PREVALENCE AND ANTIBIOGRAM OF *E.COLI* IN BROILER CHICKEN REARED IN BENI-SUEF DURING YEAR 2002

By

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ABSTRACT

One hundred and one *E. Coli* isolates were isolated from 3 – 7 weeks old broiler chickens suspected to be suffering from colibacillosis, complicated CRD, diarrhea, arthritis, slow growth rate and high mortality up to 10%. From the examined 411 broiler chickens from 68 flocks, 335 (81.5%) were infected with colibacillosis and all flocks were (100%) positive for colibacillosis where 101 *E. coli* isolates were obtained.From these isolated "101"E. coli strain, only 55 isolate were sero-grouped into:- O_{26} (4.95%), O_{55} (4.95%), O_{111}(1.98%), O_{128} (10.89%), O_{86} (6.93%), O_{126} (5.94%), O_{114} (7.92%), O_{127} (7.92%), O_{125} (2.97%) while 46 isolate cannot be serogrouped according to available antisera. The in-vitro sensitivity of the isolated strains to various chemotherapeutic agents revealed that all strains were sensitive to Kanamycin and Amoxicillin (100%). All strains were resistant to Trimethoprim, while isolated strains showed sensitivity for neomycin, Ampicillin, Doxycycline,Flumequine, Spectinomycin, Oxytetracycline, Tobramycin, Chloramphenicol, Streptomycin, Colistin, Erythromycin, Lincomycin and Nalidixic acid in the following percentage 96.36, 96.36, 83.6, 76.2, 74.5, 61.8, 50.90, 43.63, 38.2, 36.36, 32.72, 30.90 and 3.63; respectively. In vitro pathogenicity testing of the isolated *E.coli* using Congo red medium revealed that all isolated strains were pathogenic (Congo red positive).

INTRODUCTION

Escherichia coli serotypes affect nearly all species of poultry, resulting in serious disease "Colibacillosis" causing great economic losses due to high mortality, increase cost of medication and prevention **(Ibrahim *et al.*, 1998 and Radwan, 2000)**. *E.coli* is a complicating factor for other conditions including Swollen head Syndrome (SHS),Chronic Respiratory Disease(CRD) that affect mainly bird performance a specially in growing birds as slow

growth, poor feed conversion and increase rate of down graded carcasses **(Heller *et al.*, 1972 and Shane, 1983)**.

E.coli organisms characterized by its rapid ability to aquaried resistance or variable resistance to most of used antimicrobial drugs especially that used in control or prevention of *E.coli* clinical affection in poultry **(Youssef *et al.*, 1983; Ibrahim and Shahata, 1985 and Soliman, 2000)**

Therefore, the aim of the present investigation was carried out to study the present prevalence and serotyping of *E.coli* among Field clinically affected broiler chickens as well as determination of both their in vitro sensitivity to available chemotherapeutic agents and in vitro pathogenicity as compared with other previous work in a trial to make spotting on the recent situation in broiler chickens.

MATERIAL AND METHODS

Samples: Aseptically collected samples from sacrified naturally infected 411 chickens aged from 3 – 7 weeks those collected from 68 flocks (2 – 10 birds / farm. These chickens were suffering from nasal discharge, sneezing, coughing, difficult respiration, emaciation and/ or diarrhea with post mortem lesions including congestion of internal organs, pericarditis, airsaculitis and distended gall bladder.

Media used:

a- Solid media:Nutrient agar (Bioolife), MacConkey's agar (Biolife) and Trypticase Soya agar (BBI) (Oxoid) were used for isolation.

b- Fluid media:Nutrient broth (Oxoid) and MacConkey's broth (Biolife).

c- Media for preservation: Semisolid soft agar medium (0.5%) containing 1% glucose **(Cruickshank *et al.*, 1975)**.

d- Media for biochemical reactions: The following media were prepared and used according to **Cruickshank *et al.*, (1975)**Triple Sugar Iron agar (TSI)(Gibco), Christensen's Urease agar slants (Diffco), Simmons citrate agar (Diffco),Glucose phosphate peptone water (MR/VP broth) and Peptone water 2% .

e- Media for sugar fermentation: Two percent peptone water containing Andraid's indicator to which 1% of the following sugars were added. Glucose, sucrose, lactose maltose, mannitol and inositol. The medium was dispensed in 3ml amounts in test tubes containing Durham's tubes after pH adjustments to 7.2 ± 0.2.

f- Congo red medium: Congo red medium was used to distinguish between pathogenic and non- pathogenic E. coli according to **Payne and Finkelstein, (1977)**.

Reagents and Solutions: The used reagents and solutions were prepared according to **Quinn *et al.*, (1994) and Collie *et al.*, (1996)**.Oxidase reagents, Kovac's reagent for indole. **(Kovacs, 1928)**, 0.04% methyl red indicator solution. For methyl red **(Ljutov, 1961)**Andrad;s indicator (for sugar fermentation), Normal Sterile solution 0.9% and Voges- Prouskauer indicator solution **(Ljutov, 1963)**.

Stain: Gram's staining technique used for morphological identification according to **"Cruickshank *et al.*, (1975)"**.

Antisera: *E.coli* agglutinating antisera were obtained from Behring Werk Institute Germany.

Sensitivity discs: The following discs were supplied from Oxoid.Neomycin (30µg), Tobramycin (10µg), Trimethoprim (2.5µg), Lincomycin (10µg) Doxycycline (30µg),Oxytetracycline (30µg), Streptomycin (10µg), Chloramphenicol (30µg), Amoxicillin (25µg), Spectinomycin (2.5µg), Flumequine (30µg), Ampicillin (10µg), Nalidixic acid (30µg), Erythromycin(15µg), Colistin (10µg) and Kanamycin (10µg).

Bacteriological Examination: Samples for bacteriological examination were collected aseptically from heart blood, liver, gall bladder and air sacs. These samples were individually inoculated into MacConkey's broth followed by incubation for 24 h at 37° C, then subcultures onto nutrient and MacConkey's agar media and incubated at 37° C for 24 – 48 hours.

Identification:

a- Morphology and stain reaction: Bacterial colonies were purified and identified according to **Cruickshank *et al.*, (1975)**. Smears were prepared , stained with Gram's stain and examined microscopically.

b- Biochemical identification: The obtained suspected isolates were biochemically identified according to **Edwards and Ewing (1972), Cruickshank *et al.*, (1975); Koneman *et al.*, (1983); Brenner, (1984) and Carter, (1986)**.

c- Serological identification was carried according to **Neville and Bryant, (1986)**.

Antibiogram: of isolated *E.Coli*: The disc diffusion technique was adopted according to **Blair *et al.*, (1970) and Cruickshank *et al.*, (1975)**. Sensitivity of the organisms were measured and interpritated according to **manual of Bio merieux (1980)**.

In Vitro Pathogenicity:

In vitro differentiation between pathogenic and non pathogenic E. Coli isolates using Congo red medium **(Berkhoff and Vinal 1986 and Spears *et al.*, 1992)**.

RESULTS

A total of 335 *E. coli* suspected isolates forming rose pink smooth entire edge small to medium sized colonies. Smears stained by Gram's stain revealed gram negative rod- shape or coccobacilli were isolated from the tested 411 samples in a percentage of 81.5% (Table 1).

All tested flocks were positive with variable percentage according to age where it was 87.5% - 3 weeks aged birds, 78.4% - 5 weeks and 87.8% - 7 weeks old chickens.

Table (1): Incidence of suspected *E. Coli* isolates from chickens.

Age/weeks	No of flocks	No. of +ve	% of positive flocks	No of exam birds	No. of suspected isolates	% of isolates/samples
-3	3	3	100%	16	14	87.5
-4	19	19	100%	127	105	82.6
-5	18	18	100%	102	80	78.4
-6	22	22	100%	133	107	80.45
-7	6	6	100%	33	29	87.8
Total	68	68		411	335	81.5

The obtained isolates showed that (101) isolates are positive to indole, methyl red, negative to voges proskauer, Simmon's citrate, urea and TSI as well as ferment glucose, dulcitol, arabinose and lactose with production of acid and gas. So out of the tested isolates 335 only 101 isolates 30% were proved to be *E. coli*. All isolates E. coli strains 101 were Congo red positive, degree of redness was varied from one isolates to another. According to the used antisera E. coli isolates were serotyped into 9 groups in variable percentages 2 – 11% Table, (2). The O_{128} K_{67} was obtained in the highest percentage(10.89%) followed by both O_{114} and O_{127} in percentage of (7.92%), O_{86} (6.93%), O_{126} (5.94%) and both O_{26} and O_{55} in (4.95%) as well O_{111} where it was reported in lowest percentage (1.9%) while 46 isolates were untypped with available antisera in 44.5%.

Table(2): Serological identification of *E.coli* isolates (101).

No.	OK serotype	No. of isolates	Percentage of isolates %
1	O_{26}:K_{60}	5	4.95
2	O_{55}:K_{59}	5	4.95
3	O_{111}:K_{68}	2	1.98
4	O_{128}:K_{66}	11	10.89
5	O_{86}:K_{61}	7	6.93
6	O_{126}:K_{71}	6	5.94
7	O_{114}:K..	8	7.92
8	O_{127}:K_{63}	8	7.92
9	O_{125}:K_{70}	3	2.97
10	Non-typable	46	44.50

Antibiogram of *E.coli* isolates:

Results in table (3) showed that all *E.coli* isolates were resistant to Trimethoprim (0.0%). All isolates were sensitive to Amoxicillin and Kanamycin (100%) more over the tested isolates showed lowest sensitivity percentage (3.63%) to Nalidixic acid, while Lincomycin, erythromycin, colistin, streptomycin, chloramphenicol, tobramycin, oxytetracycline, spectinomycin, flumequine, doxycycline, neomycin and ampicillin showed percentages of 30.90, 32.72, 36.36, 38.02, 43.63, 50.90, 61.80, 74.50, 76.20, 83.60, 96.36 and 96.36; respectively.

The tested serotypes resulted in variable degrees of sensitivity to used antimicrobial drugs. So there was no relationship between antigenic structure and sensitivity to different or same drug.

DISCUSSION

Colibacillosis now is a big problem facing poultry industry in Egypt and allover the world because it causes many diseases in different ages such as septicaemia, omphilitis, peritonitis, panophthalmitis, cellulites, arthritis, airsaculitis and salpingitis.

E. coli could be isolated from broiler chickens aging from 20 – 45 days old. This age is the age of high incidence *E.coli*infection **(Belitskii and Paniker, 1969)**. Our observation was in agreement with **Azzam , (1983) and Raid (1994), but Awaad , (1972)** found that the highest incidence of infection was at 15 days.

The clinical signs observed in the chickens from which *E.coli*was isolated, mortality ranging from 10% to 20% similar clinical signs and lesions were described in natural outbreaks by **Awaad, (1972); Hassanain, (1977); Sarhan, (1977); Youssef *et al.*, (1983), Azzam, (1983) and Raid, (1994)**.

The serogroups recovered were:- O_{26} (4.95%), O_{55} (4.95%), O_{111} (1.98%), O_{128} (10.89%), O_{86} (6.93%), O_{126} (5.94%), O_{114} (7.92%), O_{127} (7.92%), O_{125} (3.97%) of the total isolated serotype. These percentages were calculated in relation to the No. of isolated strains. Similar *E.coli* serotypes have been previously isolated from chickens in Egypt. **Awaad, (1972); Sadek and El Jamal, (1972), Alian , (1978); Abdel-Chafar, (1979); Andraws, (1980); Farid *et al.*, (1981); Zahdeh, (1982); Aly, (1989); Khalid, (1990); Samaha and El-Bassiouny, (1991); Zouel-Fakar, (1994); El- Nasser *et al.*, (1994); Riad, (1994); Ibrahim *et al.*, (1998) and Radwan, (2000)**.

E.coli serogroup O_{128} was the most frequently isolated from broiler chickens under respiratory distress (10.89%). An incidence from 28% to 6.7% was recorded by **Awaad, (1972); Farid *et al.*, (1981); Zahdeh, (1982); Khalid, (1990); Aly, (1989) and Zouel Fakar, (1994)**.

E. coli serogroup O_{127} was isolated in 7.92% of the total isolated serotype. It was isolated also by **Awaad , (1972); Abdel-Ghafar, (1979) Alian, (1978); Zahdeh, (1982); Khalid, (1990); Zouel Fakar, (1994) and Ibrahim *et al.*, (1998).**

*E.coli*serogroup O_{114} was isolated in 7.92%. This incidence was in agreement with the observation of **Awaad, (1972); Alian, (1978); Azzam, (1983); Zouel Fakar, (1994); El- Nasser *et al.*, (1994) and Ibrahim *et al.*, (1998).**

Serogroup O_{86} was isolated in 6.93%. The same serogroup was isolated previously by **Awaad, (1972); Zouel Fakar, (1994,); Abdel- Ghafar, (1979); Zahdeh, (1982); Farid *et al.*, (1981); Azzam, (1983) and Ibrahim *et al.*, (1998).**

Serogroup O_{126} was isolatedin rate of 5.94% as previously reported by **Awaad, (1972) and Abdel-Ghafar, (1979).**

E.coli serogroup O_{55} was isolated in an incidence of 4.95% as stated before by **Awaad, (1972); Zahdeh, (1982); El-Sayed, (1987) and Ibrahim *et al.*, (1998).**

E.coli serogroup O_{26} was isolated in the present work from broiler chickens suffering from colisepticaemia and respiratory affections in an incidence of 4.95% as those reported by **Awaad, (1972); Alian, (1978); Abdel-Ghafar, (1979); Azzam, (1983); Zouel-Fakar, (1994) and Ibrahim *et al.*, (1998)** while *E. coli* serogroup O_{125} was recorded in 2.97% as previously observed by **Zahdeh, (1982); Nafie *et al.*, (1988); Zouel-Fakar, (1994); Ibrahim *et al.*, (1998) and Radwan, (2000).**

Isolation of *E.coli* serogroup O_{111} in 1.98% was also seen by **Kulkarni, (1970); Sadek and El-Jamal, (1972); Abdel-Ghafar, (1979) and Azzam, (1983).**

Studying the in vitro sensitivity of the isolated *E.coli* strains (Table 3) against different chemotherapeutic agents revealed that 100% of tested strains were sensitive to Amoxicillin and Kanamycin and resistant to trimethoprim.

In regards to colistin 3663% of serotyped isolated *E.coli* strains were sensitive. Our result is in agreement with **Abdel-Ghafar, (1979) and Azzam, (1983).** and disagreed with **Youssef *et al.*, (1983); Aly, (1989) and Khalid, (1990).**

Results of erythromycin were nearly in agreement with those reported by **Awaad, (1972); Azzam, (1983); Ibrahim and Shahata, (1985)** while **Abdel-Wahab, (1977); Youssef *et al.*, (1983)** reported that *E.coli* isolates were resistant to erythromycin.

In relation to Nalidixic acid, 3.63% of isolated serotyped strains were sensitive. **Abdel-Ghafar, (1979); Azzam, (1983); El-Sayed, (1987) and Khalid, (1990)** found that *E.coli* isolates were sensitive to Nalidixic acid but **Youssef *et al.*, (1983)** reported that *E.coli* isolates were resistant to Nalidixic acid.

In relation to Streptomycin, 38.2% of isolated strains were sensitive as stated before by **Abdel-Ghafar, (1979) and Riad, (1994)** while all isolates of **Awaad, (1972)** were sensitive but **Ibrahim and Shahata, (1985); Aly, (1989) and Khalid, (1990)** found that all tested *E.coli* isolates were resistant.

Our results revealed that 96.36% of isolates were sensitive to Neomycin as those reported by **Awaad, (1972); Abdel-Ghafar, (1979); Riad, (1994) and Saleem *et al.*, (1999)** while our result disagreed with **Aly, (1989) and Khalid, (1990)** as they reported that all isolates were resistant.

*E.coli*isolates were sensitive (50.90%) to Tobramycin, this in agreement with **El-Sayed, (1987)**.

Our results revealed that *E.coli* isolates were resistant trimethoprim. This in agreement with **Youssef *et al.*, (1983); Aly, (1989) and Khalid, (1990)** and disagreed with **El-Sayed, (1987)** who reported that all *E.coli* isolates were sensitive to trimethoprim.

Tested *E.coli* strains (100%) were sensitive to Amoxicillin, our result were in agreement with **Mosters and Palmer, (1981) and Saleem *et al.*, (1999)**.

Sensitivity to Ampicillin, (96.36%) was in agreement with **Abdel-Ghafar, (1979)** but our result disagreed with **El-Sayed, (1987); Aly, (1989) and Khalid, (1990)**.

Iisolated *E.coli* was (61.8%) sensitive to oxytetracycline,. **Awaad, (1972); Azzam, (1983); Ibrahim and Shahata, (1985)** found variable sensitivity with oxytetracycline while **Sarma *et al.*, (1981)** reported 77 – 80% of *E.coli*strains were resistant.

E.coli strains were (76.2%) sensitive to Flumequine this result agree with **El-Sayed, (1987); Riad, (1994) and Saleem *et al.*, (1999)**.

In relation to lincomycin, 30.90% of isolated *E.coli* strains were sensitive, this in agreement with **Awaad, (1972) and El-Ged *et al.*, (1985)**.

All *E.coli* serotypes were sensitive to Kanamycin, this result agree with **Awaad, (1972); Abdel-Wahab, (1977); Abdel-Ghafar, (1979) and Saleem *et al.*, (1999)** while **Azzam, (1983)** found various results with kanamycin.

In relation to chloramphenicol, 43.63% of isolated *E.coli* isolates were sensitive as reported by **Abdel-Ghafar, (1979); Karmy *et al.*, (1987) and Riad, (1994)**.

Our result revealed that 83.6% of isolated E. coli strains were sensitive to Doxycycline.

In conclusion it was clear that their was no relation between the type of antimicrobial drug and sensitivity of tested E. coli isolates as well as their was no effective drug for all tested types.

Studying in vitro pathogenicity of isolated *E.coli* revealed that all strains were Congo red positive (produce red colony) but the degree of redness differ from one isolates to another. In this investigation *E.coli*

sergroup O_{55} produce red colonies and when inoculated into broiler chickens at ages of 5, 21, 30 days old proved to be pathogenic, therefore there is direct correlation between the ability of clinical isolates of *E.coli* to bind Congo red dye (C.R) and their ability to cause septicaemic infection in chicken. This is in agreement with **Raid, (1994)** while **Panigraphy and Yushen, (1990)** reported that although Congo red dye binding did not correlate well with pathogenicity, it may be an identifiable property of some serotypes of E.coli.

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Table (3): Correlation between the antibiogram and serologically typed *E.coli* isolates (55 isolates).

Chemotherapeutic agent	O_{26}		O_{128}		O_{86}		O_{126}		O_{125}		O_{114}		O_{127}		O_{55}		O_{111}		T	%of
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Colistin CO 10 mg		5		11	7			6		3		8	8		5		-	2	20	36.36
Erythromycin E 15 mg	1	4		11	7			6	2	1		8	8			5		2	18	32.72
Nalidixic NE 30 ug		5		11		7		6		3		8		8		5	2		2	3.63
Neomycin NE 30 ug	5		11		7		6		3		8		8		5			2	53	96.36
Streptomycin S 10 ug	5			11	7		6			3		8		8	2	3	1	1	21	38.20
Tobramycin TN 10 ug	5		11		3	4	2	4	3			8	8		5		2		23	50.90
Trimethoprim TM 2.5 ug		5		11		7		6		3		8		8		5		2	00	00.00
Amoxicillin A 25 ug	5		11		7		6		3		8		8		5		2		55	100.0
Ampicillin AP 10ug	5		11		7		6		3		6	2	8		5		2		53	96.36
Oxytetracycline OT 30 ug		5	8	3		7		6	3		8		8		5		2		34	61.80
Spectinomycin SH 15 ug		5	11			7	6		3		8		8		5			2	41	74.50
Flumequine UB 30 ug		5	11			7	6		3		8		8		5		1	1	42	76.20
Lincomycin MY 10 ug		5	11		7	7	6			3		8		8		5		2	17	30.90
Chloramphenicol C 30 ug		5	7	4	7			6		3	3	5		8	5		2		24	43.63
Doxycycline DO 30 ug	5		2	9	7		6		3		8		8		5		2		46	83.60
Kanamycin K 10 ug	5		11		7		6		3		8		8		5		2		55	100.0

S = Sensitive R = Resistant T = Total

الملخص العربي

مدى انتشار وحساسية الميكروب القولوني في دجاج التسمين المربي بمحافظة بني سويف عام ٢٠٠٣

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تم عزل عدد ١٠١ معزولة من الميكروب القولوني من دجاج تسمين عمر ٣ – ٧ أسابيع تظهر أعراض الإصابة بالميكروب القولوني ، المرض التنفسي المزمن المضاعف ، الإسهال ، التهاب المفاصل ، ضعف معدلات النمو ، وارتفاع معدلات النفوق إلى ١٠%. تم فحص ٤١١ دجاجة تسمين من ٦٨ قطيع تم التعرف على ٣٣٥ دجاجة منها مصابة بالميكروب القولوني بنسبة ٨١,٥ % وكانت كل القطعان موجبة للإصابة بنسبة ١٠٠% ، حيث تم الحصول على ١٠١ معزولة من الميكروب القولوني. تم التعرف السيرولوجى لعدد ٥٥ معزولة وكانت تنتمي إلى O_{26} (٤,٩٥%) ، O_{55} (٤,٩٥%) ، O_{111} (١,٩٨%) ، O_{128} (١٠,٨٩%) ، O_{86} (٦,٩٣%) ، O_{126} (٥,٩٤%) ، O_{114} (٧ز٩٢%) ، O_{127} (٧ز٩٢%) ، O_{125} (٢ز٩٧%) ، بينما لم يتم التعرف المصلى على ٤٦ معزولة باستخدام الأمصال المتاحة.

أوضحت الدراسة حساسية المعزولات للمضادات البكتيرية الحيوية المختلفة المتاح لها أقراص أن كل المعزولات كانت حساسة لكل من الكاناميسين والأمبيسلين (١٠٠%) ومقاومة لعقار الترامبثوبريم بينما كانت حساسية المعزولات للأمبيسلين والدوكسيسلين والفلوموكوين والاسبكتينومايسين والأوكيستتراسيكلين والتوبرميسين والكلورامفينكولو الأستربتومايسين والكولستين والأريثرومايسين واللينكومايسين والنالديكسيك أسد بالنسب التالية ٩٦,٣٦، ٩٦,٣٦، ٨٣,٦ ، ٧٦,٢ ، ٧٤,٥ ، ٦١,٨ ، ٤٣,٦٣ ، ٣٨,٢ ، ٣٦,٣٦ ، ٣٢,٧٢ ، ٣٠,٩٠ ، ٣,٦٣ على التوالي.

كما أوضح اختبار ضراوة المعزولات باستزراعها على منبت Congo red إيجابية النتائج مما يشير الى أن كل المعزولات التى تم التوصل اليها من الميكروب القولونى من النوع الضاري.