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
Pathotypes of \textit{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 \textit{E. coli} infection in poultry

Early studies on avian \textit{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 \textit{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 \textit{et al.}, (2017); El-Sawah \textit{et al.}, (2018) studies showed that wide antigenic diversity existed among avian pathogenic \textit{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 \textit{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 \textit{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 \textit{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

<table>
<thead>
<tr>
<th>Geographical areas</th>
<th>Predominant sero-groups</th>
<th>References*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cairo</td>
<td>O78, O1, O2, O6, and O126</td>
<td>(Salama et al., 2007)</td>
</tr>
<tr>
<td>Beni-Suef</td>
<td>O1, O2, O6, O78 and O126</td>
<td>(Fatma-El-Zahraa, 2011)</td>
</tr>
<tr>
<td>Assiut</td>
<td>O63:K61, O78:K80, and O128:K67</td>
<td>(Mohamed et al., 2014)</td>
</tr>
<tr>
<td>Kaluobia</td>
<td>O63, O103, O125, O158, and O44</td>
<td>(AbdElTawab et al., 2015)</td>
</tr>
<tr>
<td>Sharkia, Ismailia, Sinai, Giza and Kaluobia</td>
<td>O125, O114 and O44</td>
<td>(Amer et al., 2015)</td>
</tr>
<tr>
<td>Behera</td>
<td>O2, O128, O125, and O114</td>
<td>(Abdela, 2017)</td>
</tr>
<tr>
<td>Kafr El-sheikh</td>
<td>O78, O1, O26, O2, O127, O81 and O153</td>
<td>(Abd El-Mongy et al., 2017)</td>
</tr>
<tr>
<td>Qena</td>
<td>O27, O44, O125, O132 and O159</td>
<td>(Ahmed et al., 2017)</td>
</tr>
<tr>
<td>Mansoura</td>
<td>O78, O1, O3, O91, O8</td>
<td>(Younis et al., 2017)</td>
</tr>
<tr>
<td>Giza and Kaluobia</td>
<td>O78, O24, O44, O55, O86, O124, O158 and O127</td>
<td>(Amer et al., 2018)</td>
</tr>
<tr>
<td>Alexandria</td>
<td>O109, O115, and O29</td>
<td>(Ellakany et al., 2019)</td>
</tr>
</tbody>
</table>

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

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+ , ace2c6+ ] (Schouler *et al*., 2012). Finally; the detection of at least 8 to 13 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

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

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

<table>
<thead>
<tr>
<th>Vaccine type</th>
<th>Description</th>
<th>Efficacy of vaccine</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Live attenuated vaccine | Genetically modified O<sub>78</sub> strain (Δcrp gene) | - Reduced mortality  
- Reduced pathology  
- Reduced re-isolation of the challenge strain | (Nagano et al., 2012) |
|                       | ΔtonB/Δfur mutant vaccine                         | - Reduced air sacs lesion scores in vaccinated groups.                              | (Holden et al., 2014) |
|                       | Genetically modified O<sub>78</sub> strain (ΔaroA gene) | - Protective against homologous challenge  
- Reduced organ lesion scores  
- Improved immune response  
- Reduced mortality caused by virulent *E. coli* O<sub>78</sub>  
- Better body weight gain at 35 days | (Mohamed et al., 2011) |
|                       | *E. coli* serotype O<sub>1</sub> and O<sub>78</sub> Inactivated water in oil emulsion | - Reduced mortality  
- Higher protection rate against O<sub>78</sub> and O<sub>1</sub> | (El Jakee et al., 2016) |
<p>| Autogenous inactivated vaccine | <em>E. coli</em> serotype O&lt;sub&gt;78&lt;/sub&gt;:H&lt;sub&gt;4&lt;/sub&gt;, O&lt;sub&gt;2&lt;/sub&gt;:H&lt;sub&gt;5&lt;/sub&gt;, and untypable strain Inactivated water in oil emulsion | - No significant protection against homologous or heterologous <em>E. coli</em> | (Li et al., 2017) |</p>
<table>
<thead>
<tr>
<th>Vectored, recombinant, subunit vaccines, and bacterial ghosts (BGs) vaccines</th>
<th>Vectored Δcya and Δcrp derivative of the APEC in <em>S. typhimurium</em> Expressing <em>E. coli</em> O78 LPS &amp; O antigens</th>
<th>Enhanced antibody responses against <em>E. coli</em> O78 LPS - Protective against homologous challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vectored Δcya and Δcrp derivative of the APEC in <em>S. typhimurium</em> Expressing the <em>E. coli</em> O78 LPS, O, and type 1 fimbriae antigens.</td>
<td>Protection from airsaccullitis, and weight loss. - Not protective against <em>E. coli</em> O2 or O1 serotype challenge</td>
</tr>
<tr>
<td></td>
<td><em>FimA, OmpC</em> of APEC plus dendritic cell-targeting peptide and microfold cell-targeting peptide on the surface of <em>L. saerimneri</em></td>
<td>Significant higher levels of OmpC/FimA specific IgG (serum) and IgA (cecal &amp; nasal lavage) - Effective against <em>E. coli</em> O78</td>
</tr>
<tr>
<td></td>
<td>Expressed plasmid pSS27 and pSS28-encoded the APEC O1 and O2 O-antigens, respectively in attenuated <em>Salmonella enterica</em> serovar Typhimurium</td>
<td>Stimulated opsonization and complement-mediated bactericidal activity - Protective against lethal homologous challenge</td>
</tr>
<tr>
<td></td>
<td>A recombinant antigens vaccine with the common surface proteins of ExPEC including <em>EtsC, OmpT, OmpA, and TraT</em> for broad protection</td>
<td>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</td>
</tr>
<tr>
<td></td>
<td>Glutathione S-transferase attached <em>iss</em> protein (GST-Iss) Expressed in <em>E. coli</em> BL21/water in oil emulsion</td>
<td>Humeral response to <em>iss</em> - Significant pathology reduction after challenge with <em>E. coli</em> O2 and O78</td>
</tr>
<tr>
<td></td>
<td>Glutathione S-transferase attached <em>iss</em> protein (GST-Iss) Expressed in <em>E. coli</em> BL21/QuilATM adjuvant in PBS (100 mg/ml)</td>
<td>Provided mucosal and serum antibody response (IgA &amp; IgG) against <em>iss</em> - Significant pathology reduction after challenge with <em>E. coli</em> O1, O2 and O78</td>
</tr>
<tr>
<td></td>
<td>Modified <em>E. coli</em> serotype O2 (DE17ΔluxSΔaroA) &amp; enhanced phiX174 gene E expression mediating lysis of Gram-negative bacteria</td>
<td>Protection over 90% homologous strain - No cross-protection against O78 &amp; O1 - Increased IFNγ and TNF - Reduced pathological changes</td>
</tr>
<tr>
<td></td>
<td>Modified <em>E. coli</em> O78:K80 then expression of gene E of phage φX174</td>
<td>Reduced air sac lesions - Increased levels of IFNγ, IgA and IgY. - Effective against homologous challenge</td>
</tr>
</tbody>
</table>

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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