies (Johnson et al. 2008; Kafshdouzan et al. 2013). Regarding to the occurrence of *eae*A, *stx*1, *stx*2 genes in the isolates, only one isolate carried the *eae*A gene 6.67%, whereas it was detected with higher percentages in other studies (Dutta et al., 2011 and Kobayashi et al., 2011). Furthermore, *stx*1and *stx*2 was found in 1 and 7 isolates respectively unlike other studies that found higher percentages of *stx*1 and lower percentages of *stx*2 (Stephan and Kuhn, 1999,Yngvild, 2001 and Sara et al, 2013). The gene associated with invasion *ibe*A was found with low incidence (6.67%). whereas, other studies found *ibe*A gene in *E. coli* strains exhibted high number of virulence factors with higher percentages (31%) (Cunha et al., 2014). Epidemiological survayes in many countries have classified most APEC strains into A and D (Ewers et al., 2007; Johnson

et al., 2008 and Kobayashi et al., 2011). Results of this study showed that 33.3% of the examined strains belong to group B2, followed by group B1 (26.7%), finally groups A and D were detected with (20%) each. Phylogroup B2 is considered more virulent in ExPEC infection and frequently had the greatest number of virulence genes (Smith et al., 2007 and Mellata, 2013). This group is also found to be related to the pathogenic serogroups O157and O78. In this work, we sequenced *iss* and *cva*C/*cvi* genes from *E. coli* serogroups O78 and O157. The results of the exiting study showed high homology between *iss* genes and other isolates deposited on the gene bank. Concerning *cva*C/*cvi* gene, results showed that it shared high level of identity ranged from (98-99%) with other isolates previously deposited on the gene bank. Some studies have reported that the distribution of virulence factors in APEC is independent of the host and that APEC could pose a zoonotic risk via horizontal gene transfer between different *E. coli* strains strains (Ron 2006; Johnson et al. 2008; Mellata et al. 2009).

5. CONCLUSION

High prevalence of B2 phylo group strains which also possess the highest number of virulence genes, especially those carried on large plasmids as colV plasmid such as *cva*C, *iss* and *tsh*. This large plasmid may be transmitted to other *E.coli* or different microbes by horizontal gene transfer. The strong association of predicted *iss*, *ompA* and *tsh* proteins among different *E. coli* strains suggests that it could be a good antigen to detect and control avian pathogenic *E. coli* (APEC).

6. REFERENCES

Assis, M.T.Q.M., Gruber, G.L., Hofmeister, A.W.,Guimaraes, A.M.P. 2003. Avaliação do percentual de descartenacon denaçãoparcial de frangos. RevistaNacional da Carne. 313:22-31.

Barnes, J.H., Gross, W.B.1997. Colibacillosis. In: Calnek B.W., Barnes, H. J., Beard, C.W. Mcdogald, L.R., Saif, Y.M. (Ed.).Dis. of poultry.10th ed. Ames, IA, USA: Iowa State University Press.131-139.

Barnes, H.J., Nolan, l.k., vaillancourt, J.A. 2008.Colibacillosis .in Saif, Y.M., Fadly, A.M.(Eds.), Disease of poultry. Blackwell Publishing, Ames, I.A, PP.691-732.

Basu, S., Mukherjee, S.K., Hazra,A. and Mukherjee, M. 2013.Molecular characterization of uropathogenic *Escherichia coli*:nalidixic acid and ciprofloxacin resistance, virulent factors and phylogenetic background, Journal of Clinical and Diagnostic Research, 7(12): 2727–2731.

Bergeron, C.R., Prussing, C., Boerlin, P. 2012. Chicken as reservoir for extraintestinal pathogenic *Escherichia coli* in humans, Canada," Emerging Infect. Dis., 18(3): 415–421.

Binns, M.M., Mayden, J., Levine, R.P. 1982. Further characterization of complement resistance conferred on *Escherichia coli* by the plasmid genes *tra*T of R100 and *iss* of colV, I-K94. Infect. Immun. 35: 654-659.

Bisi-Johnson, M.A., Obi, C.L., Vasaikar, S.D., Baba, K.A., Hattori, T. 2011. Molecular basis of virulence in clinical isolates of *Escherichia coli* and *Salmonella* species from a tertiary hospital in the Eastern Cape, South Africa. Gut Pathogens. 3-9.

Braun, V. 2003. Iron uptake by *Escherichia coli*. Front Biosci.8:1409-1421.

Brito, B.G., Gaziri L.C., Vidotto, M.C. 2003. Virulence factors and clonal relationships among *Escherichia coli* strains isolated from broiler chickens with cellulitis. Infect. Immun. 71:4175-4177.

Calnek, B.W., Barnc, H.J., Charles, W.B., Larry, R.M. and Saif, Y.M. 1997. Diseases of poultry, 10th Ed., Iowa State University Press, USA.

Cavard, D. and Lazdunski, C.1990. Colicin cleavage by *omp*T protease during both entry into and release from *Escherichia coli* cells. Bacteriol. J. 172 (2): 648–652.

Clermont, O., Bonacorsi, S., Bingen, E. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group Appl. Environ. Microbiol.66:4555-4558.

Cunha, M.P.V., Oliveira, M.G.X., Oliveira, M.C.V., Silva, K.C., Gomes, C.R., Moreno, A.M. and Knöbl, T. 2014.Virulence Profiles, Phylogenetic Background, and Antibiotic Resistance of *Escherichia coli* Isolated from Turkeys with Airsacculitis .The Scientific World J. Article ID 289024 (1-8).

Delicato, E.R., De Brito, B.G., Gaziri, L.C.J., Vidotto, M.C., 2003. Virulence-associated genes in *Escherichia coli* isolates from poultry with colibacillosis. Vet. Microbiol. 94:97–103.

Dho, M., Lafont, J.P. 1982. *Escherichia coli* colonization of the trachea in poultry: comparison of virulent and avirulent strains in Enterotoxigenic chickens. Avian Dis. 26:787-797.

Dipineto, L., Santaniello, A., Fontanella, M., Lagos, K., Fioretti, A. Menna, L.F. 2006. Presence of Shiga toxin-producing *Escherichia coli* O157:H7 in living layer hens. Letters in Applied Microbiology 43: 293–295.

Dutta, T.K., Roychoudhury, R., Bandyopadhyay, S., Wani, S.A., Hussain, I. 2011. Detection and characterization of shiga toxin producing *Escherichia coli* (STEC), enteropathogenic *Escherichia coli* (EPEC) in poultry birds with diarrhea. Indian Res. J. 133(5): 541-545.

Dziva F., Stevens, M.P. 2008. Colibacillosis in poultry: unravelling the molecular basis of virulence of avian pathogenic *Escherichia coli* in their natural hosts. Avi. Patho. 37(4): 355–366.

Edward, P.R., Ewing, W.H. 1972. Edward and Ewing's identification of Enterobacteriacae, 3rd Ed. Burgess, Minneapolis.

Ewers, C., Janssen, T., Kiessling, S., Philipp, H.C., Wieler, L.H. 2004. Molecular epidemiology of avian pathogenic *Escherichia coli* (APEC) isolated from colisepticemiae in poultry. Vet. Microbiol.104 (1-2): 91-101.

Ewers, C., Li, G., Wilking, H., Kiessling, S., Alt, K., Antao, E.M., Laturnus, C., Diehl, I., Glodde, S., Homeier, T., Bohnke, U., Steinruck, H., Philipp, H.C., Wieler, L.H. 2007.Avian pathogenic, uropathogenic, and newborn meningitis-causing Escherichia coli: how closely related are they? Int. J. Med. Microbiol.297:163–176.

Gilson, L., Mahanty, H.K., Kolter, R. 1987. Four plasmid genes are required for colicin V synthesis, export, and immunity. J. Bacteriol.169:2466-2470.

Gomis, S.M., Riddell, C., Potter, A.A., Allan, B.J. 2001. Phenotypic and genotypic characterization of virulence factors of *Escherichia coli* isolated from broiler chickens with simultaneous occurrence of cellulitis and other colibacillosis lesions. The Canadian J. of Vet. Res. 65 (1): 1-6.

Jakobsen, L., Garneau, P., Bruant, G. 2012. Is *Escherichia coli* urinary tract infection a zoonosis? Proof of direct link with production animals and meat. European J.ofClin.Microbiol. Infect. Dis. 31(6): 1121– 1129.

Jeong, Y.W., Kim, T.E., Kim, J.H., Kwon, H.J. 2012. Pathotyping avian

pathogenic *Escherichia col*i strains in Korea. J. Vet. Sci., 13, 145-152.

Johnson, J.R. 1991. Virulence factors in *Escherichia coli* urinary tract infection. Clin. Microbiol. Rev. 4:80–128.

Johnson, J.R., Oswald, E., O'Bryan, T.T., Kuskowski, M.A., Spanjaard, L. 2002. Phylogenetic distribution of virulence-associated genes among *Escherichia coli* isolates associated with neonatal bacterial meningitis in the Netherlands. J. Infect. Dis. 185:774–784.

Johnson, T.J., Siek, K.E., Johnson, S.J. & Nolan, L.K., 2006. DNA sequence of a ColV plasmid and prevalence of selected plasmid- encoded virulence genes among avian *Escherichia coli* strains. J. of Bacteriol.188: 745–758.

Johnson, T.J., Wannemuehler, Y., Doetkott, C., Johnson, S.J., Rosenberger, S.C., Nolan, L.K. 2008. Identification of Minimal Predictors of Avian Pathogenic *Escherichia coli* Virulence for Use as a Rapid Diagnostic Tool. Journal of Clinical Microbiology 46(12): 3987-3996.

Kafshdouzan, K., Salehi, T.Z., Nayeri, B., Madadgar, O., Yamasaki, S., Hinenoya, A. et al., 2013. Distribution of virulence associated genes in isolated *Escherichia coli* from avian colibacillosis Iranian J. of Vet. Med.7(1): 1–6.

Kaipainen, T., Pohianvitra, T.,Sphigel, N.,Pelkonen, S. 2002. Virulance factors of *E.coli* isolated from bovine clinical mastitis. Vet. Microbiol. J. 26(1): 37-46.

Kaper, J.B. 2005. Pathogenic *Escherichia coli*. Int. J. Med. Microbiol.295: 355–35.

Kobayashi, R.K.T., Aquino, I., Ferreira, A.L.D.S., Vidotto, M.C. 2011. EcoR phylogenetic analysis and virulence genotyping of avian pathogenic *Escherichia coli* strains and *Escherichia coli* isolates from commercial chicken carcasses in southern Brazil. Foodborne Path.and Dis. 8(5): 631–634.

Kwon, S.G., Cha, S. Y., Choi, E.J., Kim, B., Song, H.J. Jang, H.K. 2008. Epidemiological prevalence of avian pathogenic *E. coli* differentiated by multiplex PCR from commercial chickens and hatchery in Korea. Bacteriology and Virology J. 38(4): 179-188.

Linggood, M.A., Roberts, M., Ford, S., Parry, S.H., Williams, P.H. 1987. Incidence of the aerobactin iron uptake system among *Escherichia coli* isolates from infections of farm animals.J. Gen. Microbiol. 133: 835-842.

Lior, H. 1994. Classification of *Escherichia coli*, p.31-72. In:Gyles, C.L.(ed.), *Escherichia coli* in Domestic Animals and Humans. CAB Inter-national, Wallington, Oxon, UK.

Luria, S.E., Suit, J.L. 1987. Colicins and COL plasmids, p.1615-1624. In: Neidhardt, F.C. (ed.), *Escherichia coli* and *Salmonella Typhimurium*: Cellular and molecular biology. Vol.2. American Society of Micro-biology, Washington, DC.

McPeake, S.J.W., Smyth, J.A., Ball, H.J. 2005. Characterization of avianpathogenic *Escherichia coli* (APEC) associated with colisepticemia compared to fecal isolates form healthy birds. Vet. Microbiol. 110:245-253.

Mellata, M. 2013. Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trend Foodborne Pathogens and Diseases.10(11): 916–932.

Mellata, M., Touchman, W.J., Curtiss, R. III. 2009. Full sequence and comparative analysis of the plasmid pAPEC-1 of avian pathogenic *E.coli*

v7122 (O78:K89:H9). PLoSONE 4: 1–12.

Minarro, S., Linhares, G.F.C., Andrade, M.A., Rocha, P.T., Santana, A.P. 2001. Envolvimento de *Escherichia coli*, de *Mycoplasma gallisepticum*e de *Mycoplasma synoviae*emlesões de sacosaéreosem frangosabatidos no Estado de Goiás. Ciência Animal Brasileira. 2(2): 111-117.

Moreno, E., Andreu, A., Pigrau, Kuskowski, C.M.A., Johnson, J.R. Prats, G. 2008. Relationship between *Escherichia coli* strains causing acute cystitis in women and the fecal *E. coli* population of the host. J. of Clin. Microbiol. 46(8): 2529–2534.

Naveh, M.W., Zusman, T., Skutelsky, E., Ron, E.Z. 1984. Adherence piliin avian strains of *Escherichia coli*: effect on pathogenicity. Avian Dis. 28:651-661.

Neilands, J.B., Bindereif, A., Montgomerie, J.Z. 1985. Genetic basis of iron assimilation in pathogenic Escherichia coli. Curr. Top. Microbiol. Immunol.118:179-195.

Ngeleka, M., Brereton, L., Brown, G., Fairbrother, J.M. 2002. Pathotypes of avian *Escherichia coli* as related to *tsh-, pap-, pil-,* and *iuc-* DNA sequences, and antibiotic sensitivity of isolated from internal tissues and the cloacae of broilers. Avian Dis.46:143-152.

Pfaff-McDonough, S.J., Horne, S.M., Giddings, C.W., Ebert, J.O. Doetkott, C., Smith, M.H., Nolan. L.K. 2000. Complement resistance related traits among *Escherichia coli* isolates from apparently healthy birds and birds with colibacillosis. Avian Dis. 44:23–33.

Piva, I.C., Pereira, A.L., Ferraz, L.R., Silva, R.S.N. ,Vieira, A.C., Blanco, J.E., Blanco, M., Blanco, J., Giugliano, L.G. 2003.Virulence Markers of Enteroaggregative *Escherichia coli* Isolated from Children and Adults with Diarrhea in Brasília, Brazil. J. of clin Microbiol. 1827–1832.

Rocha, A.C., Silva, A.B., de Brito, A.B., Moraes, H.L., Pontes, A.P., Ce, M.C., Nascimento, V. Salle, C.T., 2002. Virulence factors of avian pathogenic *Escherichia coli* isolated from broilers from the south of Brazil. Avian Dis. 46:749-753.

Rodriguez-Siek, K.E., Giddings, C.W., Doetkott, C. Johnson, T.J. Fakhr, M.K., Nolan, L.K. 2005. Comparison of Escherichia coli isolates implicated in human urinary tract infection and avian colibacillosis. Microbiology. 151:2097–2110.

Rohrbach, M.R., Braun, V., Koster, W. 1995. Ferrichrome transport in *Escherichia coli* K-12: altered substrate specificity of mutated periplasmic FhuD and interaction of FhuD with the integral membrane protein FhuB. J. Bacteriol. 177: 7186-7193.

Ron, Z.E. 2006. Host specificity of septicemic Escherichia coli:human and avian pathogens. Curr. Opin. Microbiol. 9: 28–32.

Russo, T.A., Johnson, J.R. 2000. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. J. Infect. Dis. 181:1753–1754.

Sarah, A.Y., Ammar, A.M., Ahmed, D.A. 2013. Serological and Molecular Typing of Avian Pathogonic *E.coli* Origenating from Outbreaks Of Colibacllosis In Chicken Flocks. Int. J. Of Sience 6(14): 2082-2088.

Schouler, C., Schaeffer, B., Brée, A., Mora, A., Dahbi, G., Biet, F., 2012. Diagnostic strategy for identifying avian pathogenic *Escherichia coli* based on four patterns of virulence genes. J. of Clin. Microbiol. 50(5): 1673–1678.

Smith, J.L., Fratamico, P.M., Gunther, N.W. 2007. Extraintestinal pathogenic *Escherichia coli*,

Foodborne Path. and Dis. 4(2):134–163.

Someya, A., Otsuki, K., Murase,T. 2007. Characterization of *Escherichia coli* strains obtained from layer chickens affected with colibacillosis in a commercial egg-producing farm. J. Vet. Med. Sci. 69(10): 1009-1014.

Stephan, R., Kuhn, k., 1999. Prevalence of verotoxin producing *E.coli* (VTEC) in bovine coli mastitis and other antibiotic resistance patterns. Journal of Vet. Med. Seriec. B. 46(6):423-427.

Takahashi, A., Kanamaru, S. Kurazono, H. 2006. *Escherichia coli* isolates associated with uncomplicated and complicated cystitis and asymptomatic bacteriuria possess similar phylogenies, virulence genes, and O-serogroup profiles. J. of Clin. Microbiol. 44(12): 4589-4592.

Tivendale, K.A., Logue, C.M. Kariyawasam, S. 2010. 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. Infection and Immunity. 78(8): 3412–3419.

VanderWesthuizen, A.W., Bragg, R.R., 2012. Multiplex polymerase chain reaction for screening avian pathogenic *Escherichia coli* for virulence genes. Avi. Path.41(1): 33–40.

Vandekerchove, D., Vandemaele, F., Adriaensen, C., Zaleska, M. 2005. Virulence-associated traits in avian *Escherichia coli*: comparison between isolates from colibacillosis-affected and clinically healthy layer flocks. Vet. Microbiol.108:75–87.

Vincent, C., Boerlin, P. Daignault, D. 2010. Food reservoir for *Escherichia coli* causing urinary tract infections. Emerging Infec.Dis.16(1):88– 95.

Won, G., Moon, B., Oh, I., Matsuda, K., Chaudhari, A.A., Hur, J., Eo, S., Yu, I., Lee, Y., Lee, Y., Kim, B., Lee, J.H. 2009. Profiles of virulence-associated of avian pathogenic *Escherichia coli* isolates from chickens with colibacillosis. Poult. Sci. 46:260-266.

Wooley, R.E., Brown, J., Gibbs, P.S., Nolan, L.K., Turner, K.R. 1994. Effect of normal intestinal flora of chickens on colonization by virulent colicin V-producing, avirulent, and mutant colicin V-producing vian *Escherichia coli*. Avian Dis. 38:(1): 141-145.

Yaguchi, K., Ogitani, T., Osawa, R., Kawano, M., Kokumai, N., Kaneshige, T., Noro, T., Masubuchi, K., Shimizu, Y. 2007. Virulence Factors of Avian Pathogenic *Escherichia coli* Strains Isolated from Chickens with Colisepticemia in Japan. Avian Dis. J. 51(3):656-62.

Yang, C.C., Konisky, J. 1984. Colicin V treated *Escherichia coli* does not generate membrane potential. J. Bacteriol.158: 757-759.

Yngvild, W. 2001. Zoonotic *Escherichia coli*. Acta. Vet. Scand. Suppl. 95: 79-84.

Zhao, S., Maurer, J.J., Hubert S., De Villena, J.F., McDermott, P.F., Meng, J., Ayers, S., English, L., White, D.G. 2005. Antimicrobial susceptibility and molecular characterization of avian pathogenic *Escherichia coli* isolates. Vet. Microbiol. J. 107 (3-4): 215-224.

Zhao, L. Gao, S. Huan, H. Xu, X. Zhu, X. Yang, W., Gao, Q., Liu, X.2009. Comparison of virulence factors and expression of specific genes between uropathogenic *Escherichia coli* and avian pathogenic *E. coli* in a murine urinary tract infection model and a chicken challenge model Microbiology. 155:1634-1644.