Animal Health Research Journal Vol. 6, No. 4, December 2018 pp. 124-136

ISSN: 2356-7767

Relationship between virulence genes and quinolone resistance among *Escherichia coli* isolated from broilers

Ghada, A. El-gammal[*]; Nehal, A.A. Naena[*]; Atef, A. Salim[**] and Abd El-Halem, M. Hegazy[**]

Kafr El-Sheikh Regional Laboratory Animal Health Research Institute, Unit of bacteriology[*]

Kafr El-Sheikh Regional Laboratory Animal Health Research Institute, Unit of poultry diseases[**]

Received in 12/11/2018
Accepted in 18/12/2018

Abstract

A total of 100 chicken samples (apparently healthy, diseased and recently dead) broiler chickens were collected from different farms in Kafr El-Sheikh governorate. The samples were represented by liver, gall bladder, spleen, lungs and bone marrow. Bacteriological examination of the samples indicated isolation of 44 *E.coli* strains with incidence of 44%. *E.coli* isolates were characterized for O serogroupes, phenotypic pathogenicity, antimicrobial susceptibility, detection of some virulence genes and some quinolone resistance genes. *E.coli* isolates were belonged serologically to ten different serotypes (O_{18}, O_{26}, O_{25}, O_{44}, O_{126}, O_{55}, O_{111}, O_{119}, O_{125}, O_{158} and only one untyped strain). Antibiogram pattern test indicated the highest resistant rate against amoxicillin (100%) followed by naldixic acid (63.2%), cefotaxime (57.9%) and norfloxacin, flumequine, ciprofloxacin (52.6%) each followed by doxycycline (31.8%). All isolates were sensitive to amikacin and colistin sulphate. Some of the isolates were screened for presence of some virulence genes (*fim* H*, iss, tsh*) using PCR. There was a high prevalence of *fim* H and *iss* genes (100% and 85.7%) respectively but only 14.3% harbored *tsh* gene. Some isolates were subjected for screening quinolone resistance genes (*qnr* B-*qnr* S), *qnr* B gene was expressed in 42.9% of the isolates while *qnr* S was in 14.3% of the isolates. The association between virulence genes of *E. coli* and resistance to quinolones is a complex phenomenon as it was clear from this study, all quinolone susceptible strains and quinolone resistant were harboring virulence genes which resulted in no correlation between virulence genes and quinolone resistance.

Key words: *E.coli, virulence genes, phynotypic pathogenicity, quinolones resistant genes.*

Introduction

E. coli is a normal inhabitant chicken's microflora. Some avian *E. coli* serotypes are pathogenic and cause a number of diseases in domestic poultry, specially colibacillosis which continues to be one of the most economically important respiratory and systemic diseases leading to high morbidity, mortality, loss of body weight, bad feed conversion ratio (FCR) , decrease in egg and meat production and condemning or low grading of carcasses **(Satyajit *et al.*, 2013).**

Avian pathogenic *E. coli* (APEC) cause aerosacculitis, polyserositis, septicemia and other mainly extraintestinal diseases in chickens, turkeys and other avain species **(Dho-Moulin and Fairbrother, 1998).**

Control of *E. coli* is very important in poultry farms, which depends mainly on the use of certain antimicrobials to avoid hazard effect of this infection on poultry industry **(Hafez, 2008).**

Several valuable virulence genes were described in avian pathogenic *E. coli* (APEC). These genes could express different traits including adhesions, toxins production, iron uptake systems, and resistance to the host serum **(Delicato *et al*., 2003).**

Antimicrobial drug use in livestock production has been implicated as a risk factor in the development and dissemination of drug resistance from livestock production farms **(Gosh and LaPara, 2007).**

Fluoroquinolones belong to an important class of antimicrobials which used against infections caused by Gram negative bacteria with excellent activities against *E. coli* **(McDonald *et al.*, 2001)** and were approved for treatment of colibacillosis in poultry in 1995 **(Bren, 2001).**

Several studies have shown that quinolone resistant *E. coli* strains display reduced virulence **(Johnson *et al*., 2004)**.
This study was designed to study the relationship between presence of some virulence genes and quinolone resistance in *Escherichia coli* isolated from broiler chickens.

Materials and Methods

Chicken samples

A total of 100 samples were collected under aseptic conditions from apparently healthy, diseased and recently dead chickens from different chicken farms showed signs of coliseptic-emia in Kafr EL-Sheikh governorate. The samples were collected from different organs (liver, gall bladder, spleen lungs and bone marrow) from different age .Also cloacal swabs were taken. Samples were kept in ice box and transferred to the laboratory immediately.

Isolation and identification of *E. coli*:

Samples were inoculated into MacConkey's broth and incubated for 24 hours at 37ºC. Loopfuls from incubated MacConkey's broth were streaked onto MacConkey's agar plates and incubated for 24 hours at 37ºC. Suspected lactose fermented colonies were picked up and streaked on Eosin methylene blue (EMB) then incubated at 37°C overnight and then examined for the characteristic *E. coli* colonies. Pure colonies were picked up and kept in semi-solid agar for morphological and biochemical identification according to **Konemann *et al*. (1997)** and **Quinn *et al*. (2002).**

Serological typing of *E.coli*:

Nineteen isolates that were identified biochemically as *E. coli*, were chosen randomly, and subjected to serological identification **(Edward and Ewing, 1972)** in animal health research institute, Dokki, Giza using: Polyvalent and monovalent diagnostic *E.coli* antisera.

Phenotypic detection of pathogenicity of *E.coli* isolates:

Congo red dye binding test (In vitro pathogenicity test)

E.coli isolates were cultured on a separate plate of Trypticase soy agar supplemented with 0.003% Congo red dye and 0.15% bile salts. Appearance of deep brick red colonies after incubated at 37ºC for 24 hrs was recorded as positive **(Berkhoff and Vinal, 1986)**.

One day old chick lethality test (In vivo pathogenicity test)

In this test, eight groups of one-day-old chicks were used (ten chicks per group for the seven isolates &last group as a negative control). Each chick was inoculated subcutaneously with 0.2 mL of over night *Escherichia coli* suspension (1×10^8 CFU/ mL). Deaths were recorded 4 times per day, and continuing for 7 days. Clinical signs of illness were recorded daily **(Rosenberger *et al*., 1985).** Strains were classified as pathogenic when at least one chick was died.

Detection of virulence genes of *E.coli* and quinolone resistant genes by PCR (Sambrook *et al*., 1989):

DNA extraction. DNA extraction from isolates was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommen-

dations. Briefly, 200 µl of the strain suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56ºC for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer. Primers used were supplied from Metabion (Germany) are listed in Table (1).

PCR amplification. Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmolconcentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products.
The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. Gelpilot 100 bp ladder (Qiagen, Germany, GmbH) and a generuler 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1). Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences 5\-3\	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
iss	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATACCC	266	94˚C 5 min.	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Yaguchi *et al*., (2007)**
fimH	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508	94˚C 5 min.	94˚C 30 sec.	50˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	**Ghanbarpour and Salehi, (2010)**
tsh	GGT GGT GCA CTG GAG TGG AGT CCA GCG TGA TAG TGG	620	94˚C 5 min.	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	72˚C 10 min.	**Delicato *et al*., (2003)**
qnrB	GATCGTGAAAGCCAGAAAGG ACGATGCCTGGTAGTTGTCC	469	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Robicsek *et al*., (2006)**
qnrS	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	417	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	

Antimicrobial susceptibility

Antimicrobial susceptibility testing using the disk diffusion method following the Clinical and Laboratory Standards Institute, CLSI **(CLSI, 2010)** guidelines was performed. All 19 isolates were tested for 9 different antimicrobial agents (Oxoid, Basingstoke, UK): amoxicillin (30 µg), nalidixic acid (30 µg), Flumequine (30 µg), cefotaxime (30 µg), amikacin (30 µg), ciprofloxacin (5µg), doxycycline (30 µg), colistin sulphate (10 µg) and norfloxacin (5µg). The plates were incubated for 24 h at 37°C and inhibition zones were measured.

Results

1-Occurrence of *E.coli* among broiler chicken samples:

A total of 44 *E.coli* isolates were recovered from 100 chicken samples (from apparently healthy, diseased and dead chicken) collected from different farms in Kafr El-Sheikh governorate (44%).

2-Serogrouping of *E.coli* isolates recovered from chicken samples:

The serological examination of 19 randomly selected *E.coli* isolates resulted in detection of 10 different serogroups including O_{18}, O_{26}, O_{25}, O_{44}, O_{126}, O_{55}, $O_{111,}$ O_{119}, $O_{125,}$ O_{158} and one isolate untyped as shown in Table (2).

Table (2). Serogroping of some randomly selected *E. coli* isolates:

Serogroups	No. of isolates	%
O18	1	5.3%
O44	1	5.3%
O119	1	5.3%
O158	1	5.3%
Untyped	1	5.3%
O25	2	10.5%
O126	2	10.5%
O125	2	10.5%
O_{111}	2	10.5%
O55	3	15.8%
O26	3	15.8%
Total	19	100%

3- Phenotypic detection of pathogenicity of *E. coli* isolates:

3.1. Congo red dye binding test (In vitro pathogenicity test)

There were 28 isolates CR positive out of 44 *E.coli* isolates with a percentage of 63.6%.

3.2. One day old chick lethality test (In vivo pathogenicity test)

Mortality rate ranged from 50-100%. Three isolates showed 100% mortality (O_{18}, O_{111}, O_{126}), one isolate showed 70% mortality (O_{26}), two isolates showed 60% mortality (O_{25}, O_{44}) and one isolate showed 50% mortality (O_{55}).

4- Antimicrobial susceptibility of *E.coli* isolates:

Antimicrobial susceptibility test to 9 antimicrobial agents showed the highest resistant rate against amoxicillin(100%) followed by naldixic acid (63.2%), cefotaxime (57.9%) and norfloxacin, flumequine, ciprofloxacin (52.6%) each followed by doxycycline (31.8%). All isolates were sensitive to amikacin and colistin sulphate.

Table (3). Antimicrobial susceptibility of *E. coli* isolates:

Antimicrobial agents	Resistant		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Quinolones						
Norfloxacin	10	52.6	2	10.5	7	36.8
Flumequine	10	52.6	2	10.5	7	36.8
Ciprofloxacin	10	52.6	2	10.5	7	36.8
Nalidixic acid	12	63.2	2	10.5	5	26.3
Amikacin	0	0	0	0	19	100
Colistinsulphate	0	0	0	0	19	100
Doxycycline	6	31.6	6	31.6	7	36.8
Amoxicillin	19	100	0	0	0	0
Cefotaxime	11	57.9	0	0	8	42.1

5- Prevalence of some virulence genes among *E.coli* isolates:

Some of the isolates were screened for harboring (*fim* H, *iss* and *tsh*) genes. High prevalence was noted for *fim* H gene and *iss* gene with a frequency of 100% and 85.7%, respectively while only 14.3% of tested isolates contain *tsh* gene as shown in Fig (1), Fig (2) and Table (4).

Tables (4). PCR amplification of different used virulence genes of *E.coli* serogroups.

Sample	Results		
	tsh	***Fim* H**	***iss***
1 (O_{18})	+	+	+
2 (O_{26})	-	+	+
3 (O_{25})	-	+	+
4 (O_{44})	-	+	+
5 (O_{126})	-	+	+
6 (O_{55})	-	+	-
7 (O_{111})	-	+	+

5.1. Prevalence of *fim* H virulence gene among *E.coli* isolates.

Fim H gene was detected in 100% of the tested isolates.

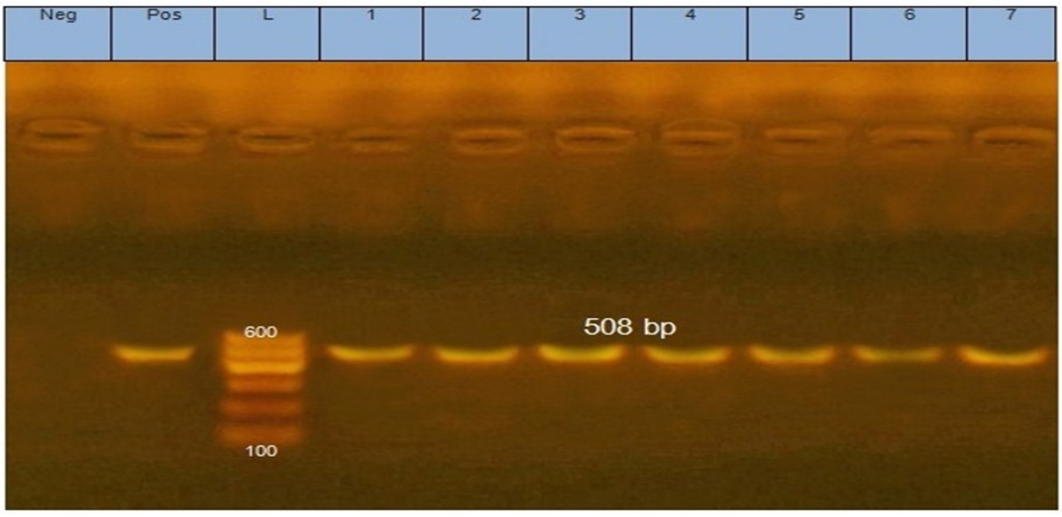

Fig. (1): Agarose gel electrophoresis of PCR amplification products of *fim* H gene for characterization of *E.coli*.
Lane L: 100bp ladder as molecular size DNA marker.
Lanes 1, 2, 3, 4, 5, 6, 7: Positive *E.coli* strains for *fim* H gene.

5.2 Prevalence of *iss* and *tsh* virulence genes among *E.coli* isolates.

The *iss* and *tsh* genes were detected in 85.7% and 14.3% of the tested isolates.

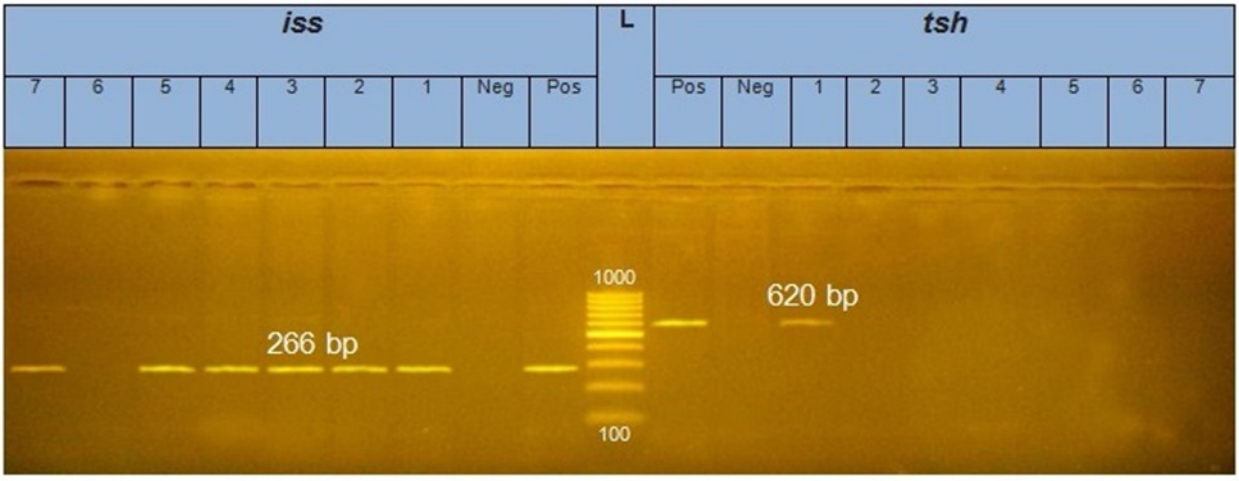

Fig. (2): Agarose gel electrophoresis of PCR amplification products of *iss* and *tsh* gene for characterization of *E.coli*.
Lane L: 100bp ladder as molecular size DNA marker.
Lanes1, 2, 3, 4, 5, 7: Positive *E.coli* strains for *iss* gene.
Lane 1: Positive *E.coli* strain for *tsh* gene.

6. Prevalence of *qnr* B and *qnr* S quinolone resistant genes among *E.coli* isolates.

It was detected that *qnr* B was expressed in 42.9% of the isolates while *qnr* S expressed in 14.3% of the isolates.

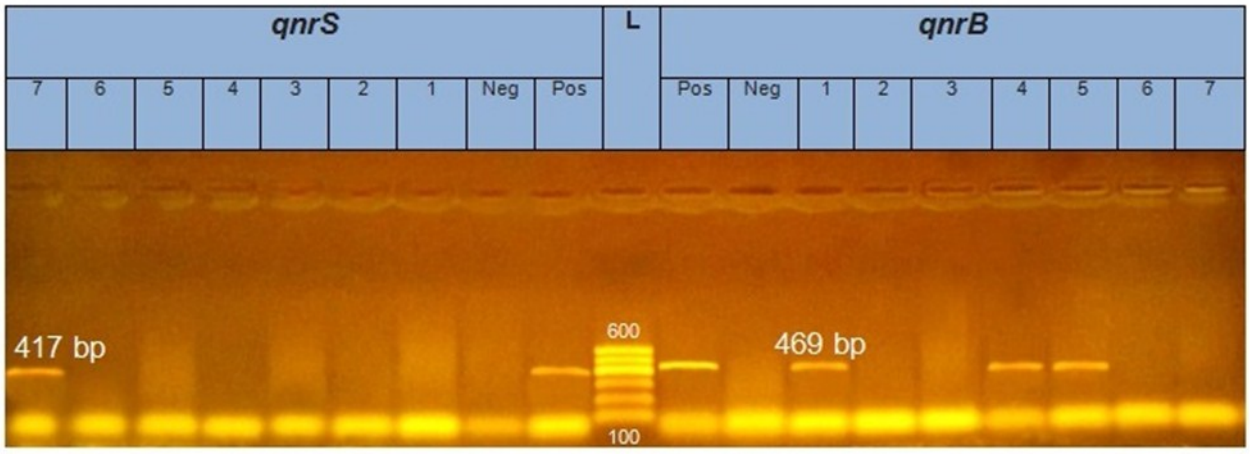

Fig. (3): Agarose gel electrophoresis of PCR amplification products of *qnr* S and *qnr* B gene for characterization of *E.coli*.
Lane L: 100bp ladder as molecular size DNA marker.
Lanes 7: Positive *E.coli* strains for *qnr* S gene.
Lane 1, 4, 5 : positive *E.coli* strain for *qnr* B gene.

Table (5). Comparison between phenotype , genotype of quinolone resistant and virulence genes among *E.coli* isolates.

Sample	Phenotypic resistance	Genotypic resistance		Virulence genes		
		qnr B	*qnr* S	fim H	*iss*	tsh
O_{18}	R	+	-	+	+	+
O_{26}	R	-	-	+	+	-
O_{25}	R	-	-	+	+	-
O_{126}	R	+	-	+	+	-
O_{44}	S	+	-	+	+	-
O_{55}	S	-	-	+	-	-
O_{111}	S	-	+	+	+	-

Discussion

Diseases caused by *E.coli* are generally known as colibacillosis which one of the major causes of morbidity and mortality with economic losses in poultry industry **Kwon *et al*., (2008).**

In the present study, out of 100 examined chicken samples 44 were positive for *E.coli* isolation (44%). Similar findings were found by **Radwan *et al*. (2014)** and **Zahid *et al.* (2016)** who isolated *E.coli* with a percentage of 41.5% and 45% respectively. Lower percentage of isolation was recorded by **Abd El-Tawab *et al*. (2014)** and **Abd El-Mongy (2018)** who isolated *E.coli* with a percentage of 28% and 27.7% respectively.

Higher percentage of isolation was recorded by **Barros *et al*., (2013); Abd El- Tawab *et al*., (2015) and Abd El- Tawab *et al*., (2016)** who isolated *E.coli* with a percentage of 75%, 82.5% and 54% respectively.

In this study, the serological examination of 19 randomly selected *E.coli* isolates revealed 10 different serogroups (O_{18}, O_{26}, O_{25}, O_{44}, O_{126}, O_{55}, O_{111},O_{119}, O_{125}, O_{158} and one untyped) with a percentage of 5.3%, 15.8%, 10.5%, 5.3%, 10.5%, 15.8%, 10.5%, 5.3%, 10.5%, 5.3% and 5.3% respectively. Some of these serogroups have been previously isolated from chickens by **Abd El-Tawab *et al*., (2016); El-Jakee *et al*., (2016) and Ramadan *et al*,. (2016)** who isolated serogroups O_{26}, O_{55} and O_{126} from chicken viscera and O_{26}, O_{44} and O_{111} from human stools**.**

It is difficult to compare between the incidences of pathogenic *E.coli* with the distribution of its serotypes among different countries due to contribution of many conditions as seasonal variation, geographic area and sampling techniques. However it might be helpful in prediction of certain outbreaks caused by these serotypes especially in countries of the same climate conditions with a sub-sequent application of suitable control regime**.**

Predominant serogroups all over the world of APEC are O_1, O_2 and O_{78} **Ramadan *et al*., (2016).** In the current study, non of these serogroups was identified and this was in agreement with **Amer *et al*. (2015)** and **Sarah *et al*. (2015).** This may be due to variation in over a period of time in a particular area **Belitski and Panika, (1969).**

Serotyping remains the most frequently used diagnostic method in laboratories, but it only allows the identification of a limited number of APEC strains so it can't be used as an effective diagnostic tool since the serotype doesn't reflect the virulence **Schouler *et al*., (2012).**

The variations in *E.coli* serotypes different from one country to another which must be taken in consideration when dealing with bacteria as it must be specific to serotypes prevalent in the locality **Abd El-Tawab *et al*., (2016)** and to develop a specific vaccine against *E.coli* infection **El-Jakee *et al*., (2016).**

There were several phenotypic methods to differentiate between pathogenic and non pathogenic *E.coli* from which lethality test **Schouler *et al*., (2012).**

Almost all strains under experiment cause mortality of one day old chicks with a mortality rate ranged from 50-100%.Death of chicks occurred within 1-4 days following subcutaneous inoculation of bacteria. These results agreed with **Nagleka *et al*. (2012)** who recorded a variable degree of virulence ranging from high to moderate. And disagree with **Zahid *et al*. (2016).**

Recently, it was suggested that the more frequently expressed virulent genes were the higher mortality rate **Oh *et al*. (2012).** And there were some specific genes can estimate this mortality as *iuc* D gene which was in association with *iss* gene which already correlated with high mortality rate as reported by **Gibbs *et al*. (2003).** These information supported our results as most strains harbor *iss* gene and show high mortality rate.

As shown in Table (3), 19 randomly selected *E.coli* were tested by disc diffusion method to 9 different antimicrobials especially quinolones group. All the tested isolates were resistant to amoxicillin (100%). This result come with accordance to that of **Ramadan *et al.* (2016)** who revealed nearly similar percentage of resistance (97.6%) and disagree with that of **Yassin *et al.* (2017)** who recorded a resistance rate of amoxicillin not exceeds 3.4%.
High resistance was observed for cefotaxime (57.9%), nearly similar results were recorded by **Abd El-Tawab *et al.* (2016)** and lower resistance was observed by **Randall *et al*. (2011)** who revealed only 0.6% resistance to cefotaxime.

The quinolone antibacterial drugs have been used widely in veterinary practice, so it was expected that high resistance to most of these drugs as resistance to nalidixic acid being the second most prevalent of all antimicrobials tested (63.2%). Similar findings was obtained by **Yassin *et al*. (2017).**

The other quinolones antimicrobials show the same pattern of resistance (norfloxacin, flumoquine, ciprocin) (52.6%) for each. These results were nearly similar to that of **Abd El- Tawab *et al*. (2015)** while dissimilar with that of **Xia *et al*. (2009)** who reported 100% resistance of *E.coli* isolates to ciprofloxacin.

All isolates were sensitive to amikacin and colistin sulphate nearly similar results were recorded by **VibhaYadav *et al*. (2018)** who reported that only one isolate from seventy tested *E.coli* isolates was resistant to amikacin and also with **Bakhshi *et al*. (2017)** who found that all *E.coli* isolates were highly susceptible (100%) to colistin suphate.

From the tested 44 *E.coli* isolates there were 28 isolates CR positive (63.6%). Nearly similar results was repored by that of **Zahid *et al*. (2016)** who found that 32 out of 53 isolates (60.4%) were CR positive and disagreed with that of **Radwan *et al*. (2014)** who found that all the tested isolates (100%) were CR positive. Binding of congo red is associated with presence of virulence genes such as *omp*A*, iss* and *fim* H and genes for multiple resistance **Fodor *et al*., (2010).**

As shown in Table (4), Fig (1) and Fig (2) of the seven PCR positive chosen *E.coli* isolates. All *E.coli* isolates (100%) were harbouring *fim* H gene followed by (85.7%) for *iss* gene while only 14.3% for *tsh* gene.

Fim H (type 1 fimbrial adhesion gene) is thought to participate in protection of *E.coli* from host heterophils **Mellata *et al*., (2003).** Similar findings were shown by many studies of **Rodriguez-Siek *et al*. (2005)** who found prevalence of 98.1% of this gene in 524 APEC isolates, and of **Vander-Westhuizen and Bragg, (2012)** who detected *fim* H gene with high incidence (88.6%). These results were dissimilar with that of **Mbanga and Nyararai**

(2015) where *fim* H prevalence was 33.3%.

The increased serum survival gene (*iss*) aids in the resistance to host serum **Chuba *et al*., (1989).** It has been proposed that *iss* gene can be used as a marker for distinguishing between APEC and commensal strains **Kwon *et al*., (2008).** Nearly similar findings for prevalence of *iss* gene was reported by those of **Ewers *et al*. (2004)** who detected 82.7% of *E.coli* isolates positive for *iss* gene from diseased chicken. Lower percentage of detection was recorded by those of **Delicato *et al.* (2003); Moon *et al*. (2006)** and **Rad and Kooshan, (2015)** who recorded it with a percentage of 38% for each.

In the united states, 85.4% of APEC strains isolated from birds clinically diagnosed with colibacillosis were positive for *iss* gene **Dissananayake *et al*. (2014).** Also *iss* gene was found in 82.7% of APEC strains isolated from chickens with colisepticemia in Germany**. Ewers *et al*., (2004).**

The temperature sensitive haemagglutinin gene (*tsh*) facilitates a haemagglutination activity in chicken **Provence and Curtiss, (1994).** *tsh* gene was detected in 14.3% of *E.coli* isolates and these results were nearly similar to that of **Vander-Westhuizan and Bragy, (2012)** who detected it in a percentage of 11.4% and these results was dissimilar to that of **Janben *et al*. (2001); Delicato *et al*. (2003); Ewers *et al*. (2004)** and **Moon *et al*. (2006)** who detected it in 85.3%, 39.5%, 53.3% and 55% respectively. There is no specific combination of virulence genes which leads to the pathogenicity of APEC has been reported till now, as APEC may possessed various virulence genes and are not homogenous **Johnson *et al*., (2008).**

The over and/or miss use of antibiotics in fields of human medicine agriculture and veterinary medicine is mainly the cause of antimicrobial resistance phenomenon **Alekshun and Levy, (2007).** Quinolones have been extensively used for treatment of a variety of systemic infection in humans and animals, wide spread use of these agents has been associated with the emergence and dissemination of quinolone resistant pathogens **Zaher *et al*., (2014).**

In this study screening of some quinolone resistant genes (*qnr* B and *qnr* S) was studied. As shown in Table (4) and Fig (3) only 3 from 7 isolates were harbouring *qnr* B gene (42.9%) while only one strain O_{111} was harbouring *qnr* S gene (14.3%). Nearly similar results was noticed by **Wang *et al*. (2008)** who detected *qnr* B in 45.4% of the tested 14 *E.coli* isolates.
Lower incidence of *qnr* gene detection were reported by **Cai *et al*. (2011)** where they examined 129 *E.coli* isolates and found *qnr* B were present in 8/129 (6.2%) and *qnr*S were present in 3/129 (2.33%) of *E.coli* tested isolates and **Chen *et al*. (2012)** who detected *qnr* B and *qnr* S in 1.3% of the isolates. **Mustak *et al*. (2012)** didn't find any of *qnr* B and *qnr* S genes in the tested 94 chicken *E.coli* isolates. Higher detection was noticed by **Samah and Ahmed(2013)** as they detected *qnr* B and *qnr* S with a percentage of 50% and 64.2% respectively.

The association between virulence characterstics of *E.coli* and quinolone resistance is a complex phenomenon as in this study all strains (quinolone susceptible and quinolone resistant) harbor virulence gene which resulted in no correlation between presence of genes of virulence and quinolone resistance and this was in agreement with that of **Rad and Kooshan, (2015)** who found no significant difference for the presence of virulence genes between quinolone susceptible and quinolone resistant isolates in diseased chicken.

In contrast with that of **Johnson *et al*. (2004)** who reported that multidrug resistant *E.coli* isolates appear to be significantly less virulent than susceptible isolates. From results shown in Table (5), there was no correlation between phenotype and genotype quinolone resistant *E.coli* isolates.

Although phenotype is not compatible with genotype in some isolates, this might be at-

tributed to that, the isolates originate from different sources of farms. Also depending on the finding of **Nathand Maurya. (2010)** `who reported that the resistance can also occur in the absence of mutations and probably due to other resistance mechanisms.

So it could be concluded that, the lack of restrictions on antimicrobial in food animals has resulted in appearance of multi-drug resistant pathogenic *E.coli* including quinolone resistant variants. Also, presence of genes of resistance is not necessary for showing resistance, as resistance can occur also when no genes of resistance can be detected. it could be concluded that, association between antimicrobial resistance with virulence may depend on types of virulence factors, types of antimicrobials and status of the host, so further studies are needed to clarify this relationship.

References

Abd El Tawab, A.A.; Ahmed, A.A.; Samir, A.A.; Fatma, I.E. and Emad, E.A. (2014). Detection of some virulence genes of avian pathogenic *E. coli* by polymerase chain reaction. Benha Vet. Med. J., 26(2): 159-176.

Abd El Tawab, A.A.; Ammar, A.M.; Nasef, S.A.; Reda, R.M. (2015). Prevalence of *E.coli* in diseased chickens with its antibiogram pattern. Benha Veterinary Medical Journal, vol. 28, no. 2: 224-230.

Abd El Tawab, A.A.; Heba B.M.; El-Hofy, F. and El-khayat, M.A. (2016). Prevalence of *bla*TEM and *bla*SHV genes in genomic and plasmid DNA of ESBL producing *Escherichia coli* clinical isolates from chicken. Benha Veterinary Medical Journal, vol. 31, no. 1: 167-177.

Abd El-Mongy, M.; Abd-El-Moneam, G.M.; Moawad, A.A.;. Abeer, M.A.B. (2018). Serotyping and virulence genes detection in *Escherichia coli* isolated from broiler chickens. J. BIOL. SCI, 18(1): 46-50.

Alekshun, M.N. and Levy, S.B. (2007). Molecular mechanisms of antibacterial multidrug resistance. Cell 128, 1037–1050.

Amer, M.M.; Bastamy, M.A.; Ibrahim, H.M. and Salim, M.M. (2015). Isolation and characterization of avian pathogenic *E.coli* from chickens in some governorates of Egypt. Vet. Med. J. Gizza (ISSN 1110-1423).

Bakhshi, M.; Fatahi Bafghi, M.; Astani, A.; Ranjba, V.R.; Zandi, H. and Vakili, M. (2017). Antimicrobial resistance pattern of *Escherichia coli* isolated from chicken with colibacillosis in Yazd, Iran. J. of food Quality and Hazards Control 4: 74-78.

Barros, L.S.S.; Silva, R.M.; Silva, I.M.; Baliza, M.D. and Virgı´lio, F.F. (2013). *Escherichia coli* from cellulitis lesions in broilers. Food Measure,7: 40–45.

Belitski, B.G. and Panika, I.I.(1969). Pathogenecity of *E.coli* isolated from broilers with septicaemia. Veterinary Moscow,12: 25-26.

Berkhoff, H.A. and Vinal, A.C. (1986). Congo red medium to distinguish between invasive and non-invasive *Escherichia Coli* for poultry. Avian Dis., 30: 117-121.

Bren, L. (2001). Antibiotic resistance from down on the chicken farm. US Food and Drug Administration, FDA Consumer Magazine, January-/February.

Cai, X.; Li, C.; Huang, J. and Li, Y. (2011). Prevalence of plasmid- mediated quinolone resistance *qnr* genes in Central China. African Journal of Microbiology Research, 5(8), 975-978.

Chen, X.; Zhang, W.; Pan, W.; Yin, J.; Pan, Z.; Gao, S. and Jiao, X. (2012). Prevalence of qnr, aac (6′)-Ib-cr, qepA, and oqxAB in *Escherichia coli* Isolates from Humans, Animals, and the Environment. Antimicrobial Agents and Chemotherapy p. 3423–3427. Volume 56 Number 6 .

Chuba, P.J.; Leon, M.A.; Banerkee, A. and Palchaudhuri, S. (1989). Cloning and DNA sequence of plasmid determinant iss, coding for increased serum survival and surface exclusion, which has homology with lambda DNA. Molecular & General Genetics, 216, 287-292.

Clinical and Laboratory Standards Institute (CLSI) (2010). Performance standard for

antimicrobial disk susceptibility testing. 26th Informational Supplement. M100S, 26th ed.

Delicato, E.R.; de Brito, B.G.; Gaziri, L.C.J. and Vidotto, M.C. (2003). Virulence-associated genes in *Escherichia coli* isolates from poultry with colibacillosis. Veterinary Microbiology, 94 97–103.

Dho-Moulin, M. and Fairbrother, J.M. (1998). Avian pathogenic *Escherichia coli* (APEC). Vet. Res. 30(2-3): 299-316.

Dissanayake, D.R.A.; Wijewardana, T.G. and Gunawardana, G.A. (2014). Distribution of lipopolysaccharide core types among Avian Pathogenic *Escherichia coli* (APEC) in relation to the major phylogenetic groups. Veterinary Microbiology. 132: 355-363.

Edward, P.R. and Ewing, W.H. (1972). Edwards and Ewing's identification of Enterobacteriacae. 3rd Ed., Burgess, Minneapolis.

El Jakee, J.K.; El Amry, G.M.; Hessain, A.M.; Hemeg, H.A.; Shafei, S.M.; Moussa, I.M. (2016). Production and evaluation of autogenous vaccine against avian colibacillosis. J. Anim. Plant Sci. 26(1): 79-87.

Ewers, C.; Janssen, T.; Kiessling, S.; Philipp, H.C. and Wieler, L.H. (2004). Molecular epidemiology of avian pathogenic *Escherichia coli* (APEC) isolated from colisepticemia in poultry. Vet. Microbiol., 104: 91-101.

Fodor, I.; Catana, N. and Herman, V. (2010). Epidemiological studies on some *E.coli* strains in broiler chickens. Bulletin UASVM, Vet. Med. 67: 74-76.

Gales, A.C.; Jones, R.N.; Kelley, G.A.; Sader, H.S.; Wilke, W.W. and Beach, M.L (2000). Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: Report from the second year of the SENTRY Antimicrobial Surveillance Program (1998). J. Antimicrob Chemother. 45: 295-303.

Ghanbarpour and Salehi (2010). Determination of Adhesin Encoding Genes in *Escherichia coli* Isolates from Omphalitis of Chicks. American Journal of Animal and Veterinary Sciences 5 (2): 91-96.

Gibbs, P.S.; Maurer, J.J.; Nolan, L.K.; Wooley, R.E. (2003). Prediction of chicken embryo lethality with the avian *Escherichia coli* traits complement resistance, colicin V production, and presence of the increased serum survival gene cluster (*iss*). Avian Dis. 47, 370–379.

Gosh, S. and La Para, T.M. (2007). The effects of subtherapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. ISME J 1: 191-203.

Hafez, A.S. (2008). Pharmacodynamic studies on drug interactions of Caphosphomycin and/or Gallipro and Enramycin in broiler. Ph.D. Pharmacology Dep., Fac. Vet. Med., Kafr El-Sheikh University.

Janben, T.; Schwarz, C.; Preikschat, P.; Voss, M.; Phillipp, H.C. and Wieler, L.H. (2001). Virulence associated genes in avian pathogenic *Escherichia coli* (APEC) isolated from internal organs of poultry having died from colibacillosis. Int. J. Med. Microbiol., 291: 371-378.

Johnson, J.R.; Kuskowski, M.A.; Gajewski, A.; Sahm, D.F. and Karlowsky, J.A. (2004). Virulence characteristics and phylogenetic background of multidrug-resistant and antimicrobial-susceptible clinical isolates of *Escherichia coli* from across the United States. J Infect Dis. 190: 1739-1744.

Johnson, T.J.; Wannemuehler, Y.; Johnson, S.J.; Stell, A.L.; Doetkott, C.; Johnson, J.R.; Kim, K.S.; Spaanjard, L. and Nolan, L.K. (2008). Comparison of Extraintestinal Pathogenic *Escherichia coli* strains from human and avian source reveal a mix subset representing potential zoonotic pathogens. Applied and Environmental Microbiology, 74(22): 7043-7050.

Konemann, E.; Allen, S.; Janda, W.; Schreckenberger, C. and Winn, W. (1997). Color Atlas and text book of Diagnostic Microbiology. Fifth Edition. Lippincott, Philadelphia, New York. 55-73.

Kwon, S.G.; Cha, S.Y.; Choi, E.J.; Kim, B.; Song, H.J. and Jung, H.K. (2008). Epidemiological prevalence of Avian Pathogenic

Escherichia coli differentiated by multiplex PCR from commercial chickens and hatchery in Korea. Journal of Bacteriology and Virology, 38(4): 179-188.

Mbanga, J. and Nyararai, Y.O. (2015). Virulence gene profiles of Avian Pathogenic *Escherichia coli* (APEC) isolated from chcikens with colibacillosis in Bulawayo, Zimbabwe. Onderstepoort Journal of Veterinary Research, 82(1), 820-850.

McDonald, L.C.; Feng-Jui, C.; Hsiu-Jung, L.O.; Hsiao-Chuan, Y.; Po-Ling, L. and Cheng-Hua, H.(2001). Emergence of reduced susceptibility and resistance to fluoroquinolones in *Escherichia coli* in Taiwan and contributions of distinct selective pressures. Antimicrob Agents Chemother. 45:/3084-/91.

Mellata, M.; Dho-Moulin, M.; Dozois, C.M.; Curtiss III, R.; Brown, P.K.; Arne`, P.; Bre`e, A.; Desautels, C. andFairbrother, J.M. (2003). Role of virulence factors in resistance of avian pathogenic *Escherichia coli* to serum and in pathogenicity. Infection and Immunity, 71, 536-540.

Moon, B.M.; Won, G.Y.; Cho, Y.Y.; Jin, J.K.; Oh, I.G.; Park, J.H.; Eo, S.K. and Lee, J.H. (2006). Isolation and characteristics of avian pathogenic *Escherichia coli* from birds associated with colibacillosis. Proceedings of AZWMP26-29 Oct, Bangkok, Thailand.

Müştak, H.K.; Ica, T.; Ciftci, A. and Diker, K.S. (2012). Plasmid-mediated quinolone resistance in *Escherichia coli* strains isolated from animal in Turkey. Ankara Üniv. Vet. FakDerg, 59, 255-258.

Nath, G. and Maurya, P. (2010). Drug resistance patterns in *Salmonella enteric* subspecies *enterica* serotype *Typhi* strains isolated over a period of two decades, with special reference to ciprofloxacin and ceftriaxone. Int. J. Antimicrob. Agents, 35: 482-485.

Nagleka, M.; Brereton, L.; Brown, G. and Fairbrother, J. (2012). Pathotypes of avian *Escherichia coli* as related to *tsh-pap- pil*-and *iuc*- DNA sequences and antibiotic sensitivity of isolates from internal tissues and the cloacae of broilers. Avian Dis.,46: 143-152.

Oh, J.Y.; Kang, M.S.; Yoon, H.; Choi, H.W.; An, B.K.; Shin, E.G.; Kim, Y.J.; Kim, M.J.; Kwon, J.H. and Kwon, Y.K. (2012). The embryo lethality of *Escherichia coli* isolates and its relationship to the presence of virulence-associated genes. Poult. Sci. 91, 370–375.

Provence, D.L. and Curtiss, R.I. (1994). Isolation and characterization of a gene involved in hemagglutination by an avian pathogenic *Escherichia coli* strain. Infection and Immunity, 62, 1369-1380.

Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W.J.C. and Leonard, F.C. (2002). Veterinary microbiology and microbial diseases. 1st Iowa State University Press Blackwell.

Rad, M. and Kooshan, M. (2015). A study on the presence of some potential virulence genes and quinolone resistance in avian pathogenic *Escherichia coli* isolated from chickens in Northeast of Iran. Iranian Journal of Veterinary Medicine. 9(4): 271-278.

Radwan, H.; Hala, S.H.S.; Soad, A.A.A. and Marwa, A.Y.A. (2014). Frequency of some virulence associated genes among multidrug-resistant *Escherichia coli* isolated form septicemic broiler chickens. International Journal of Advanced Research, 2(12): 867-874.

Ramadan, H.; Awad, A. and Ateya, A. (2016). Detection of phenotypes, virulence genes and phylotypes of avian patho-genic and human diarrheagenic *Escherichia coli* in Egypt. J. Infect. Dev. Ctries., 10(6): 584-591.

Randall, L.P.; Clouting, C.; Horton, R.A.; Coldham, N.G.; Wu, G.; Hadley, F.A.; Davies, R.H. and Teale, C.J. (2011). Prevalence of *Escherichia coli* carrying extended-spectrum b-lactamases (CTX-M and TEM-52) from broiler chickens and turkeys in Great Britain between 2006 and 2009. J. Antimicrob. Chemother., 66: 86-95.

Robicsek, A.; Strahilevitz, J.; Jacoby, G.A.; Macielag, M.; Abbanat, D.; Park, C.H.; Bush, K. and Hooper, D.C. (2006). Fluoroquinolonemodifying enzyme: a new adaptation of a common aminoglycoside

acetyltransferase. Nat Med 12:83-88.

Rodriguez-Siek, K.E.; Giddings, C.W.; Doetkott, C.; Johnson, T.J. and Nolan, L.K. (2005). Characterizing the APEC pathotype. Vet. Res., 36: 241–256.

Rosenberger, J.K.; Fries, P.A. and Cloud, S.S. (1985). In vitro and in vivocharacterization of avian *Escherichia coli*. III. Immunization. *Avian Diseases*, 29, 1108-1117.

Samah, Eid A.S. and Ahmed M. Erfan (2013). characterization of *E.coli* associated with high mortality in poultry flocks. Assiut Vet. Med. J. Vol. 59 No. 139 October 51-61.

Sambrook, J.; Fritsch, E.F. and Montias, T. (1989). Molecular Biology. In: Molecular cloning. Laboratory manual, Second Edition. Cold Spring Harbor Laboratory press, USA.P.268.

Sarah, A.Y.; Ammar, A.M. and Ahmed, D.A. (2015). Serological and molecular typing of avian pathogenic *E.coli* originating from out breaks of colibacillosis in chicken flocks. Int.J.ofSci.andRes.4(2):2082-2089.

Satyajit, G.; Deshmukh, A.A.; Amol, R.; Kadam, G. and Dnyaneshwar, B. (2013). Antibacterial efficacy study of Emblica. Officinalis against exly induced *Escherichia coli* infection in broiler chicks. Int. J. Pharmacol. Toxic. Sci., 3(1): 39-49.

Schouler, C.; Schaeffer, B.; Brée, A.; Mora, A.; Dahbi, G. and Biet, F. (2012). Diagnostic strategy for identifying avian pathogenic *Escherichia coli* based on four patterns of virulence genes. J. Clin. Microbiol., 50: 1673 –1678.

Van der -Westhuizen W.A. and Bragg R.R. (2012). Multiplex Polymerase Chain Reaction for screening Avian Pathogenic *Escherichia coli* (APEC) for virulence genes. Avian Pathology. 41(1): 33-40.

VibhaYaday, M.; Joshi, R.K. and Diwaker, R.P. (2018). Study of multiple drug resistance in *Escherichia Coli* strain of poultry origin. Int. J. Curr. Microbiol. App. Sci(7): 403-410.

Wang, A.; Yang, Y.; Lu, Q.; Wang, Y.; Chen, Y.; Deng, L.; Ding, H.; Deng, Q.; Zhang, H.; Wang, C.; Liu, L.; Xu, X.; Wang, L. and Shen, X. (2008). Presence of qnr gene in *Escherichia coli* and Klebsiellapneumoniae resistant to ciprofloxacin isolated from pediatric patients in China. BMC Infectious Diseases. 8(1): 68.

Xia, L.; Li, L.; Wu, C.; Liu, Y. and Tao, X. (2009). A survey of plasmid mediated fluoroquinolone resistance genes from *Escherichia coli* isolates and their dissemination in Shandong, China. Foodborne Path. Dis., 2009-0378.

Yaguchi, K.; Ogitani, T.; Osawa, R.; Kawano, M.; Kokumai, N.; Kaneshige, T.; Noro, T.; Masubuchi, K. and Shimizu, Y. (2007). Virulence factors of avian pathogenic *Escherichia coli* strains isolated from chickens with colisepticemia in Japan**.** Avian Dis. 51(3): 656-62.

Yassin, A.K.; Gong, J.; Kelly, P.; Lu, G.; Guardabassi . L.; Wei, L.; Han, X.; Qiu, H.; Price, S.; Cheng, D. and Wang, C. (2017). Antimicrobial resistance in clinical *Escherichia coli* isolates from poultry and livestock. China. PLOS ONE 12(9): e0185326.

Zaher, A.; Y. Artuun; A. Erdem; M. Kaan and G. Elcin (2014). Plasmid- mediated quinolone resistance in *Salmonella* serotypes isolated from chicken carcasses in Turkey. 83: 281-286.

Zahid, A.H.; AL-Mossawei, M.T.M.; Mahmood, A.B. (2016). In vitro and In vivo Pathogenicity tests of Local Isolates APEC from Naturally Infected Broiler in Baghdad. Int. J. Adv. Res. Biol. Sci., 3(3): 89-100.