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PROBLEM OF *ESCHERICHIA COLI* INFECTION IN BROILERS IN QENA PROVINCE

SHIMAA A.M. AHMED [2]; AHMED I.A.[1]; NABILA OSMAN [1] and DINA M.W. SHIBAT EL-HAMD [2]

[1] Department of Poultry Diseases, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.
[2] Department of Poultry Diseases, Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Qena Branch, Egypt.

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ABSTRACT

The present study was conducted on 81 commercial broiler chicken farms aged (1-45 days) located in five different centers at Qena province (Qena, Qift, Deshna, Qus and Nag-Hammadi), For bacteriological examination, Samples were taken from diseased and freshly dead chickens (liver; lung; intestine; trachea; nasal cavity; kidneys; yolk sacs; pericardium; and air sacs). The results revealed that 50 out of 600 samples (8.3%) were positive for *E. coli* isolation, where 30 isolates (9.0%) from 332 baby chicks (up to one week), 20 isolates (7.4%) from 268 broilers (from one week to 6 weeks) were positive for *E. coli* isolation. Thirty-seven seogroups of *E. coli* were obtained by serological identification (O1, O6, O8, O20, O26, O27, O44, O86a, O115, O125, O128, O136, O146, O152, O158, O159, O164, O166, O167,O168 and O169) represented as 4 strains were serotyped O27 (8%), 3 strains O44 (6%), 3 strains O125 (6%), 3 strains O152 (6%), 3 strains O159 (6%), 2 strains O1 (4%), 2 strains O6 (4%), 2 strains O20 (4%), 2 strains O26 (4%), 2 strains O166 (4%), 1 strain for each of O8, O86a, O115, O128, O136 ,O146, O158, O164, O167, O168, O169 with (2%) for each of them and 13 isolates were untyped (26%) by the available antisera. Multiplex PCR showed that ompA virulence gene was detected in all serogroups except O8. Moreover, papC virulence gene was detected in all serogroups except O8, O86a, O136, O146, O167 and O169. While Stx2 virulence gene was detected only in serotypes O27 & O125. The eaeA gene was amplified in serogroups O1, O20, O26, O115, O152, O166, O168 and O169. Finally, tsh virulence gene was detected in all serogroups except O20, O26, O86a, O115, O128, O167 and O169.

Key words: *Escherichia coli*, Broilers, Qena, PCR assay, Virulence.

INTRODUCTION

Colibacillosis is an economically important disease, which is prevalent in Egypt and throughout the world and may constitute a great hazard to poultry industry causing high poultry mortality, loss of weight and reduction of egg production (Bandyopadhay and Dhawedkar, 1984; Kaul *et al.*, 1992; Barnes and Gross, 1997; Margie and Lawrence, 1999; Radwan *et al.*, 2014 and Ammar *et al.*, 2015). More than 1000 *E. coli* serotypes have been reported but only small percentages have been implicated in poultry diseases. The pathogenic and non-pathogenic strains in poultry are differentiated based on the virulence. Hence, detection of these strains becomes important for effective colibacillosis treatment and control (Cloud *et al.*, 1985 and Suthantha *et al.*, 2001). *E. coli* species are divided serologically intoserogroups and serotypes on basis of their antigenic composition (somatic or O antigens for serogroups and flagella or H antigens for serotypes). Many strains express a third class of antigens (capsular or K antigens) (Compos *et al.*, 2004). Pathogenic *E. coli* strains have been divided into intestinal pathogenic *E. coli* and extra intestinal pathogenic *E. coli* (ExPEC) depending on the location of the infection. Avian pathogenic *E. coli* (APEC) strains belong to the ExPEC group is a major pathogen responsible for morbidity and mortality in chickens. It induces different syndromes in poultry, including systemic and localized infections, such as respiratory colibacillosis, acute colisepticemia, yolk sac infection, enteritis, arthritis, omphalitis, swollen-head syndrome, coli granuloma, salpingitis and oophritis accounting for about 5-50% mortality in poultry flocks. The most common form of colibacillosis is characterized by an initial respiratory disease in 3-6 week-old broiler chickens. It is usually followed by a systemic infection with characteristic fibrinous lesions (airsacculitis, perihepatitis, and pericarditis) and fatal septicemia (Ewers *et al.*, 2003; Roy *et al.*, 2006; Sharada *et al.*, 2010 and Abd El Tawab *et al.*, 2014).

Corresponding author: Dr. AHMED I.A.
E-mail address: ibrahim.ahmed@lycos.com
Present address: Department of Poultry Diseases, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

The pathogenicity of *E. coli* is generally initiated by several predisposing factors, such as mycoplasma infections, viral infections, environmental factors and immune-suppressive diseases (Ewers *et al.,* 2003; Bopp *et al.,* 2005 and Gomes *et al.,* 2005).

In addition, the zoonotic potential of APEC raises food safety concerns (Mora *et al.,* 2009; Tivendale *et al.,* 2010 and Chanteloup *et al.,* 2011). ExPECs that can cause neonatal meningitis, urinary tract infections and septicaemia in humans share high levels of genomic similarity and some key virulence factors with APECs (Moulin-Schouleur *et al.,* 2006, 2007; Johnson *et al.,* 2008; Mora *et al.,* 2012 and Olsen *et al.,* 2012).

Several virulence factors have been associated with the virulence of APEC, including those encoding for adhesions, toxins, autotransporters, iron acquisition systems, sugar metabolism, serum resistance proteins, and capsule as well as lipopolysaccharide complexes (Ewers *et al.,* 2003; Li *et al.,* 2005 and Schouler *et al.,* 2012). However, numerous studies have demonstrated that these virulence factors are rarely all present in the same isolate and that they can occur either individually or polygenically with varying frequencies in clinical isolates, indicating that APEC strains constitute a heterogeneous group (Delicato *et al.,* 2003; Vandekerchove *et al.,* 2005 and Mbanga and Nyararai, 2015). The accurate identification of virulent strains of *E. coli* and the virulence genes they possess is essential if genes that can serve as vaccine targets are to be identified. Virulence gene studies are therefore important as they not only aid in the characterization of pathogenic strains of *E. coli* but may eventually lead to the development of effective vaccines (Mbanga and Nyararai, 2015).

Epidemiological tracing of *E. coli* strains is of considerable importance in veterinary microbiology. The data can be used to monitor trends in the occurrence of pathogenic strains or to identify possible source of infection. Autologous bacterins provide limited serotype- specific protection, because multiple serogroups are associated with disease, especially O1, O2 and O78 among many others (Dziva and Stevens, 2008 and Roshdy *et al.,* 2012).

The precise set of virulence factors possessed by Avian Pathogenic *E. coli* (APEC) isolated from infected birds is not clearly established in the isolates of Qena Governorate. Detection of Avian Pathogenic *E. coli* (APEC) and their virulence factors that causes infections in poultry becomes important for effective treatment with antimicrobial therapy and control schemes resulting in reducing both the incidence and mortality. So, this study was carried out in order to record the prevalence of *E. coli* infection in Qena province by bacteriological characterization of *E. coli* isolates in chickens, identification of the prevalent serotypes of *E. coli* isolates and detection of some virulence genes of the isolated strains by using PCR.

MATERIALS AND METHODS

Collection of samples:

A total of 600 freshly dead and diseased chicken samples categorized as 332 from baby chicks, from 268 broilers were collected from 81 farms at different Governmental and private poultry farms in five different centers at Qena province (Qena, Qift, Deshna, Qus and Nag-Hammadi). Bacteriological Samples were taken from diseased and freshly dead chicken (liver; spleen; nasal cavity; trachea; lung; air sacs; kidneys; yolk sacs; pericardium; and intestine from cases exhibiting perihepatitis, pneumonia, airsacculitis, yolk sac infection, pericarditis, and enteritis) after clinical and postmortem examination. Each examined organ was taken alone in sterile plastic bag, kept in icebox and be transferred with minimum delay to the laboratory.

Bacteriological examination:

Under complete aseptic conditions, samples (after sterilization of their surfaces using heated spatula), were inoculated into nutrient broth and incubated at 37 ˚C for 24 hours. This step was followed by subculturing on *E. coli* selective media (MacConkey's agar and Eosin methylene blue (EMB) agar), with incubation at 37 ˚C for another 24 hours. Suspected purified colonies to be *E. coli* were picked up and streaked on nutrient agar slopes and kept for morphological and biochemical identification (Konemann *et al.,* 1997; Quinn *et al.,* 2002 and Lee and Nolan, 2008).

Serological typing of *E.coli*:

Fifty *E.coli* suspected isolates "biochemically identified" were subjected to serotyping according to (Edwards and Ewing, 1972 and Lee *et al.,* 2009) by Slide agglutination test, using Polyvalent and monovalent diagnostic *E.coli* antisera.

Detection of *E. coli* virulence genes by PCR:

Multiplex PCR was applied by using five sets of primers for detection of five virulence genes playing a role in the virulence of APEC. These genes were eaeA (intimin or *E.coli* attaching and effacing gene); ompA (outer membrane protein); stx2 (shiga-toxin2 gene); papC (pyelonephritis associated Pili gene); tsh (temperature sensitive heamagglutinin gene). Multiplex PCR was applied on isolated *E. coli* Following QIA amp DNA mini kit instructions (Catalogue no.51304); Dream Taq Green PCR Master Mix (2X) (Thermo Scientific) Cat No. K1081 and agarose gel electrophoreses (Sambrook *et al.,* 1989).

RESULTS

Prevalence of *E. coli* isolates among chickens:

The number of the positive samples with *E. coli* isolation was 50 out of 600 isolates (8.3%); 30 isolates (9.0%) from baby chicks (up to one week), 20 isolates (7.4%) from broilers (from one week to 6 weeks), as revealed from table (1). The bacteriological examination of studied organs revealed that *E. coli* were isolated fromlung samples (17.5%); kidney and ureter samples (16.7%); air sac samples (14%); yolk sac samples (12.5%); intestinal samples (11.8%); spleen samples (10%); heart samples (7.9%); liver samples (3.4%) and nasal cavity & tracheal samples (2.5%) as shown in table (2).

Serotyping of *E.coli* isolates:

Serological examination was carried out on biochemically identified *E. coli* isolates (50 isolates) for species identification. Among the fifty investigated isolates, 37 typable *E. coli* isolates (74%), and 13 untypable isolates (26%) were identified. Among the typable isolates, serogroup O27 was the most frequently observed (8%) followed by O44 (6%), O125 (6%), O152 (6%) and O159 (6%) followed by serogroup O1 (4%), O6 (4%), O20 (4%), O26 (4%) and O166 (4%).Then, O8 (2%), O86a (2%), O115 (2%), O128 (2%), O136 (2%), O146 (2%), O158 (2%), O164 (2%) and O167(2%), as described in table (3).

Detection of some virulence genes of *E.coli* isolates by PCR:

Multiplex PCR results shown in table 4 and figure 1 to 4, revealed that ompA virulence gene was detected in all serogroups except O8 giving a PCR product of 919 bp. Moreover, papC virulence gene was detected in all serogroups except O8, O86a, O136, O146, O167 and O169 giving a PCR product of 501 bp. While Stx2 virulence gene was detected only in serotypes O27 & O125 giving a PCR product of 779 bp. The eaeA gene was amplified in serogroups O1, O20, O26, O115, O152, O166, O168 and O169 giving a PCR product of 248 bp. Finally, tsh virulence gene was detected in all serogroups except O20, O26, O86a, O115, O128, O167 and O169 giving a PCR product of 620 bp. Although we notice some exceptions from these results, that some samples having the same strain but different in carrying the same virulence genes. For example, the strain O27, there are 4 samples represent it, samples No. 24, 15, 11 and 47. Sample No. 24 has 4 virulence genes, ompA, papC, tsh and Stx2, although sample No. 15 has only 3 virulence genes ompA, papC and tsh, on the other hand, sample No. 11 has only 2 virulence genes, ompA and papC. Interestingly, sample No.47 lacks any of them. This is the same with the strain O125 which represented with 3 samples No. 23, 22 and 48.Sample 23 has 4 virulence genes, ompA, papC, tsh and Stx2, although sample No. 22 has only 3 virulence genes ompA, papC and tsh, but the sample No.48 also lack all of them. This result makes the samples No. 47 and 48 a good chance for possibility of preparation of an effective vaccine from these samples after testing them for pathogenicity test.

Table 1: Incidence of *E. coli* recovered from examined chickens samples.

chickens	No. of chickens	No. of isolated *E.coli*	Percentage (%)
Baby chicks	332	30	9.0
Broilers	268	20	7.4
Total	600	50	8.3

Table 2: Incidence of *E. coli* in different organs of examined chicken samples (Calculated according to the total number of each samples).

Organs	No. of samples	Positive samples	Percentage (%)
Lung	40	7	17.5
Kidney&ureter	30	5	16.7
Air sacs	50	7	14
Yolk sacs	40	5	12.5
Intestine	17	2	11.8
Spleen	30	3	10
Heart	177	14	7.9
Liver	176	6	3.4
Nasal cavity& Trachea	40	1	2.5
Total	600	50	8.3

Table 3: Results of isolates serotyping.

No.	Serodiagnosis		Strain classification	Site of isolation	No. of strains	Percentage (%)
	Polyvalent sera	Monovalent sera				
1	Poly 4	O27	(ETEC)	Liver, trachea, air sacs	4	8
2	Poly 2	O44	(EPEC), (EAEC)	Heart, lung, spleen	3	6
3	Poly 2	O125	(EPEC), (EAEC)	Heart, kidney, spleen	3	6
4	Poly 8	O152	(EIEC)	Intestine, kidney,lung	3	6
5	Poly 4	O159	(ETEC), (EPEC),(EIEC)	Heart,lung	3	6
6	Poly 1	O1	(DAEC),(UPEC),(APEC), (NMEC),(SEPEC)	Air sacs, Intestine	2	4
7	Poly 4	O6	(ETEC),(UPEC), (NMEC),(SEPEC)	Heart, Kidney	2	4
8	Poly 5	O20	(SEPEC)	Yolk sac, kidney	2	4
9	Poly 1	O26	(EPEC), (VETC)	Liver, lung	2	4
10	Poly 2	O166	(EPEC)	Heart, lung	2	4
11	Poly 6	O8	(ETEC), (DAEC), (NMEC),(SEPEC)	Yolk sac	1	2
12	Poly1	O86a	(DAEC)	Air sac	1	2
13	Poly 6	O115	(EHEC)	Heart	1	2
14	Poly 1	O128	(ETEC), (EPEC), (EAEC)	Air sac	1	2
15	Poly 7	O136	(EIEC)	Liver	1	2
16	Poly 2	O146	(ETEC)	Heart	1	2
17	Poly 3	O158	(EPEC)	Yolk sac	1	2
18	Poly 8	O164	(EIEC)	Yolk sac	1	2
19	Poly 5	O167	(ETEC), (EIEC), (EPEC)	Heart	1	2
20	Poly 4	O168	(ETEC)	Lung	1	2
21	Poly 6	O169	(ETEC)	Lung	1	2
22	Untypable	------	------	------	13	26

Table 4: Results of virulence genes of *E. coli* isolates by PCR.

Serial No.	No. of sample	Strain	Virulence genes				
			papC	*ompA*	*Stx2*	*tsh*	*eaeA*
1	24	O27	+	+	+	+	-
2	15	O27	+	+	-	+	-
3	11	O27	+	+	-	-	-
4	47	O27	-	-	-	-	-
5	12	O44	+	+	-	+	-
6	14	O44	+	+	-	+	-
7	37	O44	-	+	-	+	-
8	23	O125	+	+	+	+	-
9	22	O125	+	+	-	+	-
10	48	O125	-	-	-	-	-
11	16	O152	+	+	-	+	+
12	29	O152	+	+	-	+	-
13	40	O152	-	-	-	+	+
14	25	O159	+	+	-	-	-
15	39	O159	-	+	-	+	-
16	42	O159	-	-	-	+	-
17	5	O1	+	+	-	+	+
18	6	O1	+	+	-	+	-
19	28	O6	+	+	-	+	-
20	30	O6	+	+	-	-	-
21	19	O20	+	+	-	-	-
22	38	O20	-	-	-	-	+
23	34	O26	+	+	-	-	+
24	31	O26	+	+	-	-	-
25	33	O166	+	+	-	+	+
26	44	O166	+	-	-	+	-
27	49	O8	-	-	-	+	-
28	7	O86a	-	+	-	-	-
29	18	O115	+	+	-	-	+
30	32	O128	+	+	-	-	-
31	1	O136	-	+	-	+	-
32	4	O146	-	+	-	+	-
33	43	O158	+	+	-	+	-
34	9	O164	+	+	-	+	-
35	2	O167	-	+	-	-	-
36	27	O168	+	+	-	+	+
37	36	O169	-	+	-	-	+
38	3	Untypable	+	+	-	-	-
39	8	Untypable	+	+	-	-	+
40	10	Untypable	-	+	-	+	+
41	13	Untypable	+	+	-	-	-
42	17	Untypable	+	+	-	-	+
43	20	Untypable	+	+	-	+	-
44	21	Untypable	+	+	-	+	-
45	26	Untypable	+	+	-	-	-
46	35	Untypable	+	+	-	-	-
47	41	Untypable	-	-	-	+	-
48	45	Untypable	-	+	-	-	-
49	46	Untypable	-	-	-	+	-
50	50	Untypable	-	+	-	+	-

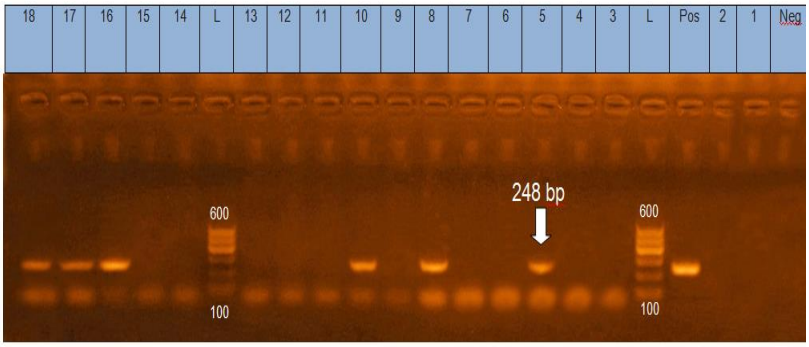

Fig. 1: Amplification of eaeA gene of *E. coli* serogroups
Lane L: 100-600bp DNA Ladder. Neg.: Negative control. Pos.: Positive control. Lane 5: *E.coli* O1 (Positive). Lane 16: *E.coli* O152 (Positive). Lane 18: *E.coli* O115 (Positive).

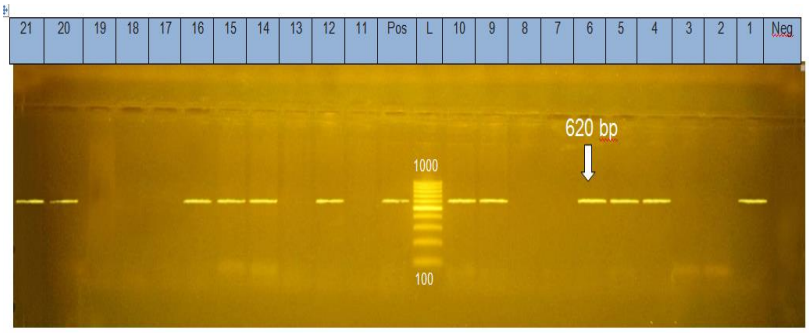

Fig. 2: Amplification of tsh gene of *E. coli* serogroups
Lane L: 100-1000 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control. Lane 5: *E.coli* O1 (Positive). Lane 12: *E.coli* O44 (Positive). Lane 15: *E.coli* O27 (Positive).

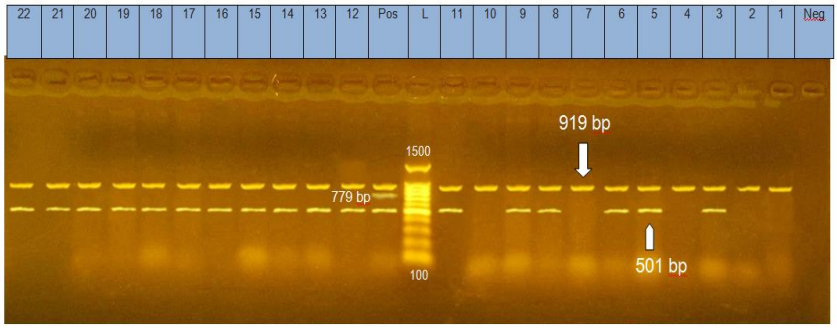

Fig. 3: Amplification of papC, stx2 and ompA genes of *E. coli* serogroups
- Lane L: 100-1500bp DNA Ladder. Neg.: Negative control. Pos.: Positive control.
- ompA gene (919 bp): Lane 5: *E.coli* O1 (Positive). Lane 12: *E. coli* O44 (Positive). Lane 15: *E. coli* O27 (Positive).
- papC gene (501 bp): Lane 5: *E. coli* O1 (Positive). Lane 12: *E. coli* O44 (Positive). Lane 15: *E. coli* O27 (Positive).

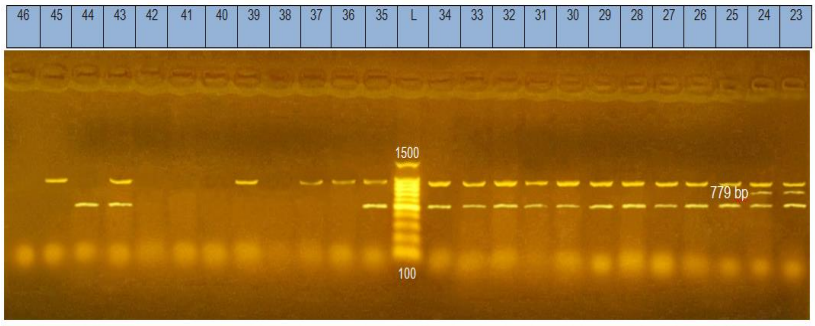

Fig. 4: Amplification of stx2 gene of *E. coli* serogroups
stx2 gene (779 bp): Lane 23: *E. coli*O125 (Positive). Lane 24: *E. coli*O27 (Positive).

DISCUSSION

The number of positive *E.coli* isolates was50 out of 600 isolates (8.3%) were collected from 81 farms at different Governmental and private poultry farms in five different centers at Qena province (Qena, Qift, Deshna, Qus and Nag-Hammadi). These finding were almost similar to those obtained by (Abd EL-Samie, 2015) who said bacteriological isolation showed that 34 out of 420 specimens was *E. coli* positive (8 %).

On the other hand, higher incidence (26.9%) was published by (Ramadan *et al.,* 2016). Interestingly, more recent investigations revealed much higher rates of *E. coli* for chicken (60%) obtained by (Eid *et al.,* 2016).

In the present study, the incidence of *E. coli* varied according to age that, 30 isolates (9.0%) were from baby chicks (up to one week) and 20 isolates (7.4%) were from broilers (from one week to 5 weeks). These results agreed with (Shahat, 2015) who found that the incidence of *E.coli* in one week old chicks was (14.3%), while in one-three weeks it was (9.8%) and in age over three weeks (8.9%). Our results also agreed with (Naliaka, 2011), who found that the prevalence of disease decreased with increase in bird age in isolates from broiler chicken, Also, This is in agreement with earlier studies (Smith *et al.,* 2006 and Moussa *et al.,* 2007) that confirmed this relation.

The bacteriological examination revealed that higher rates of isolation of *E. coli* was from lung (17.5%); followed by kidneys and ureters (16.7); air sac (14%); yolk sac (12.5%); intestinal tract (11.8%); spleen (10%); heart (7.9%); liver (3.4%); and finally nasal cavity & trachea (2.5%). These results agreed with (Abd EL-Samie, 2015) who said that the incidence of *E. coli* in lung (60%) is higher than heart (20%) and spleen (20%). Moreover, (Yousef *et al.,* 2015) disagreed this result that he found the incidence of *E. coli* in the lung was the lowest in compare with heart and liver.

In case of comparing the results of incidence of *E. coli* in liver and heart, (Abd El Tawab *et al.,* 2015) agreed with our result that the incidence of *E. coli* in heart is higher than that in liver. But, (Eid and Erfan, 2013) disagreed with this result.

In case of comparing the results of incidence of *E. coli* in intestine and liver (Abd El Tawab *et al.,* 2014) agreed with our result. But, (Sharada *et al.,* 2010) disagreed with this result.

In the present study, among the fifty investigated isolates, 37 typable *E. coli* isolates (74%) could be serotyped in 21 serogroups. The predominant serogroup was O27 (8%). These results go hand to hand with the previous study of (Shahat, 2015) who found that *E. coli* serogroup O27 was the most predominate (15%). El-jakee *et al*. (2012), identified *E. coli* serogroup O27 that recovered from diarrheic chicken from Egypt.

In the present study, other serogroups followed the O27, were O44 (6%), O125 (6%), O152 (6%) and O159 (6%). These results were agreed with that obtained with (Abd El Tawab *et al.,* 2015) who mentioned that themost commonly detected *E. coli* serogroups were O44 (11.3%), O125 (7.5%). These results also matched with those by (Suwanichkul and Panigrahy, 1988; Gross, 1991 and Bosch *et al.,* 1993), who reported that serogroup O44 and other strains were traditionally associated with colibacillosis in poultry. Also, (Roshdy *et al.,* 2012) mentioned that O44 was the predominate strain (9.3%) followed by O125 (5.5%). Moreover, (Eid and Erfan, 2013) found that O125 was the second predominate with percentage (14.29%) but O44 was found with lower percentage (3.57%). This disagreed with (Eid *et al.,* 2016) who found O125 and O44 in the lowest percentage.

E. coli serogroups O1 (4%), O6 (4%), O20 (4%), O26 (4%) and O166 (4%) was also identified in this study. This agreed with (Saif, 2003) whoreported that only 15% of the strains belonged to the serogroups O1, O2, O35, O36, and O78 that have been associated previously with avian colibacillosis were isolated from diseased birds, suggesting that this might signal the emergence of new pathogenic serotypes. Also, this is agreed with (Yousef *et al.,* 2015; Eid *et al.,* 2016 and Ramadan *et al.,* 2016) who found O1 strain in low percentages. Our result disagreed with (Hassan, 2016) who found O1 in a high percentage (23.5%).

Our results nearly matched with (Eid *et al.,* 2016) who reported that O26 found in a percentage (6.67%) and this is the same with (Ramadan *et al.,* 2016) who found O26 (6.89%). Yousef *et al.,* 2015, disagreed our result that they found O26 was the most predominate strain.

Da Silveira *et al*. 2002, identified 2.3% of APEC serogroup O6 among isolates from chicks with omphalitis, (Vandemaele *et al.,* 2003) investigated 100 APEC strains from 83 Belgian poultry farms, detecting only three serogroup O6 strains, these results agreed our results. (Knöbl *et al.,* 2012) disagreed these result that they found 31 strains (62%) belonged to serogroup O6.

In the present study, other serogroups were detected: O8 (2%), O86a (2%), O115 (2%), O128 (2%), O136 (2%), O146 (2%), O158 (2%), O164 (2%) and O167 (2%). These results agree with (Yousef *et al.,* 2015 and Eid *et al.,* 2016) that O128 present in a very low percentage.

In the present study, the percentage of Untypable *E. coli* strains was low (26%). This result close to the results of (Shahat, 2015), who found that 2 strains out of 20 strains (10%) and also (Khan *et al.,* 2005), who found that three strains of total 19 strains (15.8) isolated from poultry litter were not serogrouped to any known serogroup.

On the other hand, higher percentage (60%) was mentioned by (Zhao *et al.,* 2005) were non-typable with standard O antisera, this similar to (Cloud *et al.,* 1985 and Allan *et al.,* 1993), who found that the large percentage of Untypable *E. coli* strains was common characteristics of all groups of *E. coli* recovered from avian colibacillosis regardless of geographical location.

Enteric *E. coli* are both natural flora of humans and important pathogens causing significant morbidity and mortality worldwide. Traditionally enteric *E. coli* have been divided into 6 pathotypes, (Enteropathogenic *E. coli* (EPEC), verotoxin (Shiga-like toxin)-producing or enterohaemorrhagic *E. coli* (VETC or EHEC), Enterotoxigenic *E. coli* (ETEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC) and Diffusely adherent *E. coli* (DAEC) (Clements *et al.,* 2012). Three of these pathotypes can invade the intestine, they are EIEC, DAEC and VTEC. In EIEC, there is the invasive plasmid with the capability to invade host tissues. DAEC is also responsible for recurring urinary tract infections. VTEC travels to kidney through the bloodstream damaging renal endothelial cells resulting in renal inflammation (Hussain, 2015). So, their isolation from any organ other than intestine is very logic because they have the ability to invade the intestine and travel to any organ. But the other three pathotypes "ETEC, EPEC and EAEC" have unknown mechanisms for invasion of intestine, because their action occur only in the intestine. So, it is very interesting to isolate these strains from organs other than the intestine. This occurred with O27, O128, O146, O168 and O169 which are ETEC as shown in table (3). Also, this occurred with O44, O125, O166, O128 and O158 which are EPEC and in the same time are EAEC except O166 and O158 which only are EPEC. All of them isolated from organs other than intestine like liver, heart, lung, air sac, kidney and spleen. This is explained by (Elsas *et al.,* 2011) who mentioned that horizontal gene transfers have been involved in the spread of the virulence-related genomic islands across different *E. coli* strains.

It is difficult to compare between the incidences of pathogenic *E. coli* along with the distribution of its serotypes among different countries owing to the contribution of many conditions such as geographic area, seasonal variation, sampling techniques, and the conventional methods used for its isolation. However, it might be helpful in the prediction of certain outbreaks caused by these serotypes, especially in countries of the same geographical and climatic conditions, with a subsequent application of a suitable control regime (Ramadan *et al.,* 2016). These variations reflect that *E. coli* serogroups are country specific and also it may differ within different localities in the same country and this beneficial in bacterines preparation as it must be specific to the predominant serotypes, also it is clear that avian *E. coli* represented by known and few serotypes (Abd El Tawab *et al.,* 2015).

In the present study, Multiplex PCR results recovered that ompA virulence gene was detected in all serogroups except O8 giving a PCR product of 919 bp. This result almost similar to that of (Johnson *et al.,* 2008; Zhao *et al.,* 2009 and Abd El Tawab *et al.,* 2014) who said that ompA gene was found in all APEC isolates.

Moreover, papC virulence gene was detected in all serogroups except O8, O86a, O136, O146, O167 and O169 giving a PCR product of 501 bp. These results come in accordance with (Jan Ben *et al.,* 2001; Zhao *et al.,* 2009; Qabajah and Yaqoub, 2010 and Abd El Tawab *et al.,* 2014). Also our results agree with those by (Roussan *et al.,* 2014) who mentioned that the gene papC, indicating the existence of the papC, was present in 50% of all investigated strains.

While Stx2 virulence gene was detected only in serotypes O27 & O125 giving a PCR product of 779 bp. Our result agreed with those obtained by (Abd El Tawab *et al.,* 2014) that he found the stx2 gene was amplified in both serotypes O55 and O125 giving a PCR product of 779bp. This also nearly agree with the results obtained by (Jan Ben *et al.,* 2001; Fujioka *et al.,* 2009; Hideki *et al.,* 2009; Zhao *et al.,* 2009; Dutta *et al.,* 2011 and Al- Ajmi, 2011). On the contrary, these results disagreed with (Kobayashi *et al.,* 2005 and Shimaa, 2013) who could not detect shiga toxin genes in chicken samples.

The eaeA gene was amplified in serogroups O1, O20, O26, O115, O152, O166, O168 and O169 giving a PCR product of 248 bp. These results came in accordance with those recorded (Frank *et al.,* 1998; Osek, 2003; Ahmed *et al.,* 2007; Fujioka *et al.,* 2009; Hideki *et al.,* 2009; Dutta *et al.,* 2011; Al-Ajmi, 2011 and Abd El Tawab *et al.,* 2014). Meanwhile these results disagreed with others who found no eaeA gene detected in all APEC isolates (Olsen and Christensen, 2011 and Shimaa, 2013).

Finally, tsh virulence gene was detected in all serogroups except O20, O26, O86a, O115, O128, O167 and O169 giving a PCR product of 620 bp. This result goes in parallel with those obtained by (Gomis *et al.,* 2001; Jan Ben *et al.,* 2001; Fujioka *et al.,* 2009; Qabajah and Yaquoub, 2010 and Abd El Tawab *et al.,* 2014).

Although we notice some exceptions from these results, that some samples having the same strain but different in carrying the same virulence genes. For example, "the strain O27", there are 4 samples represent it, samples No. 24, 15, 11 and 47. Sample No. 24 has 4 virulence genes, ompA, papC, tsh and Stx2, although sample No. 15 has only 3 virulence genes ompA, papC and tsh, on the other hand, sample No. 11 has only 2 virulence genes, ompA and papC. Interestingly, sample No.47 lacks any of them. This is the same with the strain O125 which represented with 3 samples No. 23, 22 and 48.Sample 23 has 4 virulence genes, ompA, papC, tsh and Stx2, although sample No. 22 has only 3 virulence genes ompA, papC and tsh, but the sample No.48 also lack all of them. This result makes the samples No. 47 and 48 a good chance for possibility of preparation of an effective vaccine from these samples after testing them for pathogenicity test. These results typically agree with (Delicato *et al.,* 2003; Vandekerchove *et al.,* 2005 and Mbanga and Nyararai, 2015) who said that numerous studies have demonstrated that these virulence factors are rarely all present in the same isolate and that they can occur either individually or polygenically with varying frequencies in clinical isolates. This all indicates that APEC strains constitute a heterogeneous group. The accurate identification of virulent strains of *E. coli* and the virulence genes they possess is essential if genes that can serve as vaccine targets are to be identified. Virulence gene studies are therefore important as they not only aid in the characterization of pathogenic strains of *E. coli* but may eventually lead to the development of effective vaccines.

CONCLUSION

Prevalence studying of *E. coli* is very helpful for disease controlling. The combination of serotypic analysis and detection of the virulence factors of *E. coli* is more valuable as an epidemiological tool for identification of isolates. The multiplex PCR is a rapid and specific diagnostic tool used for genetic characterization of avian pathogenic *E. coli*.

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مشكلة ميكروب الايشيريشيا كولاي في قطعان التسمين في محافظة قنا

شيماء عبد الرحيم محمد ، أحمد إبراهيم أحمد ، نبيلة عثمان ، دينا محمد وحيد شيبة الحمد

Email: ibrahim.ahmed@lycos.com Assiut University web-site: www.aun.edu.eg

عدوي الميكروب القولوني من أهم الأمراض البكتيرية التي تؤثر في صناعة الدواجن والتي تسبب خسائر اقتصادية كبيرة ليس فقط نتيجة النفوق العالي للدواجن والفقد في الإنتاج والإعدامات في المجازر ولكنها عامل مساعد للإصابة بكثير من الأمراض الأخري. وعلي ذلك فإن هذه الدراسة تلقي الضوء علي ميكروب الايشيريشياكولاي المعزولة من بداري التسمين وزراعتها علي الأوساط الملائمة وكذلك الخصائص الكيميائية والخصائص السيرولوجية مع تحديد أهم الجينات الأكثر ضراوة بين العترات المعزولة. تمت هذه الدراسة علي٨١مزرعة من مزارع بداري التسمين (من عمر ١- ٤٥ يوم) من خمس مراكز مختلفة في محافظة قنا وهي قنا وقفط ودشنا وقوص ونجع حمادي، وقد تم تجميع ٦٠٠ عينة من الدجاج المريض والنافق حديثا من مختلف الأعضاء وهي الكبد والكلي والقلب والأمعاء والحويصلات الهوائية والرئتين والقصيبات الهوائية والطحال وكيس المح- ان وجد- من هذه الطيور بعد اجراء الفحص الإكلينيكي والصفة التشريحية، وتم عزل ٥٠ عينة ايجابية لميكروب الايشيريشياكولاي من اجمالي العينات بنسبة ٨,٣%، وقد سجلت أعلي معدلات عزل الميكروب القولوني من الأعضاء المختلفة كالآتي: الرئة بنسبة 17.5% يليها الكلي بنسبة ١٦,٧% والحويصلات الهوائية ١٤% وكيس المح ١٢,٥% والأمعاء ١١,٨% والطحال ١٠% والقلب ٧,٩% والكبد ٣,٤% والقصيبات الهوائية ٢,٥%. وقد أظهرت نتائج التصنيف السيرولوجي لعدد ٥٠ معزولة من الميكروب القولوني كالآتي: ٣٧ معزولة صنفت سيرولوجياً بنسبة ٧٤%بينما ١٣ معزولة بنسبة ٢٦% لم يتم التعرف عليهم سيرولوجيا. تم التعرف علي ٢١ سلالة من المعزولات المصنفة سيرولوجياً وهم O1, O6, O8, O20, O26, O27, O44, O68a, O115, O125, O128, O136, O146, O152, O158, O159, O164, O166, O167,O168 and O169 حيث مثلت ٤ معزولات من سلالة O27 بنسبة ٨ % و٣ معزولات من O44 بنسبة ٦ % و٣ معزولات من O125 بنسبة ٦ % و ٣ معزولات من O152 بنسبة ٦ % و٣ معزولات منO159 بنسبة ٦ % ومعزولتان منO1 بنسبة ٤ % ومعزولتان منO6 بنسبة ٤ % ومعزولتان منO20 بنسبة ٤ % ومعزولتان من O26 بنسبة ٤ % ومعزولتان من O166 بنسبة ٤ % ومعزولة واحدة من كلا من O8, O68a, O115, O128, O136, O146, O158, O164, O167, O168, O169 بنسب ٢ % لكلا منهم. كما أوضحت النتائج أن جميع المعزولات كان ايجابية للعامل الجيني ompA ما عدا السلالة O8، بينما وجد العامل الجيني papC في جميع العينات ما عدا O8, O86a, O136, O146, O167 O169، والعامل الجيني Stx2 وجد فقط في سلالتين فقط هما O27 & O125 ، وأيضا أوضحت النتائج أن العامل الجيني eaeA وجد فقط في السلالات O1, O20, O26, O115, O152, O166, O168, O169، وأخيراً نلاحظ وجود العامل الجيني tsh في جميع المعزولات ما عدا O20, O26, O86a, O115, O128, O167 and O169.