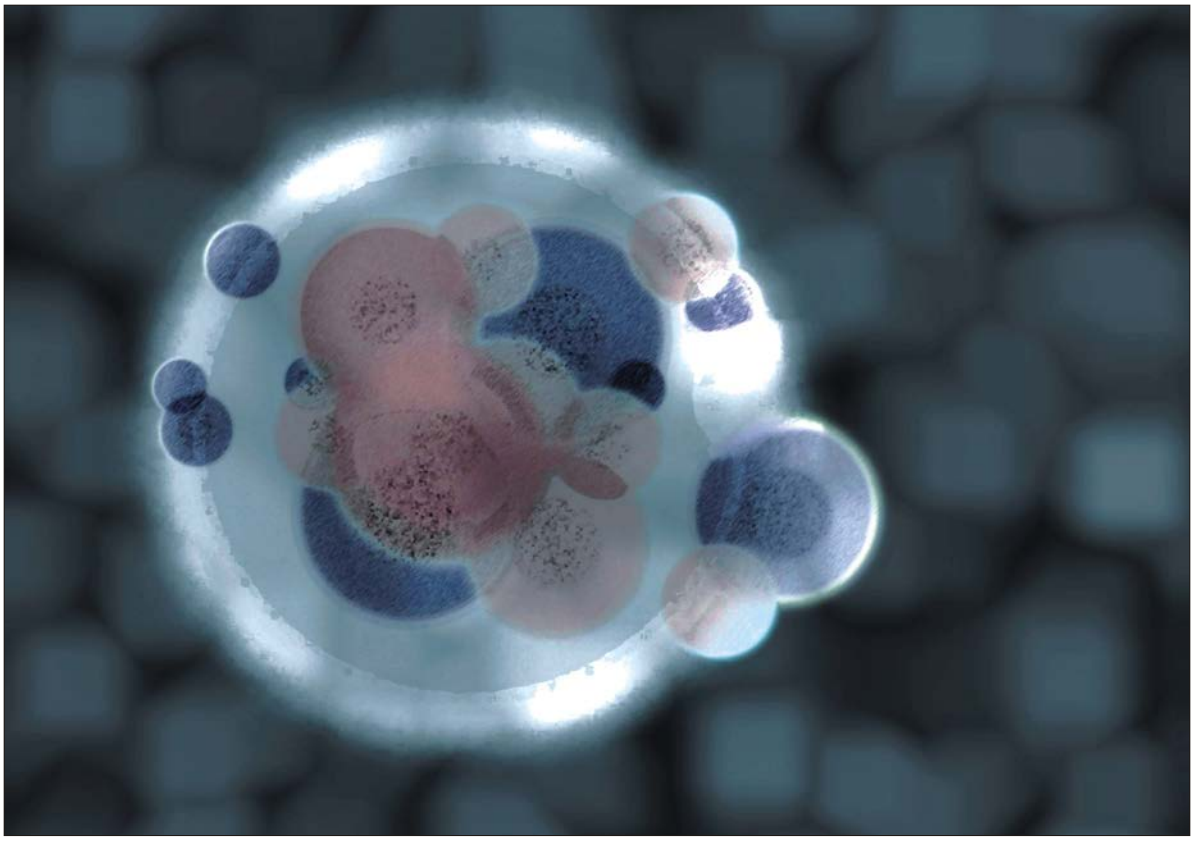

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Research Article
Serotyping and Virulence Genes Detection in *Escherichia coli* Isolated from Broiler Chickens

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Abstract

Background and Objective: *Escherichia coli* (*E. coli*) strains causing systemic disease in poultry (avian colibacillosis) are termed avian pathogenic *E. coli* (APEC). Colibacillosis is a disease of severe economic significance to all poultry producers worldwide and is characterized by a diverse array of lesions. *Escherichia coli* that cause infections usually possess one or more virulence properties that may help in establishment of the infection. The aim of this study was to investigate the virulence genes in *E. coli* isolated from broiler chickens. **Methodology:** A total number of 125 chicken samples from apparently healthy broiler chickens (25 and 15), diseased broiler chickens (25 and 15) and freshly dead ones (25 and 20) were collected in winter (from December, 2014 to February, 2015) and summer (from June, 2015 to August, 2016), respectively from Kafr El-sheikh Governorate. **Results:** In winter season, *E. coli* was recovered from 43 broiler chickens with an incidence of 57.3% and the incidence of *E. coli* in apparently healthy broiler chickens was 32%, diseased broiler chickens 64% and in freshly dead ones 76% while in summer season *E. coli* was recovered from 21 broiler chickens with an incidence 42% represented 26.6% in apparently healthy, 40% in diseased chickens and 55% in freshly dead one. The serogroups of *E. coli* that obtained by serological identification were O78, O1, O26, O2, O127, O91 and O153. The results obtained by multiplex PCR reported that eaeA (intimin *E. coli* attaching and effacing) gene detected in O2, O26, O1 and O153, ompA (outer membrane protein) gene detected in all *E. coli* serogroups that isolated O2, O26, O78, O127, O1 and O91 except O153. Stx1 gene detected in O2, O26, O78 and O91. Stx2 gene detected in O78, O127 and O91. **Conclusion:** The present study showed a higher percentage of *E. coli* isolates carrying at least one virulence gene.

Key words: *Escherichia coli*, pathogenicity, virulence genes, broiler chickens, molecular diagnosis, multiplex PCR

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Escherichia coli normally found in the digestive tract of poultry and most strains are non-pathogenic. The pathogenic capacity of *E. coli* for chickens to cause significant diarrheal and extraintestinal diseases has been associated with numerous extrinsic and intrinsic bird related factors and condition. The extrinsic factors include environment, exposure to other infectious agents, virulence and duration of exposure. The intrinsic factors affecting susceptibility includes age, rout of exposure and breed or strain of chicken[1]. Diseases caused by the bacteria species *Escherichia coli* (*E. coli*) are referred to generally as colibacillosis. Avian colibacillosis is regarded as one of the major causes of morbidity and mortality, associated with heavy economic losses to poultry industry through its association with various disease conditions, either as primary pathogen or as a secondary pathogen[2]. The symptoms of colibacillosis are non-specific and differ with age, organs involved and concurrent disease. Chickens of all ages are susceptible to colibacillosis but usually young birds are considered more susceptible[3,4]. It causes a variety of disease syndromes in poultry including yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, acute colisepticemia, coligranuloma, enteritis, cellulitis and salpingitis. Colibacillosis of poultry is characterized in its acute form by septicemia resulting death and in its subacute form by pericarditis, airsacculitis and peri hepatitis[5]. *E. coli* is serologically classified according to its antigenic composition into somatic (O) antigens, flagella (H) antigens and capsular (K) antigens[6]. Therefore, the present study was planned to determine the prevalence and serotypes of avian pathogenic *E. coli* (APEC) strains in broilers farms in winter and summer seasons in Kafr El-Sheikh Governorate, Egypt and detection of some virulence genes of the isolated strains by using polymerase chain reaction (PCR).

MATERIALS AND METHODS

Collection of samples: A total number of 125 chicken samples from apparently healthy broiler, diseased broiler and freshly dead ones chickens were collected in winter and summer, respectively from Kafr El-sheikh Governorate. The chicken samples were collected from liver, heart blood, kidneys and spleen aseptically for bacteriological isolation and identification.

Detection of *E. coli* isolates by conventional method according to Quinn *et al.*[7]

Isolation and identification of *E. coli*: Each sample was inoculated separately into buffer peptone water and incubated at 37°C for 18-24 h in aerobic condition. Then on selective differential solid media, a loopful from the broth of each sample was streaked onto MacConkey's agar and Eosin Methylene blue agar. The inoculated plates were incubated at 37°C for 24 h. Suspected *E. coli* colonies were purified and kept for additional identification.

Microscopic examination: Gram's stain was prepared and used as described by Cruickshank *et al.*[8] for morphological characterization.

Biochemical Identification: According to Kok *et al.*[9] including indole reaction, methyl red test, Voges-Proskauer test, citrate utilization test, catalase test, sugar fermentation test, oxidase test, triple sugar iron and Christensen's urea agar test.

Serological identification of *E. coli*: According to Cruickshank *et al.*[8] isolated strains were serotyped by using rapid polyvalent and monovalent diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the enteropathogenic types.

Detection of virulence genes of isolated *E. coli* strains by multiplex PCR: Application of PCR for identification of four virulence genes as shiga toxins (stx1 and stx2), intimin (eaeA) and outer membrane protein (ompA)genes that may play a role in virulence of APEC by using four sets of primers was performed essentially by using primers (Pharmacia Biotech) as shown in the Table 1.

Table 1: Primers sequences, target genes and amplicon size of the used genes

Primers	Oligonucleotide sequence (5'→3')	Product size (bp)	References
Stx1 (F)	5'ACACTGGATGATCTCAGTGG'3	614	Dhanashree and Mallya[10]
Stx1 (R)	5'CTGAATCCCCCTCCATTATG'3		
Stx2 (F)	5'CCATGACAACGGACAGCAGTT'3	779	Dhanashree and Mallya[10]
Stx2 (R)	5'CCTGTCAACTGAGCAGCACTTTG'3		
eaeA (F)	5'GTGGCGAATACTGGCGAGACT'3	890	Mazaheri *et al.*[11]
eaeA (R)	5'CCCCATTCTTTTTCACCGTCG'3		
ompA (F)	5'AGCTATCGCGATTGCAGTG'3	919	Ewers *et al.*[12]
ompA (R)	5'GGTGTTGCCAGTAACCGG'3		

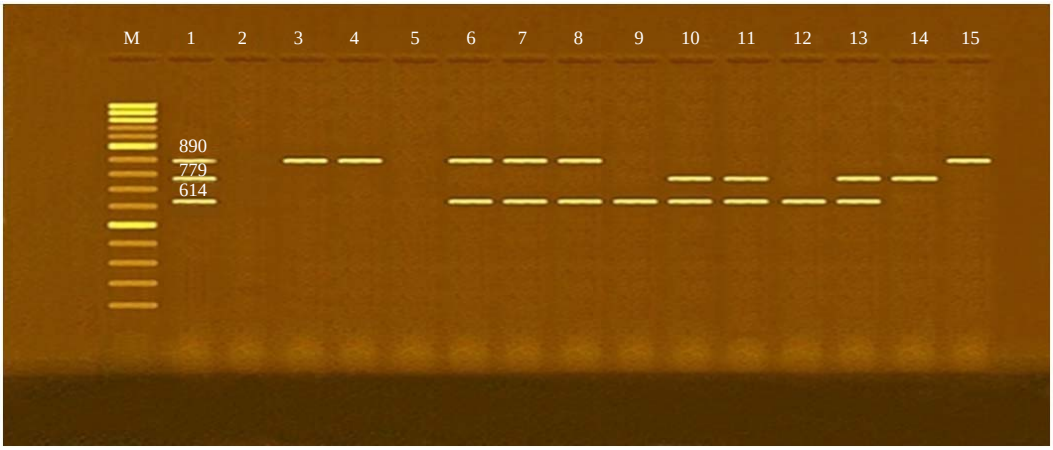

Fig. 1: Agarose gel electrophoresis of multiplex PCR of stx1 (614 bp), stx2 (779 bp) and eaeA (890 bp) genes for characterization of enteropathogenic *E. coli*

Lane M: 100 bp ladder as molecular size DNA marker, Lane 1: Control positive for stx1, stx2 and eaeA genes, Lane 2: Control negative

Table 2: Incidence of *E.coli* isolated from broiler chickens in winter and summer seasons

	Winter			Summer			Total		
	Number of examined samples	Number of +ve sample	(%) +ve	Number of examined samples	Number of +ve sample	(%) +ve	Number of examined samples	Number of %+ve sample	(% +ve)
Apparently healthy	25	8	32	15	4	26.6	40	12	30.0
Diseased birds	25	16	64	15	6	40	40	22	55.0
Freshly dead	25	19	76	20	11	55	45	30	66.6
Total	75	43	57.3	50	21	42	125	64	51.2

Table 3: Serotyping of *E. coli* isolates recovered from different examined samples

Isolated serogroups	Number of isolates	(%)
O78	6	20.0
O1:H7	5	16.6
O26:H11	3	10.0
O2:H6	2	6.6
O127:H6	2	6.6
O153:H2	1	3.3
O91:H21	1	3.3
Untyped	10	33.3
Total	30	100.0

RESULTS

The morphological characters of *E. coli* isolates were Gram-ve rods with pink colonies when cultured on MacConkey media and green metallic colonies on EMB medium. Biochemically, all *E. coli* suspected isolates were lactose fermenting colonies, positive indole, methyl red and catalase. Meanwhile all isolates were negative oxidase, urea hydrolysis, citrate utilization, Voges-Proskauer and didn't produce H_2S. The incidence of suspected *E. coli* isolates from dead chickens was 76 and 55%, followed by diseased broiler chickens was 64 and 40% and from apparently healthy broiler chickens was 32 and 26.6% in winter and summer season, respectively. This indicates that the prevalence of *E. coli* isolates is higher in winter than summer as shown in Table 2.

It is evident from this results that the high incidence of *E. coli* was recovered from liver 50 and 34.6%, followed by fresh heart blood 38.7 and 32%, spleen 22.5 and 12% and kidneys 18.7 and 12% both in winter and summer seasons, respectively. The most commonly serogroups of *E. coli* isolated from examined broiler chicken's samples were O78, O1, O26, O2, O127, O91 and O153 (Table 3).

The results of multiplex PCR of some virulence genes of *E. coli* showed that eaeA gene detected in (O26, O153, O1 and O2) that yielded the expected size of 890 bp PCR amplification products for the intimin gene, ompA gene detected in all *E. coli* serogroups that isolated except in (O153) giving a PCR product of 919 bp, stx1 detected in (O2, O26, O78 and O91) giving a PCR product of 614 bp, stx2 gene detected in (O78, O91 and O127) that yielded a consistent fragment of 779 bp (Table 4) (Fig. 1, 2).

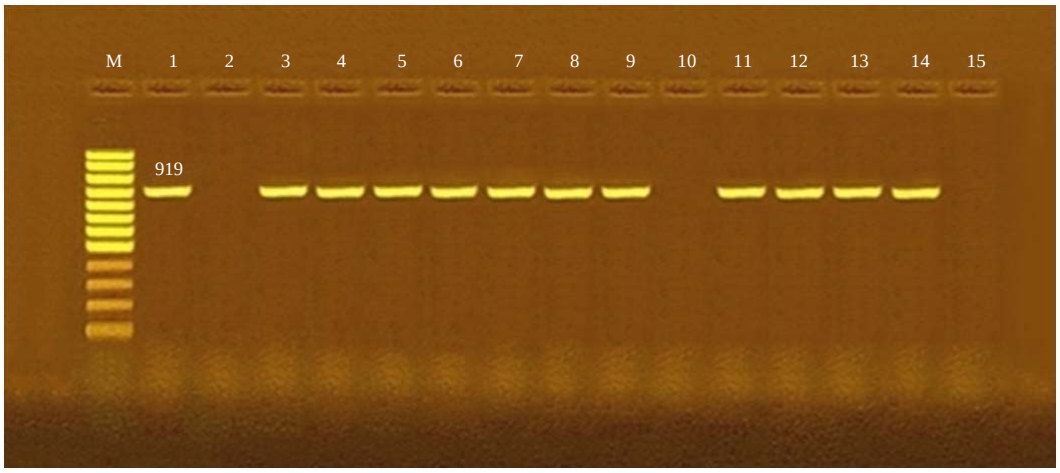

Fig. 2: Agarose gel electrophoresis of PCR of ompA gene (919 bp) gene for identification of enteropathogenic *E. coli*
Lane M: 100 bp ladder as molecular size DNA marker, Lane 1: Control positive for ompA gene, Lane 2: Control negative

Table 4: Results of PCR amplifications of different used genes of *E. coli* serogroups

	Results			
Samples	eaeA	ompA	Stx1	Stx2
(O2:H6)	+	+	+	–
(O26:H11)	+	+	+	–
(O78)	–	+	+	+
(O127:H6)	–	+	–	+
(O1:H7)	+	+	–	–
(O153:H2)	+	–	–	–
(O91:H21)	–	+	+	+

DISCUSSION

Escherichia coli typically colonize the gastrointestinal tract of warm blooded animals within a few hours after birth. However, a large number of highly adapted *E. coli* pathogens have acquired specific virulence attributes[13]. Some pathotypes of *E. coli* are capable of causing intestinal diseases, while others referred to as extra intestinal pathogenic *E. coli* (ExPEC), are responsible for extraintestinal infections. Avian pathogenic *E. coli* (APEC) is recognized by its virulence genes that enable it to live an extraintestinal life .The pathogenicity of the strain is caused by presence of at least 5 virulence genes. Regarding the morphological characters that used for identification of *E. coli*, similar results were noted by Kumar *et al.*[14], Hogan and Smith[15]. The bacteriological study revealed that *E. coli* isolates was recovered from 172. This study revealed that the *E. coli* isolates were isolated from 27.7% (172 out of 620) broiler chickens samples originated from different sources including, fresh heart blood 31.9% (55 out of 172) liver 38.3% (66 out of 172), kidneys 13.9% (24 out of 172) and spleen 15.6% (27 out of 172). These results are agreed to some extend with that obtained by El-Tawab *et al.*[16], who isolated *E. coli* at a percentage of 28%. From the above mentioned results, it is obvious that *E. coli* isolates were recovered from poultry farms with higher prevalence from liver samples followed by fresh heart blood, spleen and kidneys. Nearly similar result obtained by El-Sayed *et al.*[17]. The incidence of *E. coli* among examined chickens in winter was 60.9% and this percentage was higher than that in summer 41%. The PCR based methods, as multiplex PCR is very useful as it allows the simultaneous detection of several pathogens by introducing different primers to amplify DNA regions coding for specific genes of each bacterial strain targeted[18].

CONCLUSION

The present study showed a higher percentage of *E. coli* isolates carrying at least one virulence gene. Applying modern technique as PCR based detection of major virulence genes, shiga toxin 1 and 2 (stx1 and stx2). Shiga toxin producing *E. coli* (STEC) is a heterogeneous group of bacteria causing colibacillosis. The strains which were positive for eaeA gene which encodes intimin, an important binding protein of pathogenic STEC as *E. coli* O26, O111, O55 and O125 more virulent than other strains not carry this gene.

Escherichia coli is known as one of the most important pathogenic agents causing disease in fowls, so referred as avian pathogenic *E. coli* (APEC). APEC is recognized by its virulence genes that enable it to live an extraintestinal life. *E. coli* that cause infections usually possess one or more virulence properties that may help in establishment of the

infection. In the present study we investigate the virulence genes of *E. coli* as eaeA, stx1, stx2 and ompA genes isolated from broiler chickens.

SIGNIFICANCE STATEMENT

This study confirmed that *E. coli* is known as one of the most important pathogenic agents causing disease in fowls. The pathogenicity of the strain is caused by presence of at least five virulence genes as stx1 gene, stx2 gene, eaeA (intimin or *E. coli* attaching and effacing) gene, ompA (outer membrane protein) gene.

REFERENCES

1. Piercy, D.W.T. and B. West, 1976. Experimental *Escherichia coli* infection in broiler chickens: Course of the disease induced by inoculation via the air sac route. J. Comp. Pathol., 86: 203-210.
2. Kwon, S.G., S.Y. Cha, E.J. Choi, B. Kim, H.J. Song and H.K. Jang, 2008. Epidemiological prevalence of avian pathogenic *Escherichia coli* differentiated by multiplex PCR from commercial chickens and hatchery in Korea. J. Bacteriol. Virol., 38: 179-188.
3. Barnes, H.J. and W.B. Gross, 1997. Colibacillosis. In: Diseases of Poultry, Calnek, B.W. (Ed.). 10th Edn., Mosby-Wolf Publication Ltd., London, UK., ISBN: 0-7234-2955-3, pp: 131-139.
4. Gross, W.B., 1994. Diseases Due to *Escherichia coli* in Poultry. In: *Escherichia coli* in Domesticated Animals and Humans, Gyles, C.L. (Ed.). CAB International, Wallingford, UK., ISBN: 0-85198-921-7, pp: 237-259.
5. Calnek, B.W., H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif, 1997. Diseases of Poultry. Iowa State University Press, Ames, IA., USA., Pages: 1080.
6. Compos, L.C., M.R. Franzolin and L.R. Trabuls, 2004. Diarrheagenic *E. coli* categories among the traditional enteropathogenic *E. coli* O-serogroups. Mem. Inst. Oswald Cruz, 99: 545-552.
7. Quinn, P.J., B.K. Markey, M.E. Carter, W.J. Donnelly and F.C. Leonard, 2002. Veterinary Microbiology and Microbial Disease. Blackwell Science Publishing, Iowa, USA., Pages: 536.
8. Cruickshank, R., J.P. Duguid, B.P. Mariom and R.H.A. Swain, 1975. Medical Microbiology the Practice of Medical Microbiology. 12th Edn., Vol. 2, Churchill Livingstone, Edinburgh, pp: 434.
9. Kok, T., D. Worswich and E. Gowans, 1996. Some Serological Techniques for Microbial and Viral Infections. In: Mackie and Mccartney Practical Medical Microbiology, Collee, J., A. Fraser, B. Marmion and A. Simmons (Eds.). 14th Edn., Elsevier, India, ISBN: 9788131203934, pp: 179-204.
10. Dhanashree, B. and P.S. Mallya, 2008. Detection of shiga-toxigenic *Escherichia coli*(STEC) in diarrhoeagenic stool and meat samples in Mangalore, India. Indian J. Med. Res., 128: 271-277.
11. Mazaheri, S., S.S. Ahrabi and M.M. Aslani, 2014. Shiga toxin-producing *Escherichia coli* isolated from lettuce samples in Tehran, Iran. Jundishapur J. Microbiol., Vol. 7. 10.5812/jjm.12346.
12. Ewers, C., G. Li, H. Wilking, S. Kiessling and K. Alt *et al*., 2007. Avian pathogenic, uropathogenic and newborn meningitis-causing *Escherichia coli*: How closely related are they? Int. J. Med. Microbiol., 297: 163-176.
13. Kaper, J.B., J.P. Nataro and H.L.T. Mobley, 2004. Pathogenic *Escherichia coli*. Nat. Rev. Microbiol., 2: 123-140.
14. Kumar, K.U., R. Sudhakar and P.P. Rao, 1988. A note on *Escherichia coli* infection in poultry. Poult. Adviser, 21: 49-51.
15. Hogan, J. and K.L. Smith, 2003. Coliform mastitis. Vet. Res., 34: 507-519.
16. El Tawab, A., A. Shraf, A. Ahmed, A.A. Maarouf and Samir *et al*., 2014. Detection of some virulence genes of avian pathogenic *E. coli* by polymerase chain reaction. Benha Vet. Med. J., 26: 159-176.
17. El-Sayed, M.E., I.I. Shabana, A.M. Esawy and A.M. Rashed, 2015. Detection of virulence-associated genes of Avian Pathogenic *Escherichia Coli* (APEC) isolated from broilers. J. Genet., Vol. 1.
18. Touron, A., T. Berthe, B. Pawlak and F. Petit, 2005. Detection of *Salmonella* in environmental water and sediment by a nested-multiplex polymerase chain reaction assay. Res. Microbiol., 156: 541-553.