Characterization of *E.coli* isolated from chickens with colisepticemia in Egypt.

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Abstract

In this study, 61 tested samples were collected from different sources of poultry flocks (grandparents, layers , broilers and broiler breeders) of suspected *E. coli* septicemia in Egypt. Based on the obtained results, it was found that:

The prevalence of *E.coli* recovered from different flocks was 57 with rate of (6.22 %).and 57 isolates were distributed as 12 (21.05%) of isolates O158: K-, 8 (14.03%) of isolates O125:K70-, 6 (10.52%) of isolates O91: K-and Untypable each and finally the lowest percentage 1 (1.75%) were detected in isolates O111: K58, O86: K - and O127:K63. From testing of the phenotypic properties of the 61 isolates it was found that all tested *E. coli* isolates were Congo red positive but with different intensities, the isolates tested were positive in their motility test and haemolysis activity (100%), results showed that the two cell lines MDCK and Vero cells were infected with *E. coli.* Most of the cells were dead or indicated cytopathic changes, effect of isolates on embryonated chicken eggs (SPF) found they cause death to embryos within the 12 days post injection in a percent 80- 100 %.

This study was designed to determine the isolation rate and some serovars of *E. coli* which revealed clinical cases of colisepticaemia in poultry.

Key words:

Characterization, *E. coli,* chickens.

Introduction

Colibacillosis is one of the principal causes of morbidity and mortality in chickens and turkeys and causes significant economic losses to the poultry industry (**Dho-Moulin, 1993; Gross, 1991, 1994**). In avian species, Escherichia coli usually infects the respiratory tract, with bacteria passing though the mucosa, entering the blood stream, and causing a variety of diseases, including septicaemia, airsaculitis and Infection is generally enhanced or initiated by predisposing agents, such as mycoplasmal

or viral infection and environmental factors (**Dho and Lafont, 1982; Rosenberger *et al.,*1985; Nakamura *et al.,* 1992; Dho-Moulin, 1993; Gross, 1994**).

E. coli which inhabits the intestinal microflora or ecosystem of most mammalian and bird species is classified into 150 to 200 serotypes or serogroups based on somatic (O), capsular (K), fimbrial (F) and flagellar (H) antigens. The various pathotypes of *E. coli* tend to be clonal groups that are characterized by shared O (lipopolysaccharide, LPS) and H (flagellar) antigens that define serogroups (O antigen only) or serotypes (O and H antigens) (**Kaper *et al.,* 2004**)

Pathogenic *E. coli* are classified into categories or pathotypes based on the production of broad

classes of virulence factors and on the mechanisms by which they cause disease. Within each pathotype, strains are classified into virotypes or virulence gene profiles, based on the presence of combinations of virulence genes. Strains of a particular pathotype belong to a restricted number of serotypes or clones. Molecular genotyping or fingerprinting techniques such as pulse-field gel electrophoresis (PFGE) are being increasingly used as an adjunct or instead of serotyping, for the epidemiological monitoring of the *E. coli* in animals and their environment **(Kaper *et al.,* 2004).**

Serotyping is a common method used for the characterization of clinical isolates of *E. coli* and has a broad use in epidemiology and also in medical diagnostics . Serotyping is mainly based on the determination of the lipopolysaccharide O antigens, the flagellar H antigens and the capsular K antigens. Hence the existing association between serotype and pathotype makes this method a valuable tool for typing *E. coli* and also other bacterial species.

Pathogenic *E. coli* may also be differentiated by serotypinc, based on antigenic differences in the O antigen of the LPS, in the flagellar or H antigens, and the fimbrial or F antigens. (**Orskov and Orskov, 1984**).

For this aim, a total of 57 *E.coli* strains, from colisepticaemic birds and colibacillosis affected ones were serotyped.

In conclusion the present study demonstrate that *E.coli* is one of the major pathogen responsible for various type of diseases condition in poultry leading to economic losses in poultry industries.

Materials and methods

Bacteriological examination

Isolation and identification of pathogenic *E. coli*

Birds showing symptoms of colisepticaemia were submitted to the Reference Laboratory for Veterinary Quality Control on Poultry Production, Dokki, to be checked for the presence of *E. coli* infection in different flocks (Grandparents, broiler breeders, broilers and layers). The birds were obtained from both private and governmental farms during the period from 2009 – 2012. Organs were collected aseptically to prevent cross contamination. The collected organs (liver, spleen, ovary, and blood) were cultured within a time limit which did not exceed 24 hours from collection.

The samples (liver, spleen, ovary, and blood) were collected from live poultry under strict sterile conditions from chickens showing colisepticaemia and handled according to **Waltman *et al.,* (1998).**

All the isolates obtained from clinical coli septicaemia, in birds from Egypt were streaked on MacConkey, and eosin methylene blue agar.

The suspected colonies were picked and streaked on Tryptose soya agar plates. The purified isolates of *E. coli* were examined by the API 20 E (Enteric) system method for *E. coli* identification.

All the *E.coli* isolates were stored at -80 o C in Semi-solid nutrient agar medium (0.4%) with 20% of glycerol till use.

O-antigen serogrouping

The serotype of each isolate was determined using a slide agglutination technique on a panel of 40 different anti-O-sera, according to the laboratory methods and international literature of clinical diagnosis for poultry, rabbits and livestock **(Blanco *et al.,* 1998).** A panel of 40 different specific antisera (O1, O2, O4, O6, O8, O9, O10, O11, O15, O18, O20, O21, O22, O26, O45, O49, O64, O73, O75, O78, O83, O85, O86, O88, O92, O101, O103, O109, O111, O128, O132, O138, O139, O141, O147, O149, O153, O157). A suspension of each isolate was diluted in 1 ml PBS and heated for 1h at 100 oC. A 5µl of each sample was mixed with quantity of an O-serotype antiserum. Agglutination on a glass slide within 1 minute determined the respective serotype.

Embryo Lethality assay (pathogenisity of E.coli) (Nolan *et al.,* 1992)

Embryonated eggs were obtained from a specific-pathogen-free source and incubated in a 37oC humidified egg incubator. Each of E. coli isolate from the semisolid agar were inoculated onto a nutrient agar plates. The nutrient agar plates were incubated at 37oC, and the follow-

ing day, a single colony was selected and inoculated into a tube of Tryptose phosphate broth (TPB) and incubated overnight at 37oC. The TPB growth was washed by centrifuging the tube at 1000 xg for 15 min, after which the supernatant was decanted and replaced with an equal volume of sterile phosphate-buffered saline (PBS). The turbidity of the twice-centrifuged E. coli culture was adjusted with sterile PBS until the tube had a visual opacity equivalent to a McFarland #1 (McF #1) standard. A 10-4 dilution of the McF #1 equivalent was made in TPB and used to inoculate ten 12-day-old embryos by placing 0.1 ml into the chorioallantoic sac (CAS). All inoculated embryos were sealed and returned to incubation, then candled daily, with any lethality recorded through their 19th day of incubation. Each time an assay was run, 10 additional 12-day-old embryos of the same setting were inoculated with 0.1 ml of TPB by the same route. These embryos served as negative controls and were handled in the same manner as the E. coli inoculated eggs. Any test in which more than one negative control embryo died during the assay period was considered invalid and was repeated.

Results and discussion

The obtained results showed that the prevalence of *E.coli* isolates *were* recovered with percentage of 6.22% from the examined 61 flocks showing symptoms of coli septicaemia. The incidence of *E. coli infection* was 6.66% in the grand parent and broiler samples while the prevalence from broiler breeders and layers reached 6% and 4.76% respectively.

Table 1. Prevalence of *E. coli* from different flocks.

Type of flocks	Number of examined flocks	Number of examined samples	Results	
			+ve	%
Grand parents	2	30	2	6.66
Broilers Breeders	20	300	18	6
Broilers	32	480	32	6.66
Layers	7	105	5	4.76
Total	61	915	57	6.22

All the isolates obtained from clinical coli septicaemia, in birds from Egypt fermented lactose on Mac Conkey, and showed greenish metallic sheen on eosin methylene blue agar.

Incidence of the isolated *E. coli* recovered from internal organs and different flocks

Table (2) Incidence of the total number of *E.coli* isolates isolated from positive samples recovered from the internal organs of different flocks.

	Serovars	Types of flocks					
		Grand parents	Broilers Breeders	Broilers	Layers	number	%
1	O111: K58	0	1	0	0	1	1.75
2	O114: K90	0	3	1	1	5	8.77
3	O103: K-	0	1	1	0	2	3.5
4	O44: K74	1	0	2	0	3	5.26
5	O91: K-	0	4	2	0	6	10.52
6	O26: K60	0	0	1	1	2	3.5
7	O158: K-	0	4	6	2	12	21.1
8	O119: K69	0	2	2	0	4	7.1
9	O78: K80	1	0	2	0	3	5.26
10	O125:K70	0	0	8	0	8	14.03
11	O127:K63	0	0	1	0	1	1.75
12	O25:K11	0	0	3	0	3	5.26
13	O86:K -	0	0	1	0	1	1.75
15	Untypable	0	3	2	1	6	10.52632
	total	2	18	32	5	57	

*percentage calculated according to the total number of the isolates

From the obtained results Serotypes O1 and O78 are the most common ones among avian pathogenic *E. coli* strains **(Dho-Moulin and Fairbrother, 1999; La Ragione and Woodward, 2002; Yaguchi *et al.*, 2007; Ozaki and Murase, 2009)**. Serotyping in (Oh et al., 2012) study revealed that only 9 strains (15.51%) belonged to serogroups O1 and O78, and much more than half of the isolates could not be assigned to the common serogroups. This supports the suggestion that serotyping is not recommended as a specific diagnostic tool for the identification of avian pathogenic *E. coli* **(Ewers *et al.*, 2004; Rosario *et al.*, 2004).** One genetically distinct isolate of serotype O78 (no. 14 in this study), which is one of the major APEC serotypes, was detected among the avian pathogenic *E. coli* studied here. In the present study the serological identification of The 57 serotypes were distributed as 12 (21.05%) of serotypes O158: K-, 8 (14.03%) of serotypes O125:K70-, 6 (10.52%) of serotypes O91: K-and Untypable each and finally the lowest percentage 1 (1.75%) were detected in serotypes O111: K58, O86:K - and O127:K63.

Embryo lethality assay

Pathogenicity of E. coli recovered from grandparents

As shown in Table 3. The lethality of the embryos through their 19th day of incubation of the tested serovars were 100% highly pathogenic for the embryo lethality assay.

Table 3. The mortalities of the embryos during the incubation period against *E. coli* recovered from grand parents

Grandparents		serovars	13	14	15	16	17	18	19	20	21	22	23	24	total
	1	O78:K80	1	4	0	0	0	0	0	0	0	0	0	0	5
	2	O44:K74	0	1	2	2	0	0	0	0	0	0	0	0	5

Pathogenicity of E. coli recovered from layers

As shown in Table 4 The lethality of the embryos through their 19th day of incubation of the tested serovars were 100% highly pathogenic for the embryo lethality assay.

Table 4. The mortalities of the embryos during the incubation period against *E. coli* recovered from layers

layers	n=	serovars	13	14	15	16	17	18	19	20	21	22	23	24	total
	1	O158:K-	0	2	0	0	3	0	0	0	0	0	0	0	5
	2	O158:K-	0	4	0	0	1	0	0	0	0	0	0	0	5
	3	untypable	0	3	0	0	1	0	1	0	0	0	0	0	5
	4	O114:K90	0	1	2	0	2	0	0	0	0	0	0	0	5
	5	O26:K60	0	3	0	0	0	1	1	0	0	0	0	0	5

Pathogenicity of E. coli recovered from broilers breeders

As shown in Table 5 The lethality of the embryos through their 19th day of incubation of the tested serovars were100% highly pathogenic for the embryo lethality assay.

Table 5. The mortalities of the embryos during the incubation period against *E. coli* recovered from broilers breeders.

	n=	serovars	13	14	15	16	17	18	19	20	21	22	23	24	total
Broilers Breeders	1	O158:K-	0	2	3	0	0	0	0	0	0	0	0	0	5
	2	O158:K-	0	3	1	0	0	0	0	1	0	0	0	0	5
	3	O158:K-	0	2	3	0	0	0	0	0	0	0	0	0	5
	4	O158:K-	0	4	1	0	0	0	0	0	0	0	0	0	5
	5	O119:K69	0	2	1	1	0	0	0	1	0	0	0	0	5
	6	O119K69	0	3		0	0	0	2	0	0	0	0	0	5
	7	untypable	0	4	1	0	0	0	0	0	0	0	0	0	5
	8	untypable	1	1	1	1	0	0	0	0	0	0	0	0	4
	9	untypable	0	4	1	0	0	0	0	0	0	0	0	0	5
	10	O114:K90	1	3	0	1	0	0	0	0	0	0	0	0	5
	11	O91:K-	0	4	1	0	0	0	0	0	0	0	0	0	5
	12	O91:K-	0	2	3	0	0	0	0	0	0	0	0	0	5
	13	O91:K-	0	4	1	0	0	0	0	0	0	0	0	0	5
	14	O111:K58	0	3	1	0	0	0	0	1	0	0	0	0	5
	15	O91:K-	0	1	2	2	0	0	0	0	0	0	0	0	5
	16	O103:K-	1	3	0	0	0	0	0	1	0	0	0	0	5
	17	O114:K90	0	4	0	0	0	0	0	1	0	0	0	0	5
	18	O114:K90	0	3	2	0	0	0	0	0	0	0	0	0	5

Results of pathogenicity of *E. coli* recovered from broilers

As shown in Table 6 The lethality of the embryos through their 19th day of incubation of the tested serovars were 100% highly pathogenic for the embryo lethality assay.

Table 6. The mortalities of the embryos during the incubation period against of *E. coli* recovered from broilers.

	n =	serovars	13	14	15	16	17	18	19	20	21	22	23	24	total
broilers	1	O158:K-	0	2	0	0	0	3	0	0	0	0	0	0	5
	2	O125:K70	1	1	0	0	1	2	0	0	0	0	0	0	5
	3	O127:K63	0	3	0	0	1	1	0	0	0	0	0	0	5
	4	O44:K74	0	4	0	0	1	0	0	0	0	0	0	0	5
	5	O125:K70	0	2	2	0	1	0	0	0	0	0	0	0	5
	6	O25:K11	0	1	3	1	0	0	0	0	0	0	0	0	5
	7	O91:K-	0	1	0	2	0	2	0	0	0	0	0	0	5
	8	O25:K11	1	4	0	0	0	0	0	0	0	0	0	0	5
	9	O158:K-	0	2	1	1	0	0	1	0	0	0	0	0	5
	10	O25:K11	0	5	0	0	0	0	0	0	0	0	0	0	5
	11	O158:K-	0	5	0	0	0	0	0	0	0	0	0	0	5
	12	O125:K70	0	1	1	0	3	0	0	0	0	0	0	0	5
	13	O86:K -	0	2	3	0	0	0	0	0	0	0	0	0	5
	14	untypable	0	2	3	0	0	0	0	0	0	0	0	0	5
	15	untypable	0	4	0	0	0	0	1	0	0	0	0	0	5
	16	O125:K70	0	4	0	1	0	0	0	0	0	0	0	0	5
	17	O125:K70	0	4	0	0	0	0	1	0	0	0	0	0	5
	18	O158:K-	0	2	3	0	0	0	0	0	0	0	0	0	5
	19	O125:K70	0	3	2	0	0	0	0	0	0	0	0	0	5
	20	O125:K70	0	3	1	1	0	0	0	0	0	0	0	0	5
	21	O125:K70	0	4	0	1	0	0	0	0	0	0	0	0	5
	22	O119:K69	1	3	0	1	0	0	0	0	0	0	0	0	5
	23	O119:K69	0	5	0	0	0	0	0	0	0	0	0	0	5
	24	O91:K-	0	3	1	0	0	1	0	0	0	0	0	0	5
	25	O26:K60	0	2	1	0	0	1	1	0	0	0	0	0	5
	26	O103:K-	0	1	2	2	0	0	0	0	0	0	0	0	5
	27	O44:K74	0	2	0	3	0	0	0	0	0	0	0	0	5
	28	O78:K80	0	2	2	0	0	1	0	0	0	0	0	0	5
	29	O78:K80	0	2	3	0	0	0	0	0	0	0	0	0	5
	30	O158:K-	0	2	2	0	0	1	0	0	0	0	0	0	5
	31	O158:K-	1	3	1	0	0	0	0	0	0	0	0	0	5
	32	O114:K90	0	3	2	0	0	0	0	0	0	0	0	0	5

Discussion

Collibacillosis is caused by pathogenic Escherichia coli (APEC)

APEC belongs predominantly to three serogroups O 1, O2 and O78

Depending on the virulence status of the strain, host status and presence and type of predisposing factors

The infection manifests as an initial septecaeimia that is followed by either sudden death or localized inflammation in multiple organs (**Barnes and Gross, 1997**)

The isolated *E. coli* strains in the present investigation from the internal organs of broiler, grand parent, layers and broiler breeders chickens were of different serotypes. It was there-

fore of interest to investigate these isolates for their pathogenic ability using an experimental infection model or equivalent. One of the aims of this study was to determine if the chicken embryo lethality assay correlates with virulence. The embryo mortalities ranged from 80 to 100%; however, most of the isolates were highly virulent.

In previous studies, intravenous, subcutaneous, and intratracheal challenge of chickens was strongly correlated with ELA **(Gibbs *et al.,,* 2004).** Another study also showed that the ELA had the ability to discriminate between highly virulent, moderately virulent, and a virulent isolates of avian *E.coli* using percent morality **(Wooley *et al.,,* 2000).** Based on the criteria described by **Wooley *et al.,* (2000)**, most strains tested by **Oh *et al.,* (2012)** were highly or moderately virulent and only 4 strains (5.9%) were avirulent. This was not unexpected because all of the isolates originated from the visceral organs of chickens that died from colibacillosis. The results of **Oh *et al.,* (2012)** study and previous studies suggest that the ELA is a reliable alternative to the chicken lethality test and is preferable due to its accessibility and the fact that bacteria can be better contained at all times **(Nolan *et al.,,* 1992).**

Almost all serotypes of *E.coli* isolated have been found to be pathogenic but no particular serotype could be attributed to particular disease condition or particular age group.

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توصيف الايشريشيا كولاي المعزوله من حالات التسمم الدموي الحادمن الدجاج في مصر

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الملخص العربى

تقوم الدراسه علي تحقيق التعريف البيوكيميائى ثم التصنيف السيرولوجى لمعزولات جمعت من قطعان مختلفة (الجدود ، الامهات ، التسمين و البياض) من الدواجن و التي يشير الفحص الظاهري و التشريحي الي احتمال الاصابة بالميكروب القولوني الضارى

تم فى هذة الدراسة اختبار ٦١قطيع تم جمعها من قطعان محلية من مصادر متعددة للدواجن(الجدود و الامهات و البياض و التسمين) المشتبة في اصابتها بالايشيريشيا كولاي فى مصر. وبعد تحليل النتائج التى تم الحصول عليها، وجد أن اجراءات العزل قد اثبتت وجود ٦.٢٢% نسبة اصابة.

و تم عمل الاختبارات السيرولوجيه للمعزولات و عددها ٥٧ و كانت النتائج كالاتي ١٢ (٢١.٥%) من O158:K- و ٨ (١٤.٠٣%) من O125:K70-و اقل نسبه عزل من O111:K58 بنسبه ١.٧٥ %

و اظهرت النتائج اثر حقن المعزولات باجنه البيض أنها تسببت في موت الاجنة داخل البيض خلال ١٢ يوما بعد الحقن بنسبه ٨٠-١٠٠٪.