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Escherichia coli in broiler chickens in Egypt, its virulence traits and vaccination as an intervention strategy

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Abstract

Avian pathogenic *Escherichia coli* (APEC) is one of the extra intestinal pathogenic *E. coli* (ExPEC). Previous studies showed that O_1, O_2 and O_{78} serotypes are mostly associated with Colibacillosis outbreaks, but recently there are emergent new pathogenic serotypes that have spread worldwide. Wide antigenic diversity exists among APEC strains in Egypt; however, the involvement of a particular O serotype in the infection process appears to vary with the geographical region. Different virulence genes have been identified in APEC. Recently; the presence of these virulence genes is being employed as an indication of pathogenicity, rather than the tedious *E. coli* serotyping methods. In Egypt; several virulence genes were studied, and were found to be different based on the geographical area. However; all studies were limited to a small number of screened virulence genes, in addition to the inconsistency of these screened genes. To control APEC, antibiotics have been used for decades; however the emergence of multi-drug resistant *E. coli*, and the difficulty of discovering new antimicrobial therapies made vaccine the best choice to control *E. coli* infections in poultry farms. In this review, the various aspects of APEC infection in poultry with special focus on the epidemiology of APEC in Egypt in relation to virulence traits were discussed. In addition, the most recent vaccination trials against the APEC diseases in poultry were discussed. We concluded that the virulence gene patterns of APEC can be considered as molecular markers of pathogenicity. Although of their current limitations, some vaccine trials showed promising results as good alternative to control colibacillosis in poultry.

Keywords: Colibacillosis, *E. coli*, Epidemiology, Virulence genes, Vaccines, Egypt

1. Introduction

E. coli is a Gram-negative, rod-shaped, facultative anaerobic bacterium of the Enterobacteriaceae family. It colonizes the intestinal tract of mammals and birds (Bélanger *et al*., 2011). Pathogenic *E. coli* isolates

have been categorized into intestinal pathogenic *E. coli*, or extra intestinal pathogenic *E. coli* (ExPEC) depending on the location of the infection. The intestinal *E. coli* include; Enteropathogenic (EPEC), Enterotoxaemia (ETEC), Enteroinvassive (EIEC), Enterohaemorrhagic (EHEC), and Enteroaggregative (EAggEC) *E. coli*. Previous study of Russo and Johnson, (2000) identified several traits for distinguishing the three pathotypes of ExPEC including; avian pathogenic *E. coli* (APEC), neonatal meningitis *E. coli* (NMEC), and uropathogenic *E. coli* (UPEC).

Colibacillosis in poultry includes systemic and localized infections. The localized infections were; omphalitis, swollen head syndrome, cellulitis, and diarrhea. Whereas systemic infection including; respiratory colisepticemia, enteric colisepticemia, and neonatal colisepticemia. Ewers *et al*., (2003) reported that *E. coli* pathogenicity was generally enhanced or initiated by several influencing factors such as; environmental factors, viral infections, mycoplasma infections, and immune-suppression.

Susceptibility to APEC infection increased after exposure to many intrinsic and extrinsic factors. Extrinsic factors involve environment; exposure to other infections, virulence, duration and levels of exposure, whereas intrinsic factors involve age; route of exposure, passive and active immune status, in addition to strain and breed of chickens. Generally, young birds were more susceptible to sever infections than adult (Rodriguez-Siek *et al*., 2005). Horizontal infections occurred with *E. coli* by contact with other birds, in addition to feacal and oral routes. Nolan *et al*., (2013) added that false vertical transmission of *E. coli* was reported from breeders through egg shell contamination during hatching, or in ovo due to salpingitis.

Diagnosis of colibacillosis in chicken broilers was based on the clinical signs and the typical lesions. Moreover; molecular techniques could be used for phenotypic characterization of the bacterial isolates, by using specific Polymerase Chain Reactions (PCRs). Pathotypes of *E. coli* can be further identified depending on the presence of virulence genes (VGs). Currently; we review the various aspects of APEC infections in poultry, with special focus on the epidemiology of APEC in Egypt in relation to virulence traits. In addition, the most recent vaccination trials against the APEC diseases in poultry were discussed.

2. Avian pathogenic *E. coli* infection in poultry

Early studies on avian *E. coli* strains showed that O_1, O_2 O_{15}, O_{35} and O_{78} serotypes, were mostly associated with colibacillosis outbreaks (Dho-Moulin and Fairbrother, 1999). Later study of Nolan *et al*., (2013) revealed the presence of O_{18}, O_{81}, O_{115}, O_{116} and O_{132}, serotypes, which was a signal for the emergence of new pathogenic serotypes. Recently Younis *et al*., (2017); El-Sawah *et al*., (2018) studies showed that wide antigenic diversity existed among avian pathogenic *E. coli* strains in Egypt, and worldwide. Thus, the involvement of a particular O serotype in the infection process appeared to vary according to the geographical region (Table 1).

3. Virulence factors of the isolated avian pathogenic *E. coli*

3.1. Serum resistance

Serum resistance VGs allow the bacteria to survive exterior to the gastrointestinal tract, and overcome defense mechanisms of the host involving complement and antimicrobial peptides. Mellata *et al*., (2003) reported that the capsular K_1 and somatic O_{78} polysaccharide increased serum resistance of the APEC, which can lead to bacteremia.

A study of Nilsson *et al*., (2014) showed a strong correlation between the APEC pathogenicity and four serum resistance VGs such as; increased serum survival (iss), structural genes of Colicin V operon (cvaC), surface exclusion protein (traT), and outer membrane protein A (ompA). The iss was significantly associated with APEC than AFEC. However; individual existence of the iss gene\ or whether this iss

Table 1. Predominant APEC sero-groups in different geographic areas of Egypt

Geographical areas	Predominant sero-groups	References*
Cairo	O_{78}, O_1, O_2, O_6, and O_{126}	(Salama *et al*., 2007)
Beni-Suef	O_1, O_2, O_6, O_{78} and O_{126}	(Fatma-El-Zahraa, 2011)
Assiut	O_{86}:K_{61}, O_{78}:K_{80}, and O_{128}:K_{67}	(Mohamed *et a*l., 2014)
Kaluobia	O_{63}, O_{103}, O_{125}, O_{158}, and O_{44}	(AbdElTawab *et al*., 2015)
Sharkia, Ismailia, Sinai, Giza and Kaluobia	O_{125}, O_{114} and O_{44}	(Amer *et al*., 2015)
Behera	O_2, O_{128}, O_{125}, and O_{114}	(Abdela, 2017)
Kafr El-sheikh	O_{78}, O_1, O_{26}, O_2, O_{127}, O_{91} and O_{153}	(Abd El-Mongy *et al*., 2017)
Qena	O_{27}, O_{44}, O_{125}, O_{152} and O_{159}	(Ahmed *et al*., 2017)
Mansoura	O_{78}, O_1, O_2, O_{91}, O_8	(Younis *et al*., 2017)
Giza and Kaluobia	O_{78}, O_{24}, O_{44}, O_{55}, O_{86}, O_{124}, O_{158} and O_{127}	(Amer *et al*., 2018)
Alexandria	O_{169}, O_{115}, and O_{29}	(Ellakany *et al*., 2019)

Where; APEC: Avain pathogenic *E. coli*; * Data are based on the available published and reachable research data

gene was a marker gene for the presence of the plasmids correlating with APEC pathogenicity was not confirmed.

3.2. Adhesions

Bacterial adhesion is based on recognition between bacterial surface components and specific receptors in host tissues. ExPEC strains encode many adhesions that promote the attachment of the bacteria to cell receptors, and were very important for development of septicemia (Monroy *et al*., 2005).

Type 1 fimbriae (F_1 fimbriae) have been involved with the initial stages of upper respiratory colonization, whereas the P fimbriae were involved in colonization of the internal organs. The F_1 fimbriae were encoded by a total of nine fim genes which include a major protein named as FimA, and minor proteins named as FimF, FimG and FimH adhesions. Nevertheless, earlier study of Arne *et al*., (2000) showed that the APEC fimH mutant strain failed to adhere to the chicken trachea epithelial cells *in vitro*. The P fimbriae are hem-agglutinating fimbriae with mannose resistant properties; were found in *E. coli* strains producing human urinary tract infections as well as some APEC. Moreover; they were linked to the colonization of internal organs, which led to septicemia and lethality in one-day-old chickens. P fimbriae were encoded by pyelonephritis associated pili gene clusters (pap). This pap gene cluster involved eleven genes (papI, papB, papA, papH, papC, papD, papJ, papK, papE, papF, papG), for the biogenesis and synthesis of the P fimbriae (Dozois *et al*., 2000).

Curli fimbriae type are thin and curly appendices found on the cell surface of *Salmonella enterica* and *E. coli*; and were responsible for the bacterial linkage to proteins of the extracellular matrix, causing survival of such bacteria in the external environment. According to La Ragione and Woodward, (2002), the genes accountable for curli fimbriae expression were encoded by two types of operons: csgBAC and csgDEFG. CsgA sequence was recognized only in all APEC, recovered from chickens suffering from septicemia (Amabile de Campos *et al*., 2005). Additional adhesions recognized between APEC strains and suggested to be involved in the pathogenesis of these strains include; type 1-like fimbriae, AC/1 fimbriae, Afa, Sfa, F_{17}, and Eae fimbriae related sequences (McPeake *et al*., 2005).

4. Iron acquisition systems

Iron is essential for the persistence of bacteria due to its involvement to many cellular activities

including; nucleotide biosynthesis, peroxide reduction, and electron transport. Iron acquisition systems among APEC strains may be encoded by plasmid genes, or by chromosomal pathogenicity islands (Johnson *et al*., 2006). The common iron sequestering mechanism in iron deficient host environments is the siderophores production. In APEC, the aerobactin operon area encodes five polypeptides. Four genes (iucABCD) encode for polypeptides that contribute in aerobactin synthesis; in addition to one gene (iutA) that encodes for an outer membrane protein, which serves as a receptor (Carbonetti and Williams, 1984).

Zhu *et al*., (2005) revealed that Salmochelins siderophores system; which were the first discovered siderophores identified in *Salmonella enterica*, comprised five genes (iroB, iroC, iroD, iroE, and iroN), that have been described among APEC. The iroN gene encoding for an outer membrane siderophore, was considered as the chief receptor for transport of ferric salmochelin (Hantke *et al*., 2003). In spite of being located on plasmids, the salmochelin and aerobactin encoding operons were controlled by the chromosomally located fur gene product. This gene inhibits siderophores production when sufficient quantities of free iron were existing in the environment (Balbontín *et al*., 2016).

A study of Paixão *et al*., (2016) reported the existence of an association between the siderophore yersiniabactin firstly detected in *Yersinia enterocolitica* (encoded by irp-2 (iron-repressible) and fyuA (ferric yersinia bactin uptake genes), and pathogenicity of APEC.

5. Temperature-sensitive hemagglutinin and colicins

The temperature-sensitive hemagglutinin (tsh) gene is an auto transporter protein with double functions of proteolytic and adhesive activities. This protein stays in the outer membrane and helps the adhesion process during the early stages of the infection. Generally, this gene was identified on ColV plasmids at a greater frequency among APEC (Nakazato *et al*., 2009). The tsh gene was an important virulence markers of APEC having a strong association with internal organs colonization; septicemia and lethality in one-day-old chickens (Ngeleka *et al*., 2002), which made it a usefl target for pathotying of APEC.

Colicins are minor protein molecules secreted by *E. coli*; which were classified as bacteriocins because of their antibacterial activity toward some species of bacteria (Cascales *et al*., 2007). A study of Dias da Silveira *et al*., (2002) presented E1, E2, E3, I, K, B, Ia, Ib and V as the most predominant colicins in APEC isolates. Most of APEC strains have colicin V plasmids which harbor other pathogenicity associated genes.

6. Toxins

Cytotoxic activity in APEC was firstly studied in the early 1990's (Fantinatti *et al*., 1994). Several toxins were described in APEC strains, but with unclear roles in pathogenesis. These involve cytolethal distending toxin (cdt), enterohaemolysin (ehxA), cytotoxic necrotizing factor 1(cnf1), cytotoxin designated VT2y (Parreira *et al*., 1998), microcin ColV (cvaC), haemolysin (hly), and secreted auto transporter toxin (sat) (Tóth *et al*., 2003). According to Ewers *et al*., (2005); the vacuolating auto transporter type toxin coded on a pathogenicity island named VAT-PAI was found to have a role in the virulence of APEC, as it was identified with high frequency between APEC compared to avian faecal *E. coli* (AFEC).

The existence of ehxA, sat and cnf1 genes have also been described in APEC strains (da Silva *et al*., 2017). However, their function in pathogenesis was not fully clarified. Additionally; some of the toxins genes (hly, cdt and cvaC) have been associated with large transmissible plasmids, indicating that these VGs might be easily transmitted to other strains (Mellata *et al*., 2012). Recent study of Murase *et al*., (2016) suggested that hlyF which was one of the genes of the ColV plasmid, as a molecular indicator for APEC.

Moreover, this gene was directly included in the outer membrane vesicles production.

Shiga toxin gene had been detected in avian *E. coli* by PCR, but the proof of its expression was little. Lately; a strong mediator for apoptosis (caspase 3/7-induced) and cytotoxic action was described, following a 6-h infection assay using macrophage cell line by an APEC strain (Bastiani *et al*., 2005). Other toxins described in APEC strains involved the heat-labile enterotoxin and the heat-stable enterotoxin 1 (AstA), homologue of entero-aggregative *E. coli* (Janben *et al*., 2001).

7. Virulence gene traits in APEC isolated from broiler chickens in Egypt

The pathogenicity of APEC in relation to the presence of certain virulence gene patterns was studied. Several patterns have been suggested as diagnostic tools for rapid detection of APEC. These patterns include; pentaplex pattern containing hlyF, iutA, iroN, iss, and ompT genes (Johnson *et al*., 2008), the presence of 5 to 8 genes of the iss, tsh, papC, astA, irp2, vat, iucD, and cva/cvi genes (Kwon *et al*., 2008), the presence of crl, fimH and aer gene patterns (Ghanbarpour *et al*., 2011). Recently, the presence of one of four combination patterns of virulence genes; A [iutA +, P(F11)+], B [iutA+ , P(F11)- ,frzorf4+], C [iutA+ , P(F11)- , frzorf4- , O78+], and D [iutA- ,sitA +, aec26+] (Schouler *et al*., 2012). Finally; the detection of at least 8 to13 virulence genes, whereas intermediate pathogenic isolates contained at least about 5 to 8 virulence genes (Wang *et al*., 2015).

In Egypt, several virulence genes screened; however, the main limitation of all studies was the limited number of virulence genes screened in each study, and the inconsistency of the screened genes (Table 2).

8. Current status of vaccine development against APEC

Expanded antibiotics resistant *E. coli* are posing a zoonotic risk to humans. Meanwhile; the careless use of antimicrobials in the developing countries, and the difficulty of discovering new antimicrobial therapies for resistant *E. coli*, led to the suggestion of using the vaccines as the best choice to control *E. coli* infections

Table 2. Predominant APEC virulence genes in different geographical areas of Egypt

Geographical areas	Predominant virulence genes	References*
Dakahlia, Sharkia, Ismailia, and Damietta	*iroN, ompT, hlyF, iss, and iutA*	(Hussein *et al*., 2013)
Ismailia and North Sinai	*stx1* and *stx 2*	(Ahmed *et al*., 2013)
Kaluobia	*ompA, eaeA, tsh, kpsMTII, iss* and *iutA*	(AbdElTawab *et al*., 2014)
Beni-Suef	*hlyF, iss, iroN and ompT*	(Radwan *et al*., 2014)
Assiut	*iss, papC, tsh, and colV*	(Mohamed *et al*., 2014)
Sharkia	*Stx1, Intimin gene, and Stx2*	(Yousef *et al*., 2015)
Mansoura	*eae and Shiga* toxin	(Ramadan *et al*., 2016)
Sharkia	*stx1, stx2, eaeA* and *hly*	(Eid *et al*., 2016)
Behera	*stx2 , eae , stx1&stx2* and *stx1,*	(Abdela, 2017)
Fayoum	*ompA, iss, iutA and iroN*	(Hassan, 2017)
Qena	*ompA , papC , eaeA ,* and *tsh*	(Ahmed *et al*., 2017)
Kafr El-Sheikh	*eaeA, ompA* and *Stx1*	(Abd El-Mongy *et al*., 2017)
Gharbia	*iss* and *ompA*	(AbdEl-Tawab *et al*., 2018)

Where; APEC: Avain pathogenic *E. coli*; * Data are based on the available published and reachable research data

in poultry farms. Multiple trials have been conducted for evaluating the efficacy of using vaccination against *E. coli* infecting poultry. However, several difficulties hindered such efforts including; the capability of the vaccine to induce cross protection against various APEC sero-groups, vaccine mass delivery method, and timing of vaccination (Ghunaim *et al*., 2014).

In Table 3, some trials for development of *E. coli* vaccines in poultry in the last five years are summarized. Generally, studies revealed that the inactivated vaccines provided protection against homologous challenges only (Roland *et al*., 2004).

Meanwhile, researches on live attenuated *E. coli* vaccines resulted in the production of two commercial vaccines. Both vaccines are currently used in Egypt; however, their field efficacy against homologous and heterologous *E. coli* need to be further evaluated (Galal *et al*., 2018). Although subunit vaccines demonstrated better immune response and better protection against homologous and heterologous challenges; however, large scale experiments were not conducted.

Table 3. Vaccination trials against APEC in poultry

Vaccine type	Description	Efficacy of vaccine	Reference
Live attenuated vaccine	Genetically modified O_{78} strain (Δ*crp* gene)	- Reduced mortality - Reduced pathology - Reduced re-isolation of the challenge strain	(Nagano *et al*., 2012)
	Δ*tonB*/Δ*fur* mutant vaccine	- Reduced air sacs lesion scores in vaccinated groups.	(Holden *et al*., 2014)
	Genetically modified O_{78} strain (Δ*aroA* gene)	- Reduced pathology (airsacculitis, pericarditis, peritonitis, and perihepatitis) - No effect on the feed conversion rates	(Rawiwet and Chansiripornchai, 2009)
		- Protective against homologous challenge - Reduced organ lesion scores	(Mohamed *et al*., 2011)
		- Improved immune response - Reduced mortality caused by virulent *E. coli* O_{78} - Better body weight gain at 35 days	(Galal *et al*., 2018)
Autogenous inactivated vaccine	*E. coli* serotype O_1 and O_{78} Inactivated water in oil emulsion	- Reduced mortality - Higher protection rate against O_{78} and O_1	(El Jakee *et al*., 2016)
	E. coli serotype O_{78}:H_4, O_2:H5, and untypable strain Inactivated water in oil emulsion	- No significant protection against homologous or heterologous *E. coli*	(Li *et al*., 2017)

Vectored, recombinant, subunit vaccines, and bacterial ghosts (BGs) vaccines	Vectored Δ*cya* and Δ*crp* derivative of the APEC in *S. typhimurium* Expressing *E. coli* O_{78} LPS & O antigens	- Enhanced antibody responses against *E. coli* 0_{78} LPS - Protective against homologous challenge	(Roland *et al*., 1999; 2004)
	Vectored Δ*cya* and Δ*crp* derivative of the APEC in *S. typhimurium* Expressing the *E. coli* O_{78} LPS, O, and type 1 fimbriae antigens.	- Protection from airsacculitis, and weight loss. - Not protective against *E. coli* O_2 or O_1 serotype challenge	
	FimA, *OmpC* of APEC plus dendritic cell-targeting peptide and microfold cell-targeting peptide on the surface of *L. saerimneri*	- Significant higher levels of OmpC/FimA specific IgG (serum) and IgA (cecal & nasal lavage) - Effective against *E. coli* O_{78}	(Ma *et al*., 2018)
	Expressed plasmid pSS27 and pSS28-encoded the APEC O_1 and O_2 O-antigens , respectively in attenuated *Salmonella enterica* serovar *Typhimurium*	- Stimulated opsonization and complement-mediated bactericidal activity - Protective against lethal homologous challenge	(Han *et al*., 2018)
	A recombinant antigens vaccine with the common surface proteins of ExPEC including *EtsC*, *OmpT*, *OmpA*, and *TraT* for broad protection	- -Stimulated IgY against specific antigens and immune related mRNA expression - Reduced bacterial loads in the spleen and heart - Reduced gross lesion scores of the air sac, liver and heart	(Van Goor *et al*., 2017)
	Glutathione S-transferase attached *iss* protein (GST-Iss) Expressed in *E. coli* BL21/water in oil emulsion	- Humeral response to *iss* - Significant pathology reduction after challenge with *E. coli* O_2 and O_{78}	(Lynne *et al*., 2006; 2012)
	Glutathione S-transferase attached iss protein (GST-Iss) Expressed in *E. coli* BL21/QuilATM adjuvant in PBS (100 mg/ml)	- Provided mucosal and serum antibody response (IgA & IgG) against *iss* - Significant pathology reduction after challenge with *E. coli* O_1, O_2 and O_{78}	
	Modified *E.coli* serotype O_2 (DE17ΔluxSΔaroA) & enhanced phiX174 gene E expression mediating lysis of Gram-negative bacteria	- Protection over 90% homologous strain - No cross-protection against O_{78} & O_1 - Increased IFNγ and TNF - Reduced pathological changes	(Hoseini Shahidi *et al*., 2019; Hu *et al*., 2019)
	Modified *E. coli* O_{78}:K80 then expression of gene E of phage φX174	- Reduced air sac lesions - Increased levels of IFNγ, IgA and IgY. - Effective against homologous challenge	(Ebrahimi-Nik *et al*., 2018)

Conclusion

The epidemiology of *E. coli* serotypes in broiler chickens vary according to the geographical region in Egypt, and worldwide. The presence of individual virulence gene was not inductive to *E. coli* pathogenicity, rather than the existence of certain traits of these genes together. However, the inconsistency and incomplete screening of various virulence traits of the isolated *E. coli* in Egypt made it difficult to conclude specific virulence gene traits of the APEC. Finally, vaccines are promising strategy to control *E. coli* infections in the presence of multi-drug resistant strains; however, the availability of vaccines that provide cross protection against different APEC strains needs further investigation.

Conflict of interest

The authors declare that there is no conflict of interests.

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9. References

Abd El-Mongy, M.; Abd-El-Mon, G.M.; Moawad, A.A. and Mohammed, A.B.A. (2017). Serotyping and Virulence Genes Detection in *Escherichia coli* Isolated from Broiler Chickens. Journal of Biological Sciences. 18(1): 46-50.

AbdEl-Tawab, A.A.; El-Hofy, F.I.; Alekhnawy, k.I.; Talaie, A.T. and Ahmed, A. (2018). Insights into virulence and antimicrobial resistance plasmid associated genes of ESBL *Escherichia coli* associated with arthritis in chickens in Egypt. International Journal of Advanced Research. 7(1): 174-182.

Abdela, W. (2017). Characterization of Diarrheagenic *Escherichia coli* Serotypes Isolated from Poultry and Humans. SOJ Veterinary Sciences. 3(1): 1-8.

AbdElTawab, A.A.; Maarouf, A.A.; Abd El Al, S.A.; El Hofy, F.I. and El Mougy, E.E.A. (2014). Detection of some virulence genes of avian pathogenic *E. coli* by polymerase chain reaction. Benha Veterinary Medical Journal. 26(2): 159-176.

Ahmed, M.A.; Youssef, F.M. and Rahman, A.G.A. (2013). Differentiation Between *E. coli* strains Causing Diarrhea in Broiler Chicken by Using Multiplex PCR. Egyptian Journal of Veterinary Sciences. 44(1): 21-36.

Ahmed, S.A.M.; Ahmed, I.A.; Osman, N. and El-Hamd, D.M. (2017). Propblem of *Escherichia coli* infection in broilers in Qena province. Assiut Veterinary Medical Journal. 63(153): 252-264.

Amabile de Campos, T.; Stehling, E.G.; Ferreira, A.; Pestana de Castro, A.F.; Brocchi, M. and Dias da Silveira, W. (2005). Adhesion properties, fimbrial expression and PCR detection of adhesin-related genes of avian *Escherichia coli* strains. Veterinary Microbiology. 106(3-4): 275-285.

Amer, M.M.; Bastamy, M.A.; Ibrahim, H.M. and Salim, M.M. (2015). Isolation and characterization of avian pathogenic *Escherichia coli* from broiler chickens in some Governorates of Egypt. Veterinary Medical Journal-Giza. 61(1): 1-6.

Amer, M.M.; Mekky, H.M.; Amer, A.M. and Fedawy, H.S. (2018). Antimicrobial resistance genes in pathogenic *Escherichia coli* isolated from diseased broiler chickens in Egypt and their relationship with the phenotypic resistance characteristics. Veterinary World. 11(8): 1082-1088.

Arne, P.; Marc, D.; Bree, A.; Schouler, C. and Dho-Moulin, M. (2000). Increased Tracheal Colonization in Chickens without Impairing Pathogenic Properties

of Avian Pathogenic *Escherichia coli* MT78 with a fimH Deletion. Avian Diseases. 44(2): 343-343.

Balbontín, R.; Villagra, N.; Pardos de la Gándara, M.; Mora, G.; Figueroa-Bossi, N. and Bossi, L. (2016). Expression of IroN, the salmochelin siderophore receptor, requires mRNA activation by RyhB small RNA homologues. Molecular Microbiology. 100(1): 139-155.

Bastiani, M.; Vidotto, M.C. and Horn, F. (2005). An avian pathogenic *Escherichia coli* isolate induces caspase 3/7 activation in J774 macrophages. FEMS Microbiology Letters. 253(1): 133-140.

Bélanger, L.; Garenaux, A.; Harel, J.; Boulianne, M.; Nadeau, E. and Dozois, C.M. (2011). *Escherichia coli* from animal reservoirs as a potential source of human extraintestinal pathogenic *E. coli*. FEMS Immunology and Medical Microbiology. 62(1): 1-10.

Carbonetti, N.H. and Williams, P.H. (1984). A cluster of five genes specifying the aerobactin iron uptake system of plasmid ColV-K30. Infection and Immunity. 46(1): 7-12.

Cascales, E.; Buchanan, S.K.; Duche, D.; Kleanthous, C.; Lloubes, R.; Postle, K.; Riley, M.; Slatin, S. and Cavard, D. (2007). Colicin Biology. Microbiology and Molecular Biology Reviews. 71(1): 158-229.

da Silva, G.C.; Rossi, C.C.; Santana, M.F.; Langford, P.R.; Bossé, J.T. and Bazzolli, D.M.S. (2017). p518, a small floR plasmid from a South American isolate of *Actinobacillus pleuropneumoniae*. Veterinary Microbiology. 204129-132.

Dho-Moulin, M. and Fairbrother, J.M. (1999). Avian pathogenic *Escherichia coli* (APEC). Veterinary Research. 30(2-3): 299-316.

Dias da Silveira, W.; Ferreira, A.; Brocchi, M.; Maria de Hollanda, L.; Pestana de Castro, A.F.; Tatsumi Yamada, A. and Lancellotti, M. (2002). Biological characteristics and pathogenicity of avian *Escherichia coli* strains. Veterinary Microbiology. 85(1): 47-53.

Dozois, C.M.; Dho-Moulin, M.; Bree, A.; Fairbrother, J.M.; Desautels, C.; Curtiss, R.; Brée, A.; Fairbrother, J.M.; Desautels, C. and Curtiss, R. (2000). Relationship between the Tsh Autotransporter and Pathogenicity of Avian *Escherichia coli* and Localization and Analysis of the tsh Genetic Region. Infection and Immunity. 68(7): 4145-4154.

Ebrahimi-Nik, H.; Bassami, M.R.; Mohri, M.; Rad, M. and Khan, M.I. (2018). Bacterial ghost of avian pathogenic *E. coli* (APEC) serotype O_{78}:K_{80} as a homologous vaccine against avian colibacillosis. PLoS One. 13(3): e0194888.

Eid, H.I.; Abdelazeem, M.A.; Nasef, S.A.; Elfeil, W.K. and Mansour, G.H. (2016). Genetic Variation among Avian Pathogenic *E. coli* Strains Isolated from Broiler Chickens. Asian Journal of Animal and Veterinary Advances. 11(6): 350-356.

El-Sawah, A.A.; Dahshan, A.L.H.M.; El-Nahass, E.-S. and El-Mawgoud, A.I.A. (2018). Pathogenicity of *Escherichia coli* O_{157} in commercial broiler chickens. Beni-Suef University Journal of Basic and Applied Sciences. 7(4): 620-625.

El Jakee, j.K.; Hessain, A.M.; El Amry, G.M. and Hemeg, H.A. (2016). Production and evaluation of autogenous vaccine against avian colibacillosis. The Journal of Animal and Plant Sciences. 26(1): 79-87.

Ellakany, H.; Elhamid, H.; Ms, I.; Mostafa, N.; Elbestawy, A. and Gado, A. (2019). Isolation, Serotyping, Pathogenicity and Antibiotic Sensitivity Testing of *Escherichia coli* from Broiler Chickens in Egypt. Alexandria Journal of Veterinary Sciences. 61(2): 45-45.

Ewers, C.; Janßen, T.; Kießling, S.; Philipp, H.-C. and Wieler, L.H. (2005). Rapid Detection of Virulence-Associated Genes in Avian Pathogenic *Escherichia coli* by Multiplex Polymerase Chain Reaction. Avian Diseases. 49(2): 269-273.

Ewers, C.; Janssen, T. and Wieler, L.H. (2003). Avian pathogenic *Escherichia coli* (APEC). Berliner und Munchener tierarztliche Wochenschrift. 116(9-10): 381-395.

Fantinatti, F.; Silveira, W.D. and Castro, A.F.P. (1994). Characteristics associated with pathogenicity of avian septicaemic *Escherichia coli* strains. Veterinary Microbiology. 41(1-2): 75-86.

Fatma-El-Zahraa, G.A. (2011). A contribution towards bacteriological characterization of chicken *E. coli* isolates using recent techniques. Master thesis. Beni-Suef University, Beni-Suef, Egypt.

Galal, H.M.; Tawfek, A.M.; Abdrabou, M.I.; Hessain, A.M.; Alhaaji, J.H.; Kabli, S.A.; Elbehiry, A.; Alwarhi, W.K. and Moussa, I.M. (2018). Recent approaches for control of *E. coli* and respiratory complex in Middle East. Saudi Journal of Biological Sciences. 25(7): 1302-1307.

Ghanbarpour, R.; Sami, M.; Salehi, M. and Ouromiei, M. (2011). Phylogenetic background and virulence genes of *Escherichia coli* isolates from colisepticemic and healthy broiler chickens in Iran. Tropical Animal Health and Production. 43(1): 153-157.

Ghunaim, H.; Abu-Madi, M.A. and Kariyawasam, S. (2014). Advances in vaccination against avian pathogenic *Escherichia coli* respiratory disease: Potentials and limitations. Veterinary Microbiology. 172(1-2): 13-22.

Han, Y.; Liu, Q.; Willias, S.; Liang, K.; Li, P.; Cheng, A. and Kong, Q. (2018). A bivalent vaccine derived from attenuated Salmonella expressing O-antigen polysaccharide provides protection against avian pathogenic *Escherichia coli* O_1 and O_2 infection. Vaccine. 36(8): 1038-1046.

Hantke, K.; Nicholson, G.; Rabsch, W. and Winkelmann, G. (2003). Salmochelins, siderophores of *Salmonella enterica* and uropathogenic *Escherichia coli* strains, are recognized by the outer membrane receptor IroN. Proceedings of the National Academy of Sciences. 100(7): 3677-3682.

Hassan, H.R.M. (2017). Phenotypic and genotypic characterization of antimicrobial resistant *Escherichia coli* recovered from diseased chickens. Thesis. Ph.D. thesis. Beni-Suef University, Beni-Suef, Egypt.

Holden, K.M.; Browning, G.F.; Noormohammadi, A.H.; Markham, P. and Marenda, M.S. (2014). Avian pathogenic *Escherichia coli* Deltaton B mutants are safe and protective live-attenuated vaccine candidates. Veterinary Microbiology. 173(3-4): 289-298.

Hoseini Shahidi, R.; Hashemi Tabar, G.; Bassami, M.R.; Jamshidi, A. and Dehghani, H. (2019). The design and application of a bacterial ghost vaccine to evaluate immune response and defense against avian pathogenic *Escherichia coli* O_2:K_1 serotype. Research in Veterinary Science. 125153-161.

Hu, J.; Zuo, J.; Chen, Z.; Fu, L.; Lv, X.; Hu, S.; Shi, X.; Jing, Y.; Wang, Y.; Wang, Z., et al. (2019). Use of a modified bacterial ghost lysis system for the construction of an inactivated avian pathogenic *Escherichia coli* vaccine candidate. Veterinary Microbiology. 22948-58.

Hussein, A.H.M.; Ghanem, I.A.I.; Eid, A.A.M.; Ali, M.A.; Sherwood, J.S.; Li, G.; Nolan, L.K. and Logue, C.M. (2013). Molecular and Phenotypic Characterization of *Escherichia coli* Isolated from Broiler Chicken Flocks in Egypt. Avian Diseases. 57(3): 602-611.

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

Johnson, T.J.; Skyberg, J. and Nolan, L.K. (2006). Multiple Antimicrobial Resistance Region of a Putative Virulence Plasmid from an *Escherichia coli*

Isolate Incriminated in Avian Colibacillosis. Avian Diseases. 48(2): 351-360.

Johnson, T.J.; Wannemuehler, Y.; Doetkott, C.; Johnson, S.J.; Rosenberger, S.C. and 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.

Kwon, S.-G.; Cha, S.-Y.; Choi, E.-J.; Kim, B.; Song, H.-J. and Jang, H.-K. (2008). Epidemiological Prevalence of Avian Pathogenic *Escherichia coli* Differentiated by Multiplex PCR from Commercial Chickens and Hatchery in Korea. Journal of Bacteriology and Virology. 38(4): 179-179.

La Ragione, R.M. and Woodward, M.J. (2002). Virulence factors of *Escherichia coli* serotypes associated with avian colisepticaemia. Research in Veterinary Science. 73(1): 27-35.

Li, L.; Thofner, I.; Christensen, J.P.; Ronco, T.; Pedersen, K. and Olsen, R.H. (2017). Evaluation of the efficacy of an autogenous *Escherichia coli* vaccine in broiler breeders. Avian Pathology. 46(3): 300-308.

Lynne, A.M.; Foley, S.L. and Nolan, L.K. (2006). Immune response to recombinant *Escherichia coli* Iss protein in poultry. Avian Diseases. 50(2): 273-276.

Lynne, A.M.; Kariyawasam, S.; Wannemuehler, Y.; Johnson, T.J.; Johnson, S.J.; Sinha, A.S.; Lynne, D.K.; Moon, H.W.; Jordan, D.M.; Logue, C.M., et al. (2012). Recombinant Iss as a potential vaccine for avian colibacillosis. Avian Diseases. 56(1): 192-199.

Ma, S.T.; Ding, G.J.; Huang, X.W.; Wang, Z.W.; Wang, L.; Yu, M.L.; Shi, W.; Jiang, Y.P.; Tang, L.J.; Xu, Y.G. and Li, Y.J. (2018). Immunogenicity in chickens with orally administered recombinant chicken-borne *Lactobacillus saerimneri* expressing FimA and OmpC antigen of O_{78} avian pathogenic *Escherichia coli*. Journal of Medical Microbiology. 67(3): 441-451.

McPeake, S.J.W.; Smyth, J.A. and Ball, H.J. (2005). Characterisation of avian pathogenic *Escherichia coli* (APEC) associated with colisepticaemia compared to faecal isolates from healthy birds. Veterinary Microbiology. 110(3-4): 245-253.

Mellata, M.; Dho-Moulin, M.; Dozois, C.M.; Curtiss, R.; Brown, P.K.; Arné, P.; Brée, A.; Desautels, C. and Fairbrother, J.M. (2003). Role of virulence factors in resistance of avian pathogenic *Escherichia coli* to serum and in pathogenicity. Infection and Immunity. 71(1): 536-540.

Mellata, M.; Maddux, J.T.; Nam, T.; Thomson, N.; Hauser, H.; Stevens, M.P.; Mukhopadhyay, S.; Sarker, S.; Crabbé, A.; Nickerson, C.A., et al. (2012). New Insights into the Bacterial Fitness-Associated Mechanisms Revealed by the Characterization of Large Plasmids of an Avian Pathogenic *E. coli*. PLoS One. 7(1): e29481-e29481.

Mohamed, M.A.; Bakhit, B.M.; Ibrahim, A.A. and Saleh, M. (2011). Evaluation of The Living *Escherichia coli*-O_{78} Deleted aroA Vaccine Against Homologous and Heterologous *E. coli* Challenge in Broiler Chickens. Journal of Advanced Veterinary Research. 6(3): 89-92.

Mohamed, M.A.; Shehata, M.A. and Rafeek, E. (2014). Virulence Genes Content and Antimicrobial Resistance in *Escherichia coli* from Broiler Chickens. Veterinary Medicine International. 2014:195189.

Monroy, M.A.R.; Knöbl, T.; Bottino, J.A.; Ferreira, C.S.A. and Ferreira, A.J.P. (2005). Virulence characteristics of *Escherichia coli* isolates obtained from broiler breeders with salpingitis. Comparative Immunology, Microbiology and Infectious Diseases. 28(1): 1-15.

Murase, K.; Martin, P.; Porcheron, G.; Houle, S.; Helloin, E.; Penary, M.; Nougayrede, J.P.; Dozois, C.M.; Hayashi, T. and Oswald, E. (2016). HlyF Produced by Extraintestinal Pathogenic *Escherichia coli* Is a Virulence Factor That Regulates Outer

Membrane Vesicle Biogenesis. Journal of Infectious Diseases. 213(5): 856-865.

Nagano, T.; Kitahara, R. and Nagai, S. (2012). An attenuated mutant of avian pathogenic *Escherichia coli* serovar O_{78}: A possible live vaccine strain for prevention of avian colibacillosis. Microbiology and Immunology. 56(9): 605-612.

Nakazato, G.; Campos, T.A.; Estehling, E.G.; Brocchi, M. and Silveira, W.D. (2009). Virulence factors of avian pathogenic *Escherichia coli* (APEC). Pesquisa Veterinária Brasileira. 29(7): 479-486.

Ngeleka, M.; Brereton, L.; Brown, G. and Fairbrother, J.M. (2002). Pathotypes of avian *Escherichia coli* as related to tsh-, pap-, pil-, and iuc-DNA sequences, and antibiotic sensitivity of isolates from internal tissues and the cloacae of broilers. Avian diseases. 46(1): 143-152.

Nilsson, O.; Börjesson, S.; Landén, A.; Bengtsson, B.; Borjesson, S.; Landen, A. and Bengtsson, B. (2014). Vertical transmission of *Escherichia coli* carrying plasmid-mediated AmpC (pAmpC) through the broiler production pyramid. Journal of Antimicrobial Chemotherapy. 69(6): 1497-1500.

Nolan, L.K.; Barnes, H.J.; Vaillancourt, J.-P.; Abdul-Aziz, T. and Logue, C.M. (2013). Colibacillosis, In: Swayne, D.E. (Ed.) Diseases of Poultry. Wiley Online Library, Ames, Iowa.

Paixao, A.C.; Ferreira, A.C.; Fontes, M.; Themudo, P.; Albuquerque, T.; Soares, M.C.; Fevereiro, M.; Martins, L. and Correa de Sa, M.I. (2016). Detection of virulence-associated genes in pathogenic and commensal avian *Escherichia coli* isolates. Poultry Science. 95(7): 1646-1652.

Parreira, V.R.; Arns, C.W. and Yano, T. (1998). Virulence factors of avian *Escherichia coli* associated with swollen head syndrome. Avian Pathology. 27(2): 148-154.

Radwan, I.A.; Salam, H.S.H.; Soad, A.A. and Marwa, A.Y.A. (2014). Frequency of some virulence associated genes among multidrug resistant *Escherichia coli* isolated from septicemic broiler chicken. International Journal of Advanced Research. 2(12): 867-874.

Ramadan, H.; Awad, A. and Ateya, A. (2016). Detection of phenotypes, virulence genes and phylotypes of avian pathogenic and human diarrheagenic *Escherichia coli* in Egypt. The Journal of Infection in Developing Countries. 10(6): 584-591.

Rawiwet, V. and Chansiripornchai, N. (2009). The efficacy of *Escherichia coli* aroa-live vaccine in broilers against avian *E. coli* serotype O_{78} infection. Thai Journal of Veterinary Medicine. 39(4): 337-342.

Rodriguez-Siek, K.E.; Giddings, C.W.; Doetkott, C.; Johnson, T.J. and Nolan, L.K. (2005). Characterizing the APEC pathotype. Veterinary Research. 36(2): 241-256.

Roland, K.; Curtiss, R. and Sizemore, D. (1999). Construction and evaluation of a delta cya delta crp *Salmonella typhimurium* strain expressing avian pathogenic *Escherichia coli* O_{78} LPS as a vaccine to prevent airsacculitis in chickens. Avian diseases. 43(3): 429-441.

Roland, K.; Karaca, K. and Sizemore, D. (2004). Expression of *Escherichia coli* antigens in *Salmonella typhimurium* as a vaccine to prevent airsacculitis in chickens. Avian Diseases. 48(3): 595-605.

Russo, T.A. and Johnson, J.R. (2000). Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. The Journal of Infectious Diseases. 181(5): 1753-1754.

Salama, S.S.; K, A.A.; E, E.A. and Taha, M.M. (2007). Molecular Strategies for the differentiation and identification of local *E. coli* isolated from chicken: I. characterization of protein profile. Beni-Suef Veterinary Medical Journal. 17(1): 25-28.

Schouler, C.; Schaeffer, B.; Brée, A.; Mora, A.; Dahbi, G.; Biet, F.; Oswald, E.; Mainil, J.; Blanco,

J. and Moulin-Schouleur, M. (2012). Diagnostic strategy for identifying avian pathogenic *Escherichia coli* based on four patterns of virulence genes. Journal of Clinical Microbiology. 50(5): 1673-1678.

Tóth, I.; Hérault, F.; Beutin, L. and Oswald, E. (2003). Production of cytolethal distending toxins by pathogenic *Escherichia coli* strains isolated from human and animal sources: establishment of the existence of a new cdt variant (Type IV). Journal of Clinical Microbiology. 41(9): 4285-4291.

Van Goor, A.; Stromberg, Z.R. and Mellata, M. (2017). A recombinant multi-antigen vaccine with broad protection potential against avian pathogenic *Escherichia coli*. PLoS One. 12(8): e0183929.

Wang, J.; Tang, P.; Tan, D.; Wang, L.; Zhang, S.; Qiu, Y.; Dong, R.; Liu, W.; Huang, J.; Chen, T., et al. (2015). The Pathogenicity of Chicken Pathogenic *Escherichia coli* is associated with the numbers and combination patterns of virulence-associated genes. Open Journal of Veterinary Medicine. 05(12): 243-254.

Younis, G.; Awad, A. and Mohamed, N. (2017). Phenotypic and genotypic characterization of antimicrobial susceptibility of avian pathogenic *Escherichia coli* isolated from broiler chickens. Veterinary World. 10(10): 1167-1172.

Yousef, S.A.; Ammar, A.M. and Ahmed, D.A. (2015). Serological and Molecular Typing of Avian pathogenic *E. coli* originating from outbreaks of colibacillosis in chicken flocks. International Journal of Science and Research. 4(2): 2082-2088.

Zhu, M.; Valdebenito, M.; Winkelmann, G. and Hantke, K. (2005). Functions of the siderophore esterases IroD and IroE in iron-salmochelin utilization. Microbiology. 151(7): 2363-2372.