

Detection and antibiotic sensitivity pattern of avian pathogenic *Escherichia coli* strains isolated from different flocks from Egypt

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Abstract

Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis. Meanwhile, enormous exploitation of antibiotics in the field of poultry medicine has increased the number of resistant bacterial strains in recent years.

The present study was under taken to know the prevalence of avian pathogenic *Escherichia coli* (APEC) strains among different flocks from Egypt

Out of 61 examined samples (internal organs of birds showing colisepticaemia), 57 (93.44%) were positive for *E. coli*.

The sensitivity profile of the isolates showed complete resistance of all the isolates against oxytetracycline and streptomycin while on the other hand all the isolates showed very high susceptibility to colistin sulphate.

Keywords:

avian colibacillosis, *E. coli*, oxytetracycline, streptomycin, colistin sulphate.

Introduction

Colibacillosis is the primary cause of morbidity, mortality, and condemnation of carcasses in the poultry industry worldwide (Delicato *et al.*, 2003; Dho-Moulin and Fairbrother, 1999; Ewers *et al.*, 2004). It is an economically devastating disease for the poultry industry in many parts of the world (Dho-Moulin and Fairbrother, 1999; La Ragione and Woodward, 2002). In the past few years, both the incidence and severity of colibacillosis have increased rapidly, and current trends indicate that it is likely to continue and become an even greater problem in the poultry industry (Altekruse *et al.*, 2002; Blanco *et al.*, 1997). Although *Escherichia coli* is present in the normal microflora of the intestinal tract of chickens, a certain subset of extraintestinal patho-

genic *E. coli*, termed avian pathogenic *E. coli* (APEC) possess specific virulence attributes that have been associated with colibacillosis (Delicato *et al.*, 2003; Dho-Moulin and Fairbrother, 1999; La Ragione and Woodward, 2002; Yang *et al.*, 2004).

Antibiotics are widely used in poultry production to control infectious diseases, this consequently enhance high growth rate. This practice is reported to have caused high resistance to antibiotics by pathogenic micro-organisms in poultry (Henning and Marianne, 2001).

The development of antibiotic resistance in many bacterial organisms constitutes serious problems in the control of infectious diseases. Antibiotic misuse has been found to be the most important selecting force in bacterial antibiotic resistance (Okoli, 2006).

Materials and methods

Samples and isolates.

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 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)**.

Phenotypic characterization

Identification.

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 °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 °C. A 5µl sample of each was mixed with quantity of an O-serotype antiserum. Agglutination on a glass slide within 1 minute determined the respective serotype.

Media used for the antibiogram assay.

Muller-Hinton broth, Muller-Hinton agar.

Antibacterial discs (oxid)

Ampicillin (AMP, 10 µg), Chloramphenicol (C, 30 µg), Ciprofloxacin (CIP, 5 µg), Colistin sulphate (CL, 10 µg), Doxycycline (DO, 30 µg), Erythromycin (E, 15 µg), Gentamycin (GM, 10 µg), Nalidixic acid (NA, 30 µg), Neomycin (N, 30 µg), Norfloxacin (NOR, 10 µg), Oxytetracyclin (OT, 30µg), Streptomycin (S, 10 µg), Tetracyclin (TE, 30 µg), Trimethoprim (TR, 5 µg), Sulphamethoxazole trimethoprim (SXT, 1.25 µg + 23.75 µg).

Antibacterial sensitivity testing.

The disk diffusion technique was applied. The interpretation of inhibition zones of test culture was according to the **CLSI Standards Institute (CLSI, 2010)**.

Results and Discussion

The prevalence of isolation of *E.coli* reached 93.44% from the examined 61 flocks showing symptoms of coli septicaemia. The incidence of *E. coli* infection was 100% in the grand parent and broiler samples each while the prevalence from broiler breeders and layers reached 90% and 71.4% respectively.

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

Type of flocks	Number of examined flocks	Results	
		+ve	%
Grand parents	2	2	100
Broilers Breeders	20	18	90
Broilers	32	32	100
Layers	7	5	71.4
Total	61	57	93.44

All the isolates obtained from clinical colisepticaemia, in birds from Egypt fermented lactose on MacConkey, and showed greenish metallic sheen on eosin methylene blue agar.

Table 2. Incidence of the isolated *E. coli* recovered from internal organs and different flocks and human (feces and urine samples)

	Serovars	Types of flocks		Broilers	Layers	total	%
		Grand par- ents	Broilers Breederers				
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	

Records the total number of *E.coli* isolates isolated from 57 samples recovered from the internal organs of different flocks.

Results of antimicrobial sensitivity test of *E. coli* using the disc diffusion method.

The in vitro sensitivity of 57 *E. coli* isolates were conducted against 15 antimicrobial drugs. 1-The results of Antimicrobial sensitivity test of *E. coli* of the isolated from the internal or-

gans of Grand parents indicated that 2 (100%) were resistant to (Doxycycline - Tetracycline - Nalidixic acid - Norfloxacin- Ampicillin - Oxytetracycline - Sulpha - methoxazole trimethoprim - Streptomycin - Erythromycin) and 2 (100%) sensitive to colistin.

Table 3. Incidence of antimicrobial sensitivity test on the recovered *E. coli* from the internal organs of Grand parents.

Antimicrobial agent		R		I		S	
		n=	%	n=	%	n=	%
Nalidixic acid	NA	2	100	-	-	-	-
Tetracycline	T	2	100	-	-	-	-
Doxycycline	DO	2	100	-	-	-	-
Trimethoprim	TR	1	50	-	-	1	50
Gentamycin	G	1	50	-	-	1	50
Neomycin	N	1	50	1	50	-	-
Norfloxacin	NX	2	100	-	-	-	-
Ciprofloxacin	CF	1	50	-	-	1	50
Chloramphenicol	C	1	50	-	-	1	50
Colistin	CL	-	-	-	-	2	100
Ampicillin	A	2	100	-	-	-	-
Oxytetracycline	O	2	100	-	-	-	-
Sulpha-methoxazole trimethoprim	SXT	2	100	-	-	-	-
Streptomycin	S	2	100	-	-	-	-
Erythromycin	E	2	100	-	-	-	-

The results of Antimicrobial sensitivity test of *E. coli* isolated from the internal organs of layers

the results of antimicrobial sensitivity test on the recovered *E. coli* from the internal organs

of layers indicated that 5 (100%) were resistant to (Doxycycline- Trimethoprim - Oxytetracycline - Sulpha - methoxazole trimethoprim - Streptomycin) and 5 (100%) sensitive to 0 (colistin – Gentamycin – Chloramphenicol).

Table 4. Incidence of antimicrobial sensitivity test on the recovered *E. coli* from the internal organs of layers.

Antimicrobial agent		R		I		S	
		n=	%	n=	%	n=	%
Nalidixic acid	NA	4	80	-		1	20
Tetracycline	T	4	80	-	-	1	20
Doxycycline	DO	5	100	-	-	-	-
Trimethoprim	TR	5	100	-	-	-	-
Gentamycin	G	-	-	-	-	5	100
Neomycin	N	1	20	2	40	2	40
Norfloxacin	NX	4	80			1	20
Ciprofloxacin	CF	1	20	2	40	2	40
Chloramphenicol	C	-	-	-	-	5	100
Colistin	CL	-	-	-	-	5	100
Ampicillin	A	4	80	-	-	1	20
Oxytetracycline	O	5	100	-	-	-	-
Sulpha-methoxazole trimethoprim	SXT	5	100	-	-	-	-
Streptomycin	S	5	100	-	-	-	-
Erythromycin	E	4	80	-	-	1	20

The results of Antimicrobial sensitivity test of *E. coli* of the isolated *E. coli* from the internal organs of broiler breeders.

the results of antimicrobial sensitivity test on the recovered *E. coli* from the internal organs

of broiler breeders indicated that 18(100%) were resistant to (Oxytetracycline - Streptomycin) and 18 (100%) were sensitive to (colistin)

Table 5. Incidence of antimicrobial sensitivity test on the *E. coli* recovered from the internal organs of broiler breeders.

Antimicrobial agent		R		I		S	
		n=	%	n=	%	n=	%
Nalidixic acid	NA	16	88.9	-	-	2	11.1
Tetracycline	T	16	88.9	-	-	2	11.1
Doxycycline	DO	17	94.4	1	77.8	-	-
Trimethoprim	TR	15	83.3	-	5.6	3	16.7
Gentamycin	G	3	16.7	-	-	15	83.3
Neomycin	N	3	16.7	5	-	10	55.6
Norfloxacin	NX	11	61.1	5	27.8	2	11.1
Ciprofloxacin	CF	5	27.8	10	27.8	3	16.7
Chloramphenicol	C	5	27.8	-	55.6	13	72.2
Colistin	CL	-	-	-	-	18	100
Ampicillin	A	15	83.3	-	-	3	16.7
Oxytetracycline	O	18	100	-	-	-	-
Sulpha-methoxazole trimethoprim	SXT	15	83.3	-	-	3	16.7
Streptomycin	S	18	100	-	-	-	-
Erythromycin	E	14	77.8	-	-	4	22.2

The results of Antimicrobial sensitivity test of *E. coli* of the isolated from the internal organs of broilers.

The results of antimicrobial sensitivity test on the recovered *E. coli* from the internal organs

of broiler indicated that 32 (100%) were resistant to (Oxytetracycline -Streptomycin) and 32 (100%) were sensitive to colistin.

Table 6. Incidence of antimicrobial sensitivity test on the recovered *E. coli* from the internal organs of broilers.

Antimicrobial agent		R		I		S	
		n=	%	n=	%	n=	%
Nalidixic acid	NA	27	84.4	-	-	5	15.6
Tetracycline	T	29	90.62	-	-	3	9.4
Doxycycline	DO	31	96.8	-	-	1	3.1
Trimethoprim	TR	29	90.62	-	-	3	9.4
Gentamycin	G	3	9.4	-	-	29	90.62
Neomycin	N	9	28.2	13	40.6	10	31.3
Norfloxacin	NX	19	59.3	10	31.3	3	9.4
Ciprofloxacin	CF	12	37.5	10	31.3	10	31.3
Chloramphenicol	C	17	53.1	3	9.4	12	37.5
Colistin	CL	-	-	-	-	32	100
Ampicillin	A	27	84.4	-	-	5	15.6
Oxytetracycline	O	32	100	-	-	-	-
Sulpha-methoxazoletrimethoprim	SXT	27	84.4	-	-	5	15.6
Streptomycin	S	32	100	-	-	-	-
Erethromycin	E	22	68.75			10	31.25

Table 7. Incidence of antimicrobial sensitivity test on the *E. coli* isolates recovered from different flocks.

Antimicrobial agent		R		I		S	
		n=	%	n=	%	n=	%
Nalidixic acid	NA	49	85.9	0	0	8	14.1
Tetracycline	T	51	89.5	0	0	6	10.5
Doxycycline	DO	55	96.5	1	1.8	1	1.8
Trimethoprim	TR	0	0	0	0	6	10.5
Gentamycin	G	7	12.3	0	0	0	0
Neomycin	N	15	26.4	21	36.8	21	36.8
Norfloxacin	NX	36	63.2	15	26.3	6	10.5
Ciprofloxacin	CF	19	33.3	22	38.6	6	10.5
Chloramphenicol	C	23	40.4	3	5.3	19	33.3
Colistin	CL	0	0	0	0	57	100
Ampicillin	A	48	84.2	0	0	9	15.8
Oxytetracycline	O	57	100	0	0	0	0
Sulpha-methoxazole trimethoprim	SXT	49	85.9	0	0	8	14.1
Streptomycin	S	57	100	0	0	0	0
Erythromycin	E	42	73.7	0	0	15	26.3

Conclusion

The result of this study suggests that multiple-antimicrobial-resistant APEC isolates are present in different flocks from Egypt, introduction of surveillance programs to monitor antimicrobial resistance of pathogenic bacteria is strongly recommended in Egypt. Appropriate use of antibiotics in humans and farm animals needs to be addressed in Iran and Egypt.

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الكشف عن ونمط الحساسية للمضادات الحيوية لسلالات الإي كولاي القولونية المعزولة من الطيور من قطعان مختلفة من مصر

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الملخص العربي

لقد تم في هذه الدراسة اختبار 61 قطيع تم جمعها من قطعان محلية من مصادر متعددة للدواجن (الجدود و الامهات و البياض و التسمين) المشتبه في اصابتها بالايشيريشيا كولاي في مصر. وبعد تحليل النتائج التي تم الحصول عليها، وجد أن اجراءات العزل قد اثبتت وجود 93.44% نسبة اصابة. و باختبار خواص المعزولات و عددها 57 وجد ان جميعها المعزولات ذات تاثير علي اجنه البيض (SPF) وجد انها قتلت كل الاجنه علي مدي اثني عشر يوم بعد الحقن و قد كشفت الدراسة عن وجود تفاوت في مقاومه العترات لعدد خمسة عشره من المضادات الحيوية و دلت ايضا انه لا توجد عترة حساسه لجميع المضادات الحيوية كما ان جميع العترات حساسه بنسبه 100% لكل من الكولستين و الترايميثوبريم و مقاومه بنسبه 100% لكل من الاوكسيتتراسيكلين و الستريبتوميسين .