



***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 O₁, O₂ and O₇₈ 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), Enteroinvasive (EIEC), Enterohaemorrhagic (EHEC), and Enteroaggregative (EAaggEC) *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 fecal 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 O₁, O₂, O₁₅, O₃₅ and O₇₈ serotypes, were mostly associated with colibacillosis outbreaks (Dho-Moulin and Fairbrother, 1999). Later study of Nolan *et al.*, (2013) revealed the presence of O₁₈, O₈₁, O₁₁₅, O₁₁₆ and O₁₃₂, 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 K₁ and somatic O₇₈ 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 <i>et al.</i> , 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 <i>et al.</i> , 2014)
Kaluobia	O ₆₃ , O ₁₀₃ , O ₁₂₅ , O ₁₅₈ , and O ₄₄	(AbdElTawab <i>et al.</i> , 2015)
Sharkia, Ismailia, Sinai, Giza and Kaluobia	O ₁₂₅ , O ₁₁₄ and O ₄₄	(Amer <i>et al.</i> , 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 <i>et al.</i> , 2017)
Qena	O ₂₇ , O ₄₄ , O ₁₂₅ , O ₁₅₂ and O ₁₅₉	(Ahmed <i>et al.</i> , 2017)
Mansoura	O ₇₈ , O ₁ , O ₂ , O ₉₁ , O ₈	(Younis <i>et al.</i> , 2017)
Giza and Kaluobia	O ₇₈ , O ₂₄ , O ₄₄ , O ₅₅ , O ₈₆ , O ₁₂₄ , O ₁₅₈ and O ₁₂₇	(Amer <i>et al.</i> , 2018)
Alexandria	O ₁₆₉ , O ₁₁₅ , and O ₂₉	(Ellakany <i>et al.</i> , 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 (F₁ 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 F₁ 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, F₁₇, 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 useful target for pathotyping 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	<i>iroN</i> , <i>ompT</i> , <i>hlyF</i> , <i>iss</i> , and <i>iutA</i>	(Hussein <i>et al.</i> , 2013)
Ismailia and North Sinai	<i>stx1</i> and <i>stx2</i>	(Ahmed <i>et al.</i> , 2013)
Kaluobia	<i>ompA</i> , <i>eaeA</i> , <i>tsh</i> , <i>kpsMTII</i> , <i>iss</i> and <i>iutA</i>	(AbdElTawab <i>et al.</i> , 2014)
Beni-Suef	<i>hlyF</i> , <i>iss</i> , <i>iroN</i> and <i>ompT</i>	(Radwan <i>et al.</i> , 2014)
Assiut	<i>iss</i> , <i>papC</i> , <i>tsh</i> , and <i>colV</i>	(Mohamed <i>et al.</i> , 2014)
Sharkia	<i>Stx1</i> , <i>Intimin gene</i> , and <i>Stx2</i>	(Yousef <i>et al.</i> , 2015)
Mansoura	<i>eae</i> and Shiga toxin	(Ramadan <i>et al.</i> , 2016)
Sharkia	<i>stx1</i> , <i>stx2</i> , <i>eaeA</i> and <i>hly</i>	(Eid <i>et al.</i> , 2016)
Behera	<i>stx2</i> , <i>eae</i> , <i>stx1&stx2</i> and <i>stx1</i> ,	(Abdela, 2017)
Fayoum	<i>ompA</i> , <i>iss</i> , <i>iutA</i> and <i>iroN</i>	(Hassan, 2017)
Qena	<i>ompA</i> , <i>papC</i> , <i>eaeA</i> , and <i>tsh</i>	(Ahmed <i>et al.</i> , 2017)
Kafr El-Sheikh	<i>eaeA</i> , <i>ompA</i> and <i>Stx1</i>	(Abd El-Mongy <i>et al.</i> , 2017)
Gharbia	<i>iss</i> and <i>ompA</i>	(AbdEl-Tawab <i>et al.</i> , 2018)

Where; APEC: Avian 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 (Δcrp gene)	- Reduced mortality - Reduced pathology - Reduced re-isolation of the challenge strain	(Nagano <i>et al.</i> , 2012)
	$\Delta tonB/\Delta fur$ mutant vaccine	- Reduced air sacs lesion scores in vaccinated groups.	(Holden <i>et al.</i> , 2014)
		- Reduced pathology (airsacculitis, pericarditis, peritonitis, and perihepatitis) - No effect on the feed conversion rates	(Rawiwet and Chansiripornchai, 2009)
	Genetically modified O ₇₈ strain ($\Delta aroA$ gene)	- Protective against homologous challenge - Reduced organ lesion scores - Improved immune response - Reduced mortality caused by virulent <i>E. coli</i> O ₇₈ - Better body weight gain at 35 days	(Mohamed <i>et al.</i> , 2011) (Galal <i>et al.</i> , 2018)
Autogenous inactivated vaccine	<i>E. coli</i> serotype O ₁ and O ₇₈ Inactivated water in oil emulsion	- Reduced mortality - Higher protection rate against O ₇₈ and O ₁	(El Jakee <i>et al.</i> , 2016)
	<i>E. coli</i> serotype O ₇₈ :H ₄ , O ₂ :H ₅ , and untypable strain Inactivated water in oil emulsion	- No significant protection against homologous or heterologous <i>E. coli</i>	(Li <i>et al.</i> , 2017)

Vectored, recombinant, subunit vaccines, and bacterial ghosts (BGs) vaccines	Vectored Δcya and Δcrp derivative of the APEC in <i>S. typhimurium</i> Expressing <i>E. coli</i> O ₇₈ LPS & O antigens	- Enhanced antibody responses against <i>E. coli</i> O ₇₈ LPS - Protective against homologous challenge	(Roland et al., 1999; 2004)
	Vectored Δcya and Δcrp derivative of the APEC in <i>S. typhimurium</i> Expressing the <i>E. coli</i> O ₇₈ LPS, O, and type 1 fimbriae antigens.	- Protection from airsacculitis, and weight loss. - Not protective against <i>E. coli</i> O ₂ or O ₁ serotype challenge	
	<i>FimA</i> , <i>OmpC</i> of APEC plus dendritic cell-targeting peptide and microfold cell-targeting peptide on the surface of <i>L. saerimneri</i>	- Significant higher levels of OmpC/FimA specific IgG (serum) and IgA (cecal & nasal lavage) - Effective against <i>E. coli</i> O ₇₈	(Ma et al., 2018)
	Expressed plasmid pSS27 and pSS28-encoded the APEC O ₁ and O ₂ O-antigens, respectively in attenuated <i>Salmonella enterica</i> serovar <i>Typhimurium</i>	- 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 <i>EtsC</i> , <i>OmpT</i> , <i>OmpA</i> , and <i>TraT</i> 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 <i>iss</i> protein (GST-Iss) Expressed in <i>E. coli</i> BL21/water in oil emulsion	- Humeral response to <i>iss</i> - Significant pathology reduction after challenge with <i>E. coli</i> O ₂ and O ₇₈	(Lynne et al., 2006; 2012)
	Glutathione S-transferase attached <i>iss</i> protein (GST-Iss) Expressed in <i>E. coli</i> BL21/QuilATM adjuvant in PBS (100 mg/ml)	- Provided mucosal and serum antibody response (IgA & IgG) against <i>iss</i> - Significant pathology reduction after challenge with <i>E. coli</i> O ₁ , O ₂ and O ₇₈	
	Modified <i>E. coli</i> serotype O ₂ (DE17 Δ luxS Δ aroA) & enhanced ϕ X174 gene E expression mediating lysis of Gram-negative bacteria	- Protection over 90% homologous strain - No cross-protection against O ₇₈ & O ₁ - Increased IFN γ and TNF - Reduced pathological changes	(Hoseini Shahidi et al., 2019; Hu et al., 2019)
	Modified <i>E. coli</i> O ₇₈ :K80 then expression of gene E of phage ϕ X174	- Reduced air sac lesions - Increased levels of IFN γ , 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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