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INCIDENCE AND CHARACTERIZATION OF SOME VIRULENCE GENES IN MULTI-DRUG RESISTANT *E. COLI* ISOLATED FROM BROILER CHICKENS

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ABSTRACT

A total of 22 Avian pathogenic multi-drug resistant *E.coli* strains isolated from broiler chickens suffering from colibacillosis obtained from Poultry diseases Department, Animal Health Research institute, Dokki, Giza. *E. coli* strains were subjected to molecular characterization of some virulence genes (*iroN, ompT, hlyF, iss and iutA*) by using the Polymerase Chain Reaction (PCR), the results revealed that all these genes were detected in both chromosomal DNA (77.27%) and plasmid (59.09%) of the examined multidrug-resistant *E. coli* strains. *iroN* was detected in (45.45% and 36.36%), *ompT* in (50% and 36.36%), *hlyf* in (54.55% and 54.55%), *iss* in (63.64**%** and 54.55%) and *iutA* in (36.36% and 36.36%) in chromosomal DNA and plasmid respectively.It was concluded that,the misuse of antimicrobials without veterinary supervision and consultation may lead to selection of multidrug resistant *E. coli*. There is a relationship between the presence of MDR *E. coli* and the virulence genes which lead to increased virulence and fail of treatment of pathogenic *E.coli* which in turn increase the economic losses among broiler chickens.

Key words:

E.coli, Multi-Drug resistant, Broilers

INTRODUCTION

Escherichia coli is a commensal bacterium of the bird's intestinal tract, but it can invade different tissues resulting in systemic symptoms (colibacillosis) .This disease occurs only when the *E. coli* infecting strain presents virulence factors (encoded by specific genes) that enable the adhesion and proliferation in the host organism. Thus, it is important to differentiate avian pathogenic *E. coli* (APEC) and non-pathogenic or avian fecal *E. coli* (AFEC) isolates **(Carli *et al.,* 2015).** The association of the increased serum survival gene, ***iss*** **(**with the *E. coli* implicated in avian diseases) may mean that *iss* and/or, perhaps, the genes

associated with it are important contributors to avian *E. coli* virulence **(Johnson *et al.,* 2002).** Avian colibacillosis causes significant economic losses, either as a primary disease or as a secondary infection, to broilers chickens, youth, of laying hens and of breeding hens. It is produced by strains of *E. coli* falling within APEC pathotypes (with the penetration site, the body's respiratory mucosa), being considered systemic extra intestinal infection **(Iancu *et al.,* 2015)**. Avian pathogenic *E. coli* strains harbor chromosomal and plasmid pathogenicity-related genes. The presence of resistance plasmids in avian *E. coli* strains could facilitate horizontal transfer of virulence gene between pathogenic and non-pathogenic strains **(Barros, 2012).Ammar *et al.* (2015)** reported that, the association of antimicrobial resistance and virulence traits in multidrug resistant (MDR) *E. coli* strains highlight the risk of promoting the spread of virulence through the extensive use of antibiotics in Egypt .Several factors have been shown to contribute to the virulence of avian *E. coli* ,and many of the genes encoding these factors have been found on large conjugative plasmids. Because of the occurrence of antimicrobial resistance genes on these same plasmids, it is possible that, the use of antimicrobial agents may select for persistence of *E .coli* containing such plasmids **(**Johnson ***et al.,* 2004).** The aim of the present work is to detect some of the virulence genes in both chromosomal DNA and plasmid by polymerase Chain Reaction (PCR) and to determine the relationship between these genes and the multi-drug resistant *E.coli.*

MATERIAL AND METHODS

Sampling:

22 strains of known multi-drug resistant *E.coli* were obtained from Poultry Diseases Department, Animal Health Research Institute (AHRI), Dokki, Giza

Molecular Detection of virulence genes by PCR:

The molecular detection of some virulence genes (*iroN, ompT, hlyF, iss and iutA*) in the chromosomal DNA and plasmid of multi-drug resistant *E.coli* was undergone by using specific primers and cyclic conditions according to **Timothy *et al.* (2008)** is demonstrated in (Table 1 and 2).

RESULTS

Results of molecular detection of virulence genes in multi-drug resistant *E. Coli.*

The molecular characterization of some virulence genes of *E. coli* (*iron, ompT, hlyF, iss* and *iutA*) in the chromosomal DNA reviled that these genes detected in 17/22 strains at a percentage of 77.27%, while these genes found in the plasmid in 13/22 strains at a

percentage 59.09%. The results of the molecular detection of the mentioned virulence genes are tabulated in (Table 3). Regarding the incidence of these genes in both chromosomal DNA and plasmid, *iroN* was detected in (45.45% and 36.36%), *ompT* in (50% and 36.36%), *hlyf* in (54.55% and 59.09%), *iss* in (63.64% and 54.55%) and *iutA* in (36.36% and 36.36) in chromosomal DNA and plasmid respectively. Combinations between 5, 4, and 3 genes were detected in 4, 4 and 3 strains in both chromosomal DNA and plasmid respectively, while a combination between 2 genes was detected in 4 strains in the chromosome and in the plasmid of one strain. Also one or more virulence genes charred 13 (59.09%) strains in both chromosomal DNA and plasmid, one or more genes found in the chromosomal DNA only but not detected in plasmid in 4 strains, while none of the virulent genes could be detected in both DNA and plasmid in 5 strains (Table 4).

DISCUSSION

Avian pathogenic *E. coli* strains fall under the category of extra intestinal pathogenic *E. coli*, which are characterized by the possession of virulence factors that enable them to live in extra intestinal life **(Dozois *et al.*, 2000)**. Multidrug resistant *E.coli* is defined as the organism which is resistant to 3 or more antibacterial groups **(Magiorakos *et al.*, 2012)**. All the strains used in this study expressed multiple antimicrobial phenotypes (greater than or equal to 3 antimicrobials). This pattern of *E. coli* resistance was reported by **(Savita *et al.* 2007; Wang *et al.* 2010; Foder *et al.* 2011 and Meena 2015)**. The results reported in this study may be due to the misuse of antimicrobial agents in poultry farms for treatment of infections caused by pathogenic bacteria, this widespread usage of large quantities of antimicrobials in poultry farms in Egypt without veterinary consultation together with using antibiotics as growth promoters create a great problem in controlling diseases. These finding agreed with that recorded by **(Aarestrup, 2005).** This study targeted the detection of 5 virulence genes (*iron, ompT, hlyF, iss and iutA*) in the multidrug resistant *E. coli* in both chromosomal DNA and plasmid as shown in (Table3), these genes were recovered from 10 (45.45%), 11 (50%), 12 (54.55%), 14 (63.64%) and 8 (36.36) of chromosomal DNA respectively and from 8 (36.36%),8 (36.36%),12 (54.55%), 12 (54.55) and 8 (36.36%) of plasmid DNA respectively. 17/22 (77.27%) of the isolated *E. coli* at least had one of the virulence genes either in the chromosomal DNA or in the plasmid. Many studies undergone in Egypt and worldwide supported our recorded results which demonstrated in (Table 3), these results proved that

there is a relationship between virulence genes content and multidrug resistance of *E. coli.* **Hussein *et al.* (2013)** found more than 90% of the examined *E. coli* that collected from 84 flocks in Egypt possessed *iroN, ompT, hlyF, iss* and *iutA*. **Ahmed *et al.* (2013)** characterized the virulence and antimicrobial resistance of multidrug-resistant *E.coli* isolated from septicemic broilers in Egypt at the molecular level, Among 91 non-repetitive *E. coli* isolates, 73 (80.2%) carried three or more of the APEC virulence genes *(iroN, ompT, iss, iutA,* and *hlyF)*. All 73 APEC isolates showed multidrug resistance phenotypes. **Ismail *et al.* (2014)** found 90.4% of 83 *E.coli* isolates recovered from 200 broiler chickens suffering colibacillosis in Egypt to be multidrug resistant. The study targeted the detection of some virulence genes from these isolates. The highest prevalence was for *iroN, ompT, hylF* genes that were equally detected in (80%) of isolates followed by *iss* gene, which was harbored by (75%) isolates. In contrast, the *iutA* gene was only detected in (5%) isolates. **Kafshdouzan *et al.* (2013)** detected *hlyF, ompT, iss, iutA* and *iroN* in 77.3%, 73%, 68.2%, 67.4% and 65.2% respectively in their *E. coli* isolates, several combination patterns of these genes were detected, the most prevalent one was between *hlyF* and *ompT* (70.8%). **Jin *et al.* (2008)** detected ***iss*** gene in 58.8% of 216 avian pathogenic *E.* coli obtained from poultry with colibacillosis in China. **Rocha *et al.* (2008)** recovered *iss* and *iutA* from 73.8% and 45.9% of 61 *E. coli* isolates obtained from chickens with respiratory symptomatology. In his study on *E. coli* strains collected from chickens in 2007 and 2013in Brazil **Koga (2015)** could detected *iroN, ompT, hlyF, iss* and *iutA* virulence genes with the *iutA* gene being the most prevalent.

CONCLUSION

The misuse of antimicrobials without veterinary supervision and consultation may lead to selection of multidrug resistant *E. coli*. There is a relationship between the presence of MDR *E. coli* and the virulence genes which lead to increased virulence and fail of treatment of pathogenic *E.coli* which in turn increase the economic losses among broiler chickens.

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Table (1): Primers Used For Molecular Detection of Virulence Genes by PCR.

Primer	Sequence		Amplified product/ bp	Reference
IroN	F	AAT CCG GCA AAG AGA CGA ACC GCC T	553	Timothy *et al.*,(2008)
	R	GTT CGG GCA ACC CCT GCT TTG ACT TT		
ompT	F	TCA TCC CGG AAG CCT CCC TCA CTA CTA T	496	Timothy *et al.*,(2008)
	R	TAG CGT TTG CTG CAC TGG CTT CTG ATA C		
hlyF	F	GGC CAC AGT CGT TTA GGG TGC TTA CC	450	Timothy *et al.*,(2008)
	R	GGC GGT TTA GGC ATT CCG ATA CTC AG		
iss	F	CAG CAA CCC GAA CCA CTT GAT G	323	Timothy *et al.*,(2008)
	R	AGC ATT GCC AGA GCG GCA GAA		
iutA	F	GGC TGG ACA TCA TGG GA ACT GG	302	Timothy *et al* (2008)

Table (2): cycling conditions of PCR for amplification of virulence genes of *E. coli*.

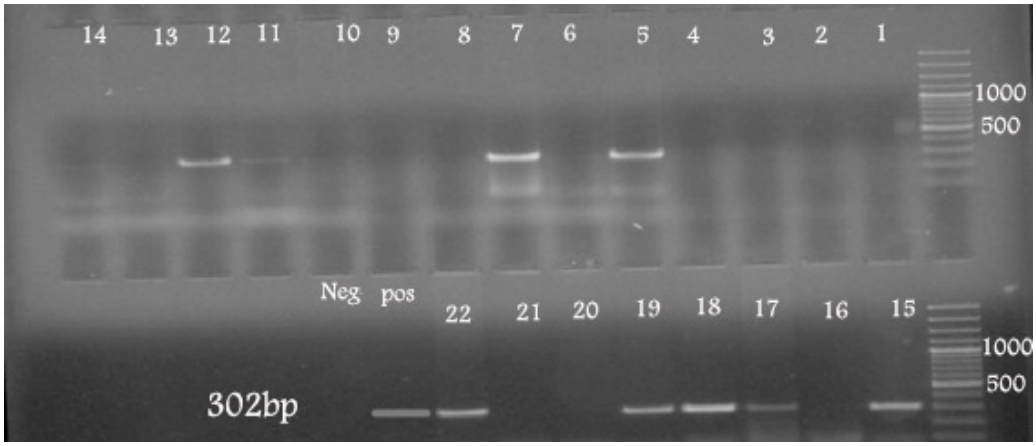
Gene	Initial denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension	Size of ampified product	Referance
iroN	94 °C 2 minutes	94 °C 30 seconds	63°C 30 seconds	72 °C 30 seconds	25	72 °C 10 minutes	553	Timothy *et al.*,(2008)
ompT	94 °C 3 minutes	94 °C 60 seconds	50-52 °C 60 seconds	72 °C 60 seconds	25	72 °C 10 minutes	496	Timothy *et al.*,(2008)
hlyF	94 °C 2 minutes	94 °C 30 seconds	63°C 30 seconds	72 °C 30 seconds	25	72 °C 10 minutes	450	Timothy *et al.*,(2008)
iss	94 °C 2 minutes	30 °C 30 seconds	63°C 30 seconds	72 °C 30 seconds	25	72 °C 10 minutes	323	Timothy *et al.*,(2008)
iutA	94 °C 2 minutes	94 °C 30 seconds	63°C 30 seconds	72 °C 30 seconds	25	72 °C 10 minutes	302	Timothy *et al.*,(2008)

Table (3): Results of Virulence genes by PCR

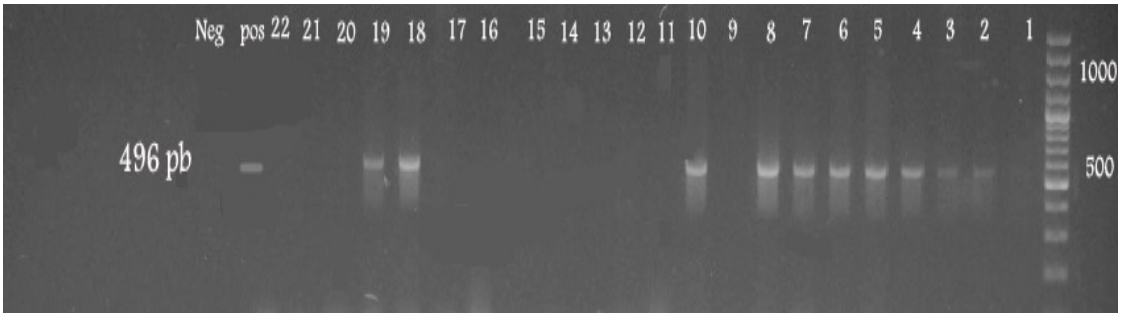
Isolate No.	*Virulence gene*									
	DNA					*Plasmid*				
	iroN	*ompT*	*hlyF*	*iss*	*iutA*	*iroN*	*ompT*	*hlyF*	*iss*	*iutA*
1	+	+	+	+	-	-	+	+	+	-
2	+	+	-	-	-	-	-	+	+	+
3	+	+	+	+	-	+	+	+	+	+
4	+	+	+	+	-	+	+	+	+	
5	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	-	+	+	+	+	+
7	+	+	+	+	+	-	-	+	+	
8	-	+	+	+	-	+	-	+	+	+
9	+	-	-	-	-	-	-	-	-	-
10	-	+	+	+	-	-	-	+	+	-
11	-	-	-	-	-	-	-	-	-	-
12	-	-	+	+	+	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-
14	-	-	+	+	-	-	-	-	-	-
15	-	-	-	+	+	-	-	+	+	+
16	-	-	-	-	-	-	-	-	-	-
17	-	-	-	+	+	+	+	+	-	+
18	+	+	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+	+	
20	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	+	-	-	-	-	-
Total	**10**	**11**	**12**	**14**	**8**	**8**	**8**	**13**	**12**	**8**
%	**45.45**	**50**	**54.55**	**63.64**	**36.36**	**36.36**	**36.36**	**59.09**	**54.55**	**36.36**

Table (4): Rate of virulence genes in positive *E. coli* isolate

No. of detected genes	**Chromosome**	**Plasmid**
	Virulence genes in Isolate No.	**Virulence genes in Isolate No.**
5	**5,7,18,19**	**3,5,6,18**
4	**1,3,4,6**	**4,8,17,19**
3	**8,10,12**	**1,2,15**
2	**2,14,15,17**	**7**
1	**9,22**	**10**
0	**11,13,16,20,21**	**9,11,12,13,14,16,20,21,22**



Fig(1): Detection of *iutA* gene in DNA; DNA Ladder,1Kb; lanes 5,7,12,15,17,18,19 and 22 were the positive samples produced band (302 bp).



Fig(2): Detection of *ompT* gene in DNA; DNA Ladder,1Kb; lanes 1,2,3,4,5,6,7,8,10,18,and 19 were the positive samples produced band (496 bp).

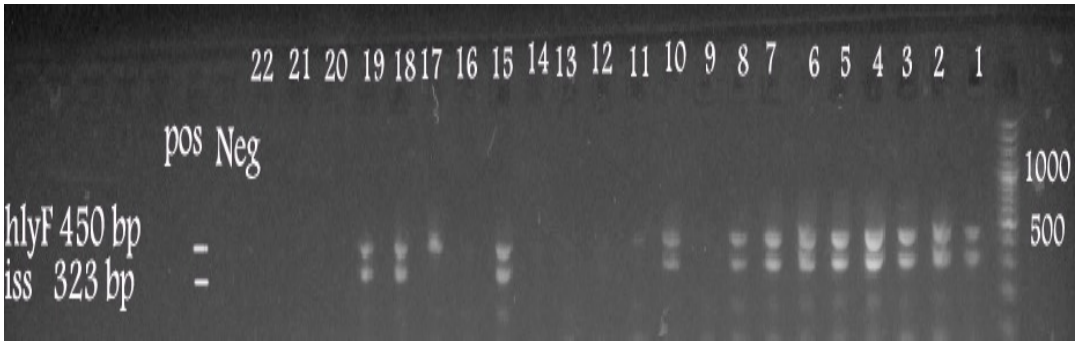


Fig. (3): Detection of iss and hlyF genes in Plasmid; DNA Ladder, 1Kb; lanes 1,2,3,4,5,6,7,8,10,15, 18 and 19 were the positive samples produced band (323 bp) for iss gene, while lanes: 1,2,3,4,5,6,7,8,10,15,17,18 and 19 were the positive samples produced band (450 bp) for hlyF gene.ss