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

Mahmoud, Abd El-Mongy[1], Abeer, M. Bayme[1], Ghada, M. Abd –El-Moneam[2], and Amgad, A. Moawad[2]

[1] Genetic Engineering and Biotechnology Research Institute" Sadat City University"
[2] Bacteriology, Immunology and Mycology Dep., Fac.Vet. Med. Kafr –Elsheik University.

Abstract: 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 to February) and summer (from June to August), respectively from Kafr –Elsheik Governorate. 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, O91and O153.* The results obtained by multiplex PCR reported that *eaeA* (intimin *or E. coli attaching and effacing*) gene detected in O2,O26,O1and O153, *ompA* (*outer membrane protein*) gene detected in all *E. coli* serogroups that isolated O2,O26,O78,O127,O1and O91 except O153. *Stx1* gene detected in O2, O26, O78and O91. *Stx2* gene detected in O78,O127 and O91.
[Mahmoud, Abd El-Mongy, Abeer, M. Bayme, Ghada, M. Abd –El-Moneam, and Amgad, A. Moawad. **Serotyping and Virulence Genes Detection in *Escherichia coli* Isolated from Broiler Chickens.** *Biomedicine and Nursing* 2018;4(1): 38-41]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org. 7. doi:10.7537/marsbnj040118.07.

Keywords: Serotyping; Virulence; Gene; Detection; *Escherichia coli;* Isolated; Broiler Chicken

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 **(Piercy and West, 1976).** 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 **(Kwon et.,2008).** The symptoms of colibacillosis are non-specific and differ with age, organs involved, and concurrent disease. Chickens of all ages are susceptible to colibacilliosis, but usually young birds are considered more susceptible **(Barnes and Gross, 2003)**. 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 peri-carditis, airsacculitis and peri-hepatitis **(Calnek *et al.*, 1997).** *E. coli* is serologically classified according to its antigenic composition in to somatic (O) antigens, flagella (H) antigens and capsular (K) antigens **(compos *et al*., 2004).** 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 –Elsheik Governorate, Egypt and detection of some virulence genes of the isolated strains by using Polymerase chain reaction (PCR)**.**

2. Material And Methods

2.1 *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 –Elsheik Governorate. The chicken samples were collected from liver, Heart blood, kidneys and spleen aseptically for bacteriological isolation and identification.

2.2 Detection of *E. coli* Isolates by conventional method according to **Quinn et al., (2002).**

2.2.1. Isolation and identification of *E. coli:*

Each sample was inoculated separately into buffer peptone water and incubated at 37˚C for 18 -24 hrs 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 hours. Suspected *E. coli* colonies were purified and kept for additional identification.

2.2.2. Microscopic examination:

Gram's stain was prepared and used as described by **Cruickshank *et al*., (1975)** for morphological characterization.

2.2.3. Biochemical Identification:

According to **Quinn *et al.*, (2002)** including Indole reaction, Methyl red test, Voges Proskauer test, Citrate utilization test, Catales test, Sugar fermentation test, Oxidase test, Triple sugar iron and Christener's urea agar test.

2.3. Serological identification of *E. coli:*

According to **Kok *et al.*, (1996)** 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.

2.4. Detection of Virulence genes of isolated *E. coli* strains by mutiplex PCR:

Application of PCR for identification of four virulence genes as shiga toxins (stx1 & 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

Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
stx1 (F)	**5′** ACACTGGATGATCTCAGTGG **′3**	614	**Dhanashree and Mallya (2008)**
Stx1 (R)	**5′** CTGAATCCCCCTCCATTATG **′3**		
Stx2 (F)	**5′** CCATGACAACGGACAGCAGTT **′3**	779	**Dhanashree and Mallya (2008)**
Stx2 (R)	**5′** CCTGTCAACTGAGCAGCACTTTG **′3**		
eaeA (F)	**5′** GTGGCGAATACTGGCGAGACT **′3**	890	**Mazaheri *et al*. (2014)**
eaeA (R)	**5′** CCCCATTCTTTTTCACCGTCG **′3**		
ompA (F)	**5′** AGCTATCGCGATTGCAGTG **′3**	919	**Ewers *et al.* (2007)**
ompA (R)	**5′** GGTGTTGCCAGTAACCGG **′3**		

3. 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 H2S. 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).

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

	Winter			Summer			Total		
	No. of examined samples	No. of +ve sample	% +ve	No. of examined samples	No. of +ve Sample	% +ve	No. of examined samples	No. of %+ve sample	% +ve
Apparently healthy	25	8	32	15	4	26.6	40	12	30
Diseased birds	25	16	64	15	6	40	40	22	55
Freshly dead	25	19	76	20	11	55	45	30	66.6
Total	75	43	57.3	50	21	42	125	64	51.2

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,O1and 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 919bp, *stx1* detected in (O2,O26,O78,O91) giving a PCR product of 614bp, *stx2* gene detected in (O78,O91 and O127) that yielded a consistent fragment of 779 bp. (Table 4) (Fig. 1,2).

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

Isolated serogroups	No. of isolates	%
O78	6	20
O1:H7	5	16.6
O26:H11	3	10
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

Table (4): The results of PCR amplifications of different used genes of *E. coli* serogroups.

Sample	Results			
	eaeA	*ompA*	*Stx1*	*Stx2*
1(O2:H6)	+	+	+	_
2(O26:H11)	+	+	+	_
3(O78)	_	+	+	+
4(O127:H6)	_	+	_	+
5(O1:H7)	+	+	_	_
6(O153:H2)	+	_	_	_
7(O91:H21)	_	+	+	+

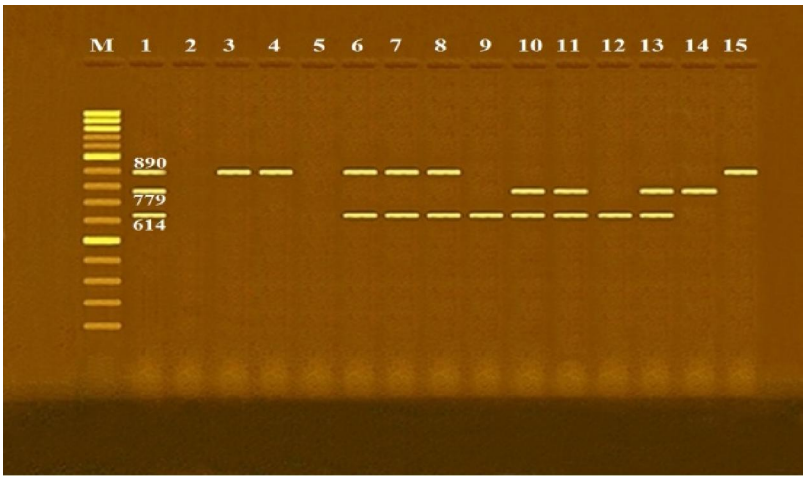

Figure (1): Results of PCR for amplification of *eaeA, stx1, stx2* genes of *E. coli* serogroups.

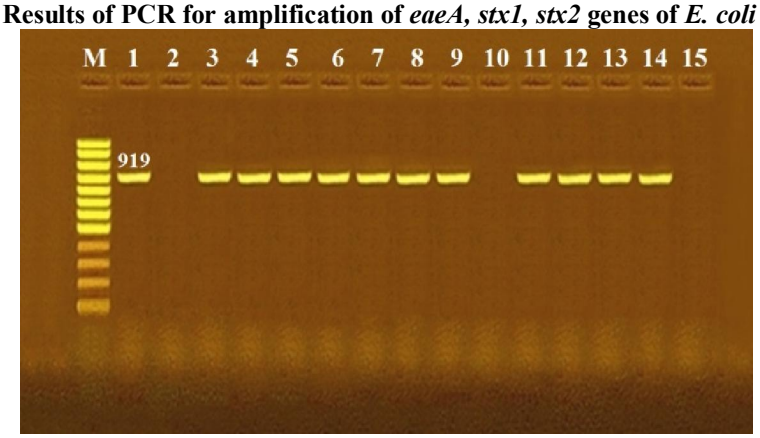

Figure (2): Results of PCR for amplification of omp Agene of *E. coli* serogroups.

Discussion

E. 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 (**kaper *et al.,* 2004**). 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 extra intestinal infections. Avian pathogenic *E. coli* (APEC) is recognized by its virulence genes that enable it to live an extra-intestinal life. The pathogenicity of the strain is caused by presence of at least five virulence genes. Regarding the morphological characters that used for identification of *E. coli*, similar results were noted by **Kumar *et al., (*1996) and Hogan and larry (2003).** 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% (55out 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 **Abd El Tawab, (2014)** 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., (2015).** The incidence of *E. coli* among examined chickens in winter was 60.9% and this percentage was higher than that in summer 41%. 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 **(Touron et al., 2005)**. 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.

Reference:

1. Abd El Tawab, A. shraf, A., Ahmed, A. A. Maarouf, Samir, A. Abd El Al, Fatma, I. El Hofy and Emad, E. A. El Mougy. 2014. Detection of Some Virulence Genes of Avian Pathogenic *E. Coli* by Polymerase Chain Reaction. Benha Veterinary Medical Journal, 26(2):159 - 176.
2. Barnes, H. A. and Gross, W. B (2003): Colibacillosis. In: Barners *et al.,* editors. Disease of poultry. London: Mosby-Wolf Publication Ltd;. p. 131-139.
3. Calnek, B. W., Barnes, H. J., Beard, C. W., McDougald, L. R., Saif, Y. M. 1997. Diseases of Poultry. Iowa State University Press, Ames, Iowa, USA.
4. Compos, L. C., Franzolin, M. R., Trabuls, L. R. (2004): Diarrheagenic *E. coli* Categories among the traditional enteropathogenic *E. coli* O-serogroups. Mem. Inst. Oswald Cruz; 99(6):545-552.
5. Cruickshank, R., Duguid, J. P., Marmoin, B. P. and Swain, R. H. A. (1975): Medical Microbiology. The practice of Medical Microbiology. 12 Edition, Vol. II. Churchill, Edinburgh. P.434.
6. El- sayed, M. E., Shabana, I. I., Esawy, A. M., Rashed, A. M. (2015): Detection of Virulence-Associated Genes of Avian Pathogenic *Escherichia Coli* (APEC) Isolated from Broilers. J. Genetics.2015, 1(1):004.
7. Hogan, J. and Larry S. K. (2003): Coliform mastitis. Vet. Res., 34(5): 507- 519.
8. Kaper, J. B., Nataro, J. P. and Mobley, H. L. T. (2004): Pathogenic *Escherichia coli*. Nature Reviews Microbiology, 2: 123-140.
9. Kok, T.; Worswich, D. and Gowans, E. (1996): Some serological techniques for microbial and viral infections. In Practical Medical Microbiology (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A., eds.), 14th ed., Edinburgh, Churchill Livingstone, UK.
10. Kumar, K. U., Sudhakar, R. and Rao, P. P. (1988): A note on *Escherichia coli* infection in poultry. Poultry Adviser., 21(6): 49-51.
11. 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. Virul, 38: 179-188.
12. Piercy, D. W. T and West, B. (1976): "Experimental *E. coli* infection in broiler chickens: Course of the disease induced by inoculation via the air Sac route." J. Comp. Pathol., 86:203-210.
13. Quinn, P. J., Markey, B. K., Carter, M. E., Donnelly, W. J. C. and Leonard, F. C. (2002): Veterinary microbiology and microbial diseases.1st Iowa State University Press Blackwell Science.
14. Touron, A., Berthe, T., Pawlak, B. and Petit, F. (2005): Detection of Salmonella in environmental water and sediment by a nested-multiplex polymerase chain reaction assay. Res, Microbiol., 156:541–53.

3/24/2018