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Original Research Article

Serogrouping and resistance gene detection in avian pathogenic *E.coli* isolated from broiler chickens

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ABSTRACT

E coli infection in poultry is one of the principle causes of mortality and morbidity in chickens and turkeys resulting in retardation of growth and decreased feed conversion rate . The most common form of collibacillosis is characterized as an initial respiratory infection (air sacculitis) followed by generalized septicemia, perihepatitis and pericarditis. The present study aimed to identify the bacteria associated with pericarditis , airsacculitis and perihapatitis in Egyptian broiler chickens. A total 300 samples of diseased and dead broilers from 3-6 weeks age were collected. The isolated bacteria included *E. coli*, *Klebsiella* spp., *Shigella* spp. And *Enterobacter* spp. Sero-grouping of the isolated *E. coli* strains revealed O125, O158, O55,O129, O20 , O6 , O8 , O27, O115, O142 and un-typed strains with prevalence of 16%, 12%, 8%, 8%, 8% , 8% , 8% ,4%,4% and 16% respectively. The majority of E coli isolates were sensitive to colistin sulphate (38%) and Norofloxacin (38%) followed by ciprofloxacin (19%), cefotaxim (19%) and Ofloxacin (19%). On the other hand, E coli were resistant (100%)to amoxicillin, clindamycin , Erythromycin and streptomycin. PCR analysis for antibiotic resistance genes of E coli detected that 12 serogroups isolates were positive using the specific primers for Aada2 , BlaCTX and TetA(A) genes. The current study demonstrated the high prevalence of *E. coli* indeed broilers suffered from pericarditis , perihepatitis and airsacculitis . Measures are needed to control *E. coli* contamination in poultry farms to reduce economic losses caused by infection of *E.coli.*

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Introduction

E. coli is a member of the family Enterobacteriaceae, which may constitute a great hazard to poultry industry causing high mortality, loss of weight and reduction of egg production (Bandyopadhay and Dhawedkar, 1984). E. coli infection is one of the serious problems that cause a great threat to the profitability of birds' enterprises allover the word. The most common form of collibacillosis is characterized as an initial respiratory infection (air sacculitis) in 3 -12 weeks old broiler chickens and turkeys, which is frequently followed by generalized septicemia, perihepatitis and pericarditis.

Vandekerchove et al. (2004 a) observed outbreaks of acute mortality in layer flocks in Europe due to coli septicemia. They described the disease and identified the serotypes of the APEC present in these outbreaks. Salama et al., (2007) recovered 5 serogroups of E. coli identified as O1, O2, O6, O78 and O126. Out of 33 isolates derived from a total of 60 samples collected from colisepticemic chickens isolated on nutrient agar and MacConkey agar and colonies examined for their colonial morphology, microscopically and biochemically using API 20E identification system. El-Jakee et al., (2012) identified 28 isolates recovered from cloacal swabs of diarrheic chicken; they were O2, O6, O8, O26, O27, O78, O86, O111, O128,

O136 and O157. Sarah et al ., (2015): recovered E coli serovars from different sources of poultry broiler farms which were; untypable E coli isolates followed by O26; then O2, O124, O125, and O115. Food animals and their production environments are source and reservoirs of both resistant bacteria and resistance genes that could be transferred to.

E coli bacteria often carry multiple drug resistance plasmids and mixing of species in the intestines allows E coli to accept and transfer plasmids from one to other bacteria, [salyers et al 2004]. Many authors had clarified a lot about the antibiotic resistance profile of bacteria including E coli in poultry farms in Egypt [Yousef et al 2013 – Moawad et al 2017 found that 87 APEC isolates from septicaemic broilers at the molecular level showed multidrug-resistant phenotypes, particularly against ampicillin, kanamycin, ciprofloxacin, levofloxacin, streptomycin, gentamycin, ofloxacin, norfloxacin, and ceftriaxone. Hering et al 2016; Suggested that the analysis of fecal samples in sufficient to determine cefotaxim-resistant E coli in broiler farm but that cefotaxime resistance is a good proxy for the presence of ESBL- or plasmidic AmpC-beta-lactamases and the prevalence of broiler farms with cefotaxime-resistant E. coli in Germany is very high. Nawaz et al (2016) studied that the efficacy of two probiotics of different origins (yeast and bacterial based) on the growth performance, immune response, carcass characteristics and nutrient digestibility of broilers.

The aim of the present study was to isolate and identify the bacteria associated with mortality and poor feed conversion from broiler chickens

2. Materials and methods

2.1 Case history: Recently dead or diseased broilers (3-4weeks) were collected from farms in Elminya Province suffer from pericarditis, perihepatitis and air sacculitis .

2.2 Chickens Samples

A total of 300 samples were collected from different organs (liver ,heart and lung) were transferred immediately to sterile buffered peptone water, then wrapped with ice, kept in box and transferred to the laboratory to be submitted to bacteriological examination **.**

2.3. Detection of E. coli by conventional method: it was done according to Quinn et al. (2002)

2.3.1 Selective enrichment of E. coli

For bacteriological examination 0.5 gm of liver, heart and lung were inoculated into tryptone soya broth. All inoculated media were incubated aerobically at 37°C for 16-18 hrs.

2.3.2. Colonization of E. coli on selective differential solid media

Loopfuls from the inoculated broth were streaked onto SS-agar, XLD agar, MacConkey agar and EMB agar (Cheesbrough, 2000) Suspected E. coli colonies were purified and kept for further morphological and cultural identification. .

2.3.3. Microscopic examination

Gram's stain was prepared and used as described by (Cruickshank et al. 1975) for morphological characters (Koneman *et al.,* 1992; Quinn *et al.,* 2002)).

2.3.4 Confirmatory API20 E biochemical test

Analytical profile index 20 E (API 20 E Biochemical rapid tests, Bio-Meraux, France) was used for identification of *Enterobacteriaceae*. The test was carried out according to the instructions of the manufacturer (Bio Meraux, France). Only pure cultures of a single organism were used.

2.3.5 Serological identification of E. coli

Isolated E. coli were serogrouped at the Animal Health Research Institute, Dokki, Giza according to (Koneman *et al.,* 1992). Suspected microorganisms were subjected to serological typing by slide agglutination test using standard polyvalent and monovalent E. coli antisera.

2.4 Virulence testing of isolated bacteria

2.4.1. Congo red binding assay

Selected organisms were tested for virulence using Congo red binding assay and detection of hemolytic activity (Berkhoff and Vinal, 1986). Briefly, isolates were tested for its growth status on Congo red medium after incubation for 24 hours at 35°C then left at room temperature for additional two days for better results

2.4.2. Hemolytic activity on Blood Agar

The test was conducted according to (Marilda *et al.,* 1990), where overnight cultures of tested organisms were streaked onto Blood agar base containing 10 % defibrinated sheep blood and incubated at 37°C for 24 hr. Complete hemolysis

was recognized as β- hemolysis while, weak incomplete hemolysis was recognized as ∝- hemolysis.

2.5.Antibiogram sensitivity test :

Twenty one serogrouped E. coli were tested by the single-disc diffusion method according to Mary and Usha (2013). Sensitivity discs and Muller–Hinton agar (Oxoid Limited, Basingstoke, Hampshire, UK) . The tested antimicrobials included Amoxicillin (25µg AMX), Colistin (10µg Ct), Ciprofloxacin (5µg CP), Erythromycin (15µgE), Enerofloxacin (5µgK), Cefotax (50µg), clindamycin (2µg), Ofloxacin (5µg), Norfloxacin (10µg NOR), Doxycycline (30 µg Do), Streptomycin (10µg S) and Sulphamethoxazol &trimethoprim (25µg SXT) **table (1).** The interpretation of inhibition zones of tested culture was according to NCCLS, 2002).

Table (1): Antimicrobial susceptibility profile of E coli isolates

Antimicrobial discs	conc	Sensitive		Intermediate		Resistant	
		No	%	No	%	No	%
Doxycycline	Do30	1	4.7	2	9.5	18	85.7
Ciprofloxacin	Cip5	3	14.2	2	9.5	16	76
Amoxicillin	Ax25	0	0	0	0	21	100
Cefotaxime	Ctx30	4	19	0	0	17	80.9
Colistin sulphate	Ct10	8	38	0	0	13	61.9
Sulphamethoxazole/trimethoprime	Sxt25	3	14.2	5	23.8	13	61.9
Clindamycin	Da2	0	0	0	0	21	100
Erythromycin	E15	0	0	0	0	21	100
Streptomycin	S10	0	0	0	0	21	100
Ofloxacin	Ofx5	2	19	3	14.2	16	76
Norofloxacin	Nor10	3	14.2	6	28.5	12	57

2.6. Detection of antibiotic resistance gene

Twelve E coli were tested for presence or absences of resistance genes mainly resistance to streptomycin (Aada 2) , tetracycline (TetA (A) and cefotaxime (Bla CTX) were determined by PCR and the set of primers used for each gene is shown in **Table 2** at the Animal Health Research Institute, Dokki, Giza.

Table (2). Gene primer sequence

Gene	Primer Sequence 5'-3'	Amplified product	References
TetA(A)	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	576bp	Randall et al. 2004
AadA2	TGTTGGTTACTGTGGCCGTA GATCTCGCCTTTCACAAAGC	622 bp	Walker et al. (2001)
Bla_{CTX}	ATG TGC AGY ACC AGT AAR GTK ATG GC TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593 bp	Archambault et al., 2006

3. Results

3.1. Incidence of E. coli infection in chicken

Out of 300 recently dead broilers from commercial farm were subjected to postmortem examination to detect birds that show pericarditis , perihepatitis and airsacculitis (72) different bacterial isolates of Enterobacteriaceae suspected to be *E. coli* which identified as (56)fifty six *E. coli*,(1) one shigella,(1) one *Enterobacte*r and (14) fourteen *kluyvera* **(Table 2 &figure (1)**

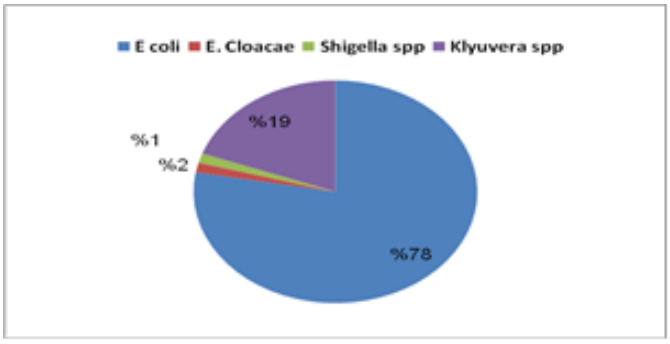

Fig. 1. Total bacteriological isolates of samples

3.2. Serogrouping of E. coli isolates recovered from chicken samples

The most commonly E. coli isolated were 4 (O125), 3(O158) ,2(O55) , 2(O129) ,1(O27), 1(O115), 2(O8), 1(O142), 2(O20) ,2(O6) and 4 untyped **(Table 3**) .

3.3. *Virulence test results* :

3.3.1. *Conge red binding ability*

Congo red binding ability test were revealed that all of 56 E coli isolates showed Congo red binding activity giving red colonies and considered Congo red positive (CR+).

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3.3.2. *Hemolytic activity on Blood Agar*

Out of 56 *E. coli* isolates, only one isolate gave beta hemolysis

3.4. Antibiogram sensitivity test

3.5. Antibiotic resistance genes detection of E. coli:

Twelve sero groups isolates of *E. coli* were differed about prescence or absence of resistance gene as mentioned in **table 5 . fig (2) , (3) &(4)** using the specific primers for Aada2 , BlaCTX and TetA(A) genes respectively.

Isolate resistance rate was 100 % to AX, DA, E and S; 85,7% to DO ; 80% CTX , 76% to OFX and CIP; 61.8 % to CT and SXT and 57% to Nor (Table 4)

Table (3): Results of sero-grouping of E coli isolates.

ID of the samples	E coli sergroupes	Number of isolates	(%)
1	O_{125}	4	16
2	O_{158}	3	12
3	O_{55}	2	8
4	O_{129}	2	8
5	O_{20}	2	8
6	O_{6}	2	8
7	O_{8}	2	8
8	O_{27}	2	8
9	O_{115}	1	4
10	O_{142}	1	4
	Un typable	4	16
	Total	25	100

Table (4): Antibiogram result of E coli serogroupes :

E. coli serotypes	Antibiotics											
	CIP 5	AX25	CTX30	NOR10	OFX5	DA2	CT10	SXT25	E15	DO30	ENR	S
O20	R	R	R	R	R	R	S	R	R	S	S	R
O20	S	R	R	S	S	R	S	R	R	R	S	R
O125	S	R	I	R	I	R	S	R	R	R	I	R
O125	I	R	R	R	I	R	S	R	R	R	I	R
O55	R	R	R	R	R	R	S	S	R	I	R	R
O55	R	R	R	R	R	R	S	S	R	I	R	R
O115	R	R	R	R	R	R	R	R	R	R	R	R
O142	R	R	R	R	R	R	R	S	R	S	R	R
O142	R	R	R	R	R	R	R	S	R	S	R	R
O6	S	R	R	I	S	R	R	R	R	I	R	R
O8	S	R	R	I	I	I	R	R	R	R	R	R
O8	R	R	R	I	R	R	R	R	R	R	R	R
O158	R	R	S	I	R	R	R	R	R	R	R	R
O158	R	R	S	I	R	R	R	R	R	R	R	R
O158	S	R	S	R	R	R	S	I	R	R	R	R
O27	R	R	R	S	R	R	R	R	R	R	R	R
O27	R	R	R	S	R	R	R	R	R	R	R	R
O129	R	R	S	R	R	R	S	I	R	R	R	R
O125	R	R	R	R	R	R	R	R	R	R	R	R
O125	R	R	R	R	R	R	S	R	R	R	R	R

Table (5): PCR analysis for antibiotic resistance genes (aaDA2 – blaCTX – tetA(A)

S: sensitive R: resist

ID of the samples	*E. coli* serotypes	Results		
		Aada2	*BlaCTX*	*TetA(A)*
1	O20	Positive ®	Positive ®	Positive ®
2	O125	Positive ®	Negative(S)	Positive ®
3	O20	Positive®	Positive ®	Positive ®
4	O125	Positive®	Positive ®	Positive ®
5	O55	Negative (S)	Positive ®	Positive ®
6	O55	Negative(S)	Positive ®	Positive®
7	O158	Negative(S)	Positive ®	Positive®
8	O158	Negative(S)	Negative(S)	Positive®
9	O158	Positive ®	Negative(S)	Positive®
10	O27	Negative(S)	Negative(S)	Positive®
11	O27	Positive ®	Negative(S)	Positive®
12	O129	Positive ®	Negative(S)	Positive®

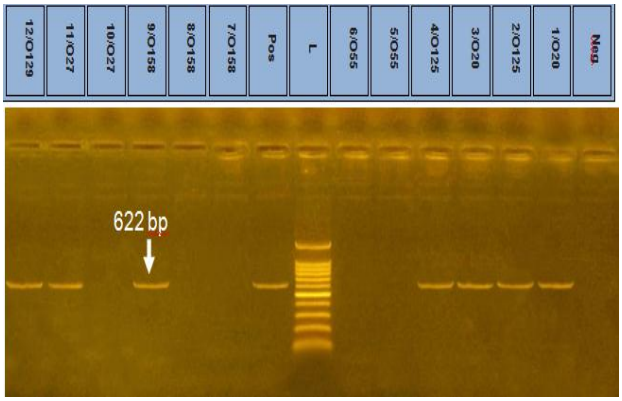

Fig. 2. Electrphoretic pattern of PCR products (622 bp) specific for aada2 gene of E. coli in agarose gel stained with ethidium bromide

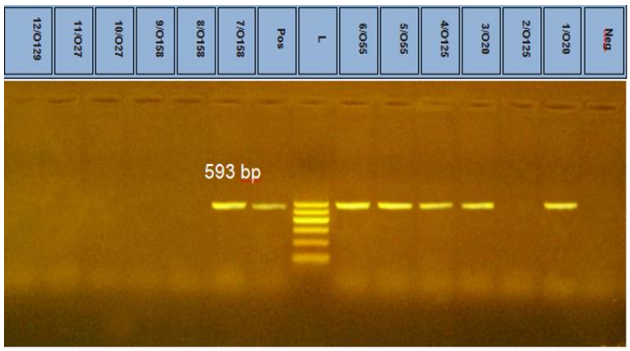

Fig. 3. Electrphoretic pattern of PCR products (593 bp) specific for blaCTX gene of E. coli in agarose gel stained with ethidium bromide.

4. Discussion

In this study, E. coli was detected after pre-enrichment on BPW. Then inoculated direct on agar medium (MacConky agar, VRBL and EMB agar), Typical colonies on TSA were used for further morphological and biochemical identification The typical E. coli colonies were typing by antisera. In the present work, all E. coli strains showed lactose fermentation (pink colonies) on MacConkey agar and green metallic sheen colonies on EMB .Out of 300 recently dead broilers from commercial farms were (72) different bacterial isolates of Enterobacteriaceae suspected *E. coli* were identified as (56) fifty six *E. coli*,(1) one *shigella*,(1) one *Enterobacter* spp and (14) fourteen *kluyvera spp.*

Congo red binding ability test revealed that all of 56 E coli isolates showed Congo red binding activity giving red colonies and considered Congo red positive (CR+). The degree of redness of the colonies varied from one isolate to another. Only The strains which gave more powerful results (dark red colony) are selected for sero grouping The results were in agreement with Berkhoff and Vinal [1986], who reported a strong correlation between expression of CR phenotype and virulence in avian *E. coli*. Pathogenic *E. coli* can be identified by their ability to bind CR and produce brick red colonies .

The most prevalent serogroups were 4 (O125), 3(O158) ,2(O55) , 2(O129) ,1(O27), 1(O115), 2(O8), 1(O142), 2(O20) ,2(O6) and 4 untyped (table ,4) A wide variety of *E. coli* serogroupes and non-subtypes from broiler in Egypt were also reported Taha et al 2002.

Antibiogram test was 100 % to AX, DA, E and S; 85,7% to DO ; 80% CTX , 76% to OFX and CIP; 61.8 % to CT .

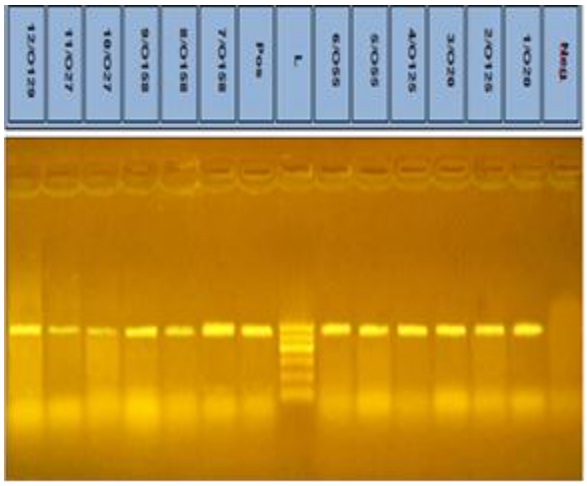

Fig. 4. Electrphoretic pattern of PCR products (bp) specific for tetA gene of E. coli in agarose gel stained with ethidium bromide.

SXT and 57% to Nor . Similar results were reported by Ozawa et al [2008]. The present study detected most of isolates was resistance to doxycycline with percent 85.7% by disc diffusion which agree with 'Moon et al (2011) that studied the actual frequency of antimicrobial resistance in fecal Escherichia coli isolated from .One hundred and nine E.coli isolates were higher resistant to ampicillin (68.8%) streptomycin (60.6%), ciprofloxacin (65.1%), and tetracycline (96.3%).

PCR analysis for antibiotic resistance genes of E coli. Twelve sero groups isolates of E coli were differed on result using the specific primers for aaDa2 , blaCTX and tetA(A) genes respectively. The obtained results were recorded (Table 5) .

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