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ORIGINAL ARTICLE

Molecular Characterization of Antimicrobial-resistant *Escherichia coli* Isolated from Broiler Chickens

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Abstract
Avian colibacillosis is one of the most important diseases of chickens resulting in significant economic losses as well as high morbidity and mortality. In the current study, the prevalence of avian colibacillosis was studied in different farms of broiler chickens in Beni-Suef, EL-Minia, ElFayoum, Assiut and Sohag Governorates. A total of 300 pooling samples were collected aseptically from heart blood as well as the affected organs including air sacs, pericardial sac, and liver of slaughtered diseased and freshly dead broiler chickens. Bacteriological examination of the collected samples showed that a total number of 80 *E. coli* isolates were recovered with an overall prevalence of 26.7. The highest prevalence was recorded in El-Fayoum (33.3%) followed by El-Minia (25%), Beni-Suef and Assiut (22.5% for each) while the lowest prevalence was in Sohag (17.5%). The invitro antimicrobial susceptibility testing revealed that *E. coli* isolates showed high sensitivity to colistin sulphate only (70%). Meanwhile, high resistances were recorded against other antimicrobials including amoxicillin (97.5%), cefotaxime sodium and florfenicol (95% for each), apramycin, ciprofloxacin and gentamicin (92.5% for each), streptomycin (90%), enrofloxacin (87.5%), trimethoprim-sulphamethoxazol and doxycycline HCl (77.5% for each). All *E. coli* isolates were MDR (100%). PCR was applied on 10 MDR *E. coli* isolates to detect the 3 resistance-associated genes (qnrA, tetA and aac(6')- Ib-cr and 5 virulence-associated genes (iutA, hly, sta, lt and astA). The results showed that all the tested isolates (100%) harbored qnrA, tetA, iutA and astA genes meanwhile aac(6')-Ib-cr, hly, sta and lt genes were not detected in any isolate.

Keywords Broiler chickens, *E. coli*, Multidrug resistance (MDR), Resistance genes

1. Introduction
One of the major problems in the poultry farms is the control of infectious diseases causing high economic losses in the poultry industry **(McKissick 2006).** Avian colibacillosis; caused by avian pathogenic *E. coli* (APEC), has been recognized as bacterial infection of major importance. Colibacillosis is a widespread disease causing severe economic losses in the aviculture all over the world and is often the most frequent cause of carcass condemnation at processing **(Barnes et al. 2008).** It indicates localized or systemic infections caused by APEC including many forms as

colisepticemia, coligranuloma, chronic respiratory disease (CRD, airsacculitis), enteritis, swollen-head syndrome, cellulitis, salpingitis, omphalitis/yolk sac infection, panophthalmitis**,** osteomyelitis, pericarditis, synovitis and peritonitis which often occur concurrently with other bacteria, viruses, protozoa, and fungi **(Yue et al. 2018).** Chickens of all ages are susceptible to colibacillosis but more common in young birds which are severely affected **(Barnes et al. 2003).** Colisepticemia is the most common form of colibacillosis which is responsible for high economic losses in aviculture in many parts worldwide **(Saif 2003).** *E. coli* infections are of great concern in the poultry industry. It is one of the most important and frequently encountered avian bacterial pathogens causing a wide range of disease syndrome in birds which causes up to 30% of poultry mortality **(Geornaras et al. 2001).** *E. coli* strains were classified by **Russo and Johnson (2000)** into 3 major groups including intestinal pathogenic strains, commensal strains and, extra-intestinal pathogenic *E. coli* (ExPEC) strains. Serological and bacteriological methods are not sensitive enough to differentiate all bacterial isolates. Molecular differentiation of *E. coli* strains and other bacteria may help in differentiation of those are specifically pathogenic for a certain host, these techniques of molecular and genetic differentiation involve polymerase chain reaction (PCR) **(Whittam and Wilson 1988).** Although *E. coli* infections have been costly to the poultry industry, the exact virulence mechanisms used by these organisms to cause disease in birds remain an interesting point of research. The presence of several virulence genes has been positively linked to the pathogenicity of APEC strains **(Ewers et al. 2005).** The establishment of PCR assays was to facilitate detection of the frequency with which the various virulence-associated genes occur in the resident APEC population; subsequently, the isolates identified as the most highly pathogenic *E. coli* by PCR technique are used as the basis for the production of a powerful vaccine to be used against APEC infections. By researching the chain of infection, new and effective controls can be put in place to prevent the rapid spread of APEC **(Ewers et al. 2004).** Increasing of antimicrobial resistance is a very important public health concern, and the emergence and spread of antimicrobial resistance is a complex problem driven by numerous interconnected factors. In-vitro antimicrobial sensitivity testing of veterinary pathogens provides valuable guidance to the veterinarian in the choice of appropriate drug treatment **(Radwan et al. 2016).** Moreover, it is very useful to detect the multidrug-resistant (MDR) isolates. Therefore, the appropriate antibiotic should be selected on the basis of its sensitivity that could be detected by laboratory examination. Resistance of *E. coli* species to antimicrobials is widespread and of concern to poultry veterinarians. This increasing resistance has received considerable attention in Egypt and worldwide. Plasmids are the major vector in the spreading of resistance genes through the bacterial population **(Radwan et al. 2016).** There is a wide variety of *E. coli* resistant to multiple antimicrobials, and PCR can be used to detect antimicrobial resistance genes in *E. coli* isolates.

The present study was designed to investigate the molecular characterization of antimicrobial resistant *E. coli* isolated from broiler chickens achieved by the detection of some virulence and antimicrobial resistance associated genes in the MDR isolates using PCR technique.

2. Material and Methods

2.1. Ethical approval

The approval from the Institutional Animal Ethics Committee to carry out this study was not required as no invasive technique was used.

2.2. Chickens Samples

A total of 300 samples were collected aseptically from 300 diseased Hubbard and Ross broiler chickens aged from 2-5 weeks from different farms in Beni-Suef, EL-Minia, El-Fayoum, Assiut and Sohag Governorates during the period from January 2016 up to December 2016. These chickens were subjected to clinical and postmortem examinations. Pooling swab samples were collected aseptically from heart blood as well as the lesions in the internal organs; air sacs, pericardial sac, liver, and lung of slaughtered diseased and freshly dead chickens.

2.3. Bacteriological isolation

The collected pooling samples were aseptically inoculated into MacConkey broth (Oxoid) and incubated aerobically at 37°C for 24 hrs. Then a loopful of the broth culture was streaked onto MacConkey agar (Oxoid) and incubated aerobically at 37°C for 24-72hr. The lactose fermenting (pink) colonies were inoculated onto eosin methylene blue agar medium (Oxoid) and confirmed as *E. coli* using the standard biochemical tests according to **Collee et al. (1996).**

2.4. Identification of *E. coli* isolates.

2.4.1. Morphological and biochemical identification

All the recovered isolates were identified morphologically and biochemically according to **Collee et al. (1996)** and **Quinn et al. (2002)** using the following tests; oxidase, catalase, urease, H2S production on TSI, and citrate utilization. Other non-biochemical tests including motility test and hemolysis onto blood agar were applied.

2.4.2. Identification by using an API20E kit

The appropriate API kit (API20E, Oxoid) was used. API strips should only be used to identify pure cultures. It was used according to the manufacturer's instructions.

2.5. Antimicrobial susceptibility testing

All *E. coli* isolates were tested for their antimicrobial susceptibility to eleven of the most important antimicrobials used in the field. Antimicrobial discs included amoxicillin (10µg), apramycin (15µg), cefotaxime sodium (30µg), ciprofloxacin (5µg), enrofloxacin (5µg), sulphamethoxazol-trimethoprim (25µg), colistin sulphate (10µg), doxycycline HCl (30µg), gentamycin (10µg), florfenicol (30µg) and streptomycin (10µg) (Oxoid, Basing Stoke, UK). Antimicrobial susceptibility testing was applied using the disc diffusion method on Muller Hinton agar according to Clinical and Laboratory Standards Institute **(CLSI 2016).** The antimicrobial susceptibility was based on the induced inhibition zones according to the guidelines of the CLSI (2016). Resistance to three/or more antimicrobials of different categories was taken as multidrug resistance (MDR) according to **Chandran et al. (2008).**

2.6. Polymerase chain reaction (PCR) for *E. coli* isolates

PCR was applied on 10 MDR *E. coli* isolates for detection of 8 genes; 3 resistance-associated genes (qnrA, tetA and *aac (6')*-Ib-cr) as well as 5 virulence-associated genes (iutA, hly, sta, lt, and astA). Genomic DNA was extracted by QIAamp DNA extraction Mini prep Kit from confirmed cultures in accordance with the manufacturer's instructions. Extracted DNA was kept at -80°C until used in PCR amplification. Primers sequences and amplified products for the targeted genes for *E. coli* isolates were illustrated in **Table (1).** Temperature and time conditions of the primers during PCR were shown in **Table (2)** according to specific authors and Emerald Amp GT PCR master-mix Takara) kit.

Table (1). Primers of resistance and virulence genes used in PCR.

	Primer		Primer sequence (5'-3')	Amplified product	Reference
Resistance genes	***qnr*A**	***F***	*ATTTCTCACGCCAGGATTTG*	516 bp	**Robicsek *et al*. (2006)**
		R	*GATCGGCAAAGGTTAGGTCA*		
	***tet*A(A)**	***F***	*GGTTCACTCGAACGACGTCA*	576 bp	**Randall *et al.* (2004)**
		R	*CTGTCCGACAAGTTGCATGA*		
	aac(6')-Ib-cr	***F***	*CCCGCTTTCTCGTAGCA*	113 bp	**Lunn *et al*. (2010)**
		R	*TTAGGCATCACTGCGTCTTC*		
Virulence genes	***iut*A**	***F***	*GGCTGGACATGGGAACTGG*	300 bp	**Yaguchi *et al*. (2007)**
		R	*CGTCGGGAACGGGTAGAATCG*		
	hly	***F***	*AACAAGGATAAGCACTGTTCTGGCT*	1177 bp	**Piva *et al*. (2003)**
		R	*ACCATATAAGCGGTCATTCCCGTCA*		
	sta	***F***	*GAAACAACATGACGGGAGGT*	229 bp	**Lee *et al.* (2008)**
		R	*GCACAGGCAGGATTACAACA*		
	lt	***F***	*GGTTTCTGCGTTAGGTGGAA*	606 bp	
		R	*GGGACTTCGACCTGAAATGT*		
	***ast*A**	***F***	*CCATCAACACAGTATATCCGA*	110 bp	**Piva *et al*. (2003)**
		R	*GGTCGCGAGTGACGGCTTTGT*		

Table (2): Cycling conditions of the different primers during PCR.

Gene	Primary denaturing	secondary denaturing	Annealing	Extension	No. of cycles	Final extension
- ***qnr*A**	94C/5min.	94C/30sec.	55C/40sec.	72C/45sec.	35cycles	72C/10min.
- ***tet*A**	94C/5min.	94C/45sec.	50C/40sec.	72C/45sec.	35cycles	72C/10min.
- ***aac(6')-Ib-cr***	94C/10min	94C/30sec.	52C/30sec.	72C/30sec.	35cycles	72C/7min.
- ***iut*A**	94C/10min	94C/30sec.	63C/30sec.	72C/30sec.	35cycles	72C/7min.
- ***hly***	94C/10min	94C/45sec.	60C/40sec.	72C/1min.	35cycles	72C/12min.
- ***sta***	94C/10min	94C/1min.	57C/30sec.	72C/30sec.	35cycles	72C/7min.
- ***lt***	94C/5min.	94C/45sec.	57C/40sec.	72C/45sec.	35cycles	72C/10min.
- ***ast*A**	94C/5min.	94C/45sec.	55C/30sec.	72C/30sec.	35cycles	72C/7min.

3. Results

3.1. Prevalence of *E. coli* isolation in the diseased broiler chickens in different Governorates.

Out of 300 diseased broiler chickens, 80 *E. coli* isolates were recovered with an overall prevalence rate of 26.7%. The highest prevalence was recorded in El-Fayoum Governorate as 33.3% (40 isolates/120 bird) followed by El-Minia Governorate as 25% (15/60). Then, both of Beni-Suef and Assiut Governorates as 22.5% (9/40). Meanwhile, the lowest prevalence was recorded in Sohag Governorate as 17.5% (7/40) **(Table 3).**

Table (3): Prevalence of *E. coli* isolation in the diseased broiler chickens in different Governorates.

Governorates	No. of diseased broilers (samples)	*E. coli* isolation	
		No.	%
El-Fayoum	120	40	33.3
EL-Minia	60	15	25
Beni-Suef	40	9	22.5
Assiut	40	9	22.5
Sohag	40	7	17.5
Total	300	80	26.7

%: was calculated according to the corresponding number (No.) of samples.

3.2. Antimicrobial susceptibility testing

Results of in-vitro susceptibility testing showed that *E. coli* isolates were highly sensitive to colistin sulphate only (70%). On the other hand, they were highly resistant to all other antimicrobials. The highest resistance was recorded against amoxicillin (97.5%) followed by cefotaxime sodium and florfenicol (95% for each). Then, apramycin, ciprofloxacin, and gentamicin (92.5% for each) and streptomycin (90%), enrofloxacin (87.5%). And finally, both of trimethoprim-sulphamethoxazol and doxycycline HCl were recorded as 77.5% for each (Table 4). MDR was detected in all the tested isolates (100%).

2.4. Polymerase chain reaction (PCR) analyses of *E. coli* isolates

Regarding the resistance-associated genes, PCR results revealed that all the tested isolates (n=10) harbored both qnrA and tetA genes (100%) meanwhile none of them (0%) harbored *aac (6')*-Ib-cr gene (Tables 5& 6 and Figs. 1-3). On the other hand, PCR results of virulence associated genes revealed that all the tested isolates (n=10) harbored both iutA and astA genes (100%) meanwhile none of them (0%) harbored hly, sta and lt genes **(Tables 5-6 and Figs. 4-8).**

Table (4): Results of antimicrobial susceptibility testing of *E. coli* recovered from diseased broiler chickens.

Antimicrobial disc	Disc content (µg)	*E. coli* (*n=80*)					
		R		I		S	
		No	%	No	%	No	%
Amoxicillin	10	78	97.5	0	0	2	2.5
Apramycin	15	74	92.5	6	7.5	0	0
Ciprofloxacin	5	74	92.5	4	5	2	2.5
Cefotaxime sodium	30	76	95	2	2.5	2	2.5
Colistin sulphate	10	16	20	8	10	56	70
Sulfamethoxazole-trimethoprim	25	62	77.5	8	10	10	12.5
Doxycycline HCl	30	62	77.5	12	15	6	7.5
Enrofloxacin	5	70	87.5	4	5	6	7.5
Gentamicin	10	74	92.5	6	7.5	0	0
Florfenicol	30	76	95	2	2.5	2	2.5
Streptomycin	10	72	90	4	5	4	5

% was calculated according to the number of the tested isolates (n=80).

Table (5): Distribution of virulence and resistance-associated genes in the examined *E. coli* isolates.

Gene / Sample	*iut*A	*ast*A	*hly*	*sta*	*lt*	*aac(6')-Ib-cr*	*tet*A(A)	*qnr*A
1	+	+	-	-	-	-	+	+
2	+	+	-	-	-	-	+	+
3	+	+	-	-	-	-	+	+
4	+	+	-	-	-	-	+	+
5	+	+	-	-	-	-	+	+
6	+	+	-	-	-	-	+	+
7	+	+	-	-	-	-	+	+
8	+	+	-	-	-	-	+	+
9	+	+	-	-	-	-	+	+
10	+	+	-	-	-	-	+	+
Total	**10**	**10**	**0**	**0**	**0**	**0**	**10**	**10**

Table (6): Prevalence of resistance and virulence-associated genes in the examined *E. coli* isolates.

Result / Gene		No. of tested isolates	Positive	
			No.	%
Virulence genes	***iut*A**	**10**	10	100
	***ast*A**		10	100
	hly		0	0
	sta		0	0
	lt		0	0
Resistance genes	***aac(6')-Ib-cr***		0	0
	***tet*A(A)**		10	100
	***qnr*A**		10	100

%: was calculated according to the number (No.) of the tested isolates.

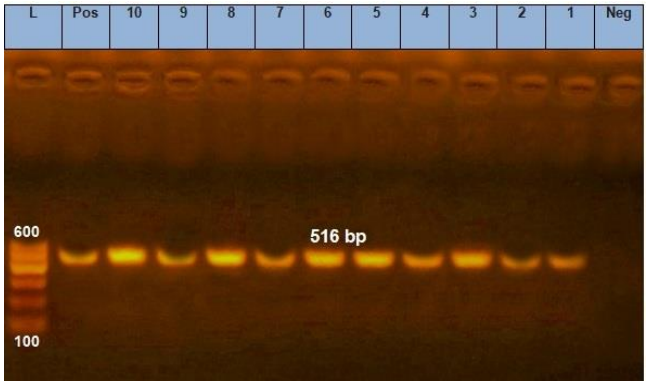

Fig. 1. PCR amplification of the 516bp fragment of *qnr*A resistance gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

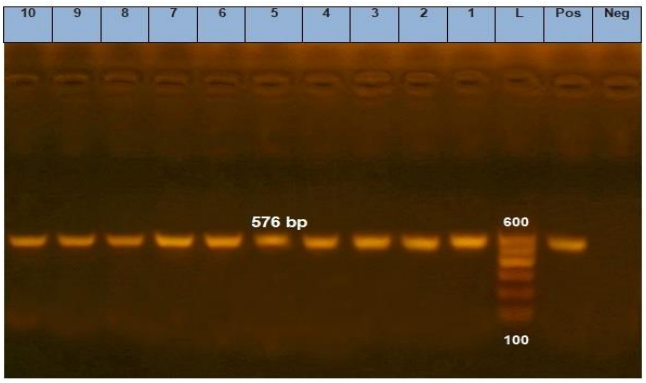

Fig. 2. PCR amplification of the576bp fragment of *tet*A(A) resistance gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

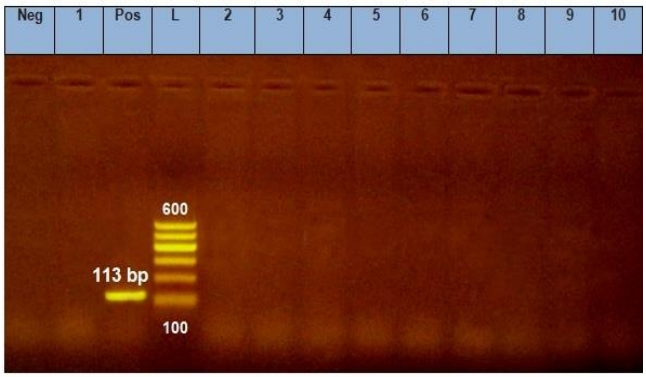

Fig. 3. PCR amplification of the 113bp fragment of *aac(6')-Ib-cr* resistance gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

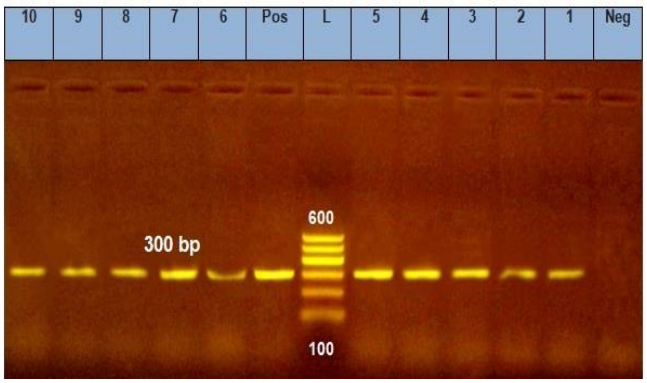

Fig. 4. PCR amplification of the 300bp fragment of *iut*A virulence gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

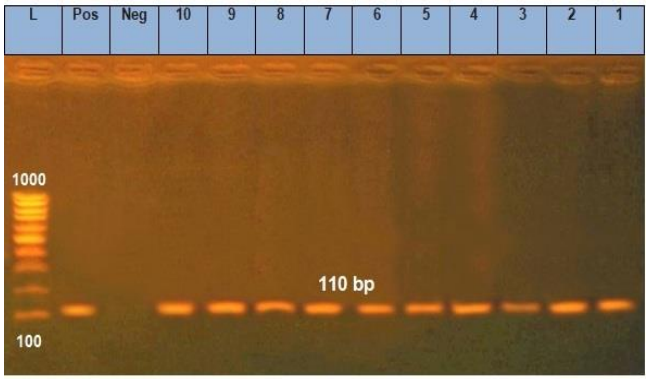

Fig. 5. PCR amplification of the 110bp fragment of *ast*A virulence gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

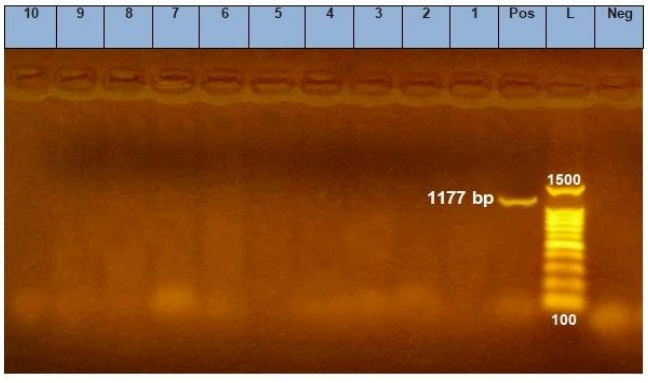

Fig. 6. PCR amplification of the 1177bp fragment of *hly* virulence gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

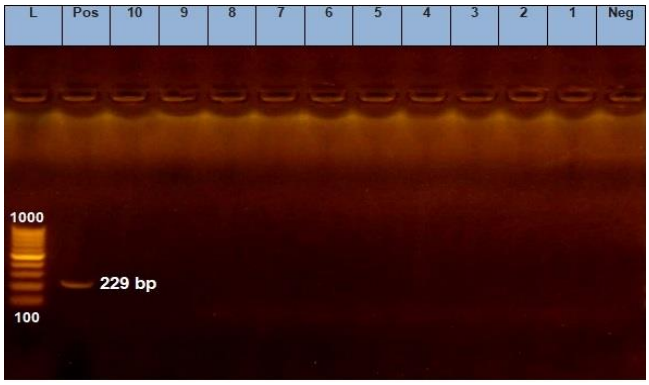

Fig. 7. PCR amplification of the 229bp fragment of *sta* virulence gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

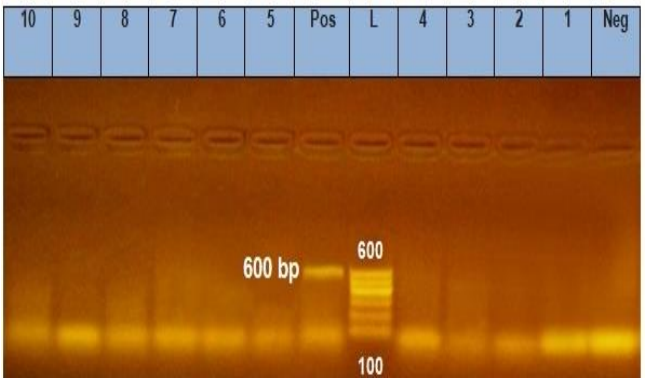

Fig. 8. PCR amplification of the 600bp fragment of *lt* virulence gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

4. Discussion

Poultry are regarded; without any doubt, the most appropriate source of animal protein supply of high nutritive value for humans all over the world. This is due to the efficiency cost of production and its short life cycle. In Egypt, a great attention was directed to the poultry industry to meet the increasing demand for animal protein. Infectious diseases are important in the broiler industry due to high mortality, retardation of growth, as well as the preventive and therapeutic use of antimicrobials. Moreover, economic losses may result from the loss of uniformity of the flock and condemnations in the slaughterhouse **(Vandemaele et al. 2002; McKissick 2006).** Avian colibacillosis is one of the most important diseases of chickens, resulting in significant economic losses as well as high morbidity and mortality among baby chicks, broilers, and layers **(Ewers et al. 2004; Paixão et al. 2016).** This syndrome is characterized by acute septicemia with considerable death rates as well as sub-acute forms characterized by multiple organ lesions including airsacculitis and associated pericarditis, perihepatitis, and **peritonitis (Ewers et al. 2003; Huja et al. 2015; Younis et al. 2017).** Stress seemed to cause invasion of APEC from intestine into blood stream and spreads into various internal organs and typically causes pericarditis, perihepatitis, peritonitis, salpingitis, and other extra-intestinal diseases **(Leinter and Heller 1992).** In the current study, the prevalence of avian colibacillosis was studied in broiler chickens in 5 Governorates. The data illustrated in the table (3) revealed that the overall prevalence of *E. coli* in the diseased broiler chickens in different Governorates was 26.7% where 80 *E. coli* isolates were recovered from 300 diseased broiler chickens. Regarding Governorates, the highest prevalence was recorded in El-Fayoum as 33.3% followed by El-Minia as 25% and then, both of Beni-Suef and Assiut as 22.5% while the lowest prevalence was recorded in Sohag as 17.5%. In Egypt, these results were nearly similar to those obtained by **Abd El Tawab et al. (2014)** who recorded a prevalence of 24.7% in diseased chickens and **El-Seedy et al. (2019);** who remarked that prevalence rate was 23%. Also, **Ammar et al. (2011)** collected 204 samples from broiler chickens in El-Sharkia Governorate and recovered 100 *E. coli* isolates (24%). This observation was also slightly lower than those previously described by **Younis et al. (2017);** 36.5%, and **Qurani (2019);** 33%. Higher results were obtained by **Roshdy et al. (2012);** 43.1%, and **Yahia (2014);** 41.5%, Meanwhile, much higher prevalences were recorded including **ElSukhon et al. (2002);** 88.2%, **Abd El-Latif (2004);** 78.7%, **Abd El Aziz et al. (2007);** 90%, and **Radwan et al. (2016**); 56%. These variations in the *E. coli* prevalence in broilers may be attributed to the difference in strains pathogenicity and virulence beside the severity of the cases as well as the immunological status of the host **(Heba et al. 2012).** Moreover, **Ashraf et al. (2015)** attributed the variation in *E. coli* prevalence to the difference in the seasons as they recorded higher prevalence in winter (60.9%) than that in summer (41%). This difference might be due to the lower environmental and hygienic conditions in poultry farms in winter such as overcrowding, bad ventilation, and higher ammonia level in the air. Also, it might be due to a higher percentage of *E. coli* in feed, water, litter, and air in winter than in summer. Also, **Abd El Tawab et al. (2015)** recorded prevalences of *E. coli* in samples from apparently healthy, diseased, and freshly dead broiler chickens in winter and summer seasons. In winter season they were 15.7%, 37.1%, and 55%, respectively while in summer season they were

15.8%, 17.5%, and 18.7%, respectively. Stress may cause invasion of pathogenic *E. coli* from intestine into blood stream and spreads into different visceral organs causing peritonitis, perihepatitis, pericarditis, salpingitis, and other extra-intestinal diseases **(Leinter and Heller 1992).** Antimicrobial therapy is considered one of the worldwide primary controls for the reduction of both incidence and mortality associated with avian colibacillosis therefore, reducing their great losses in the poultry industry **(Radwan et al. 2016).** Although antimicrobials are considered valuable tools prevent and treat infectious bacterial diseases and as growth promoters at sub-therapeutic levels in feeds to maintain health and productivity of birds, its use in livestock production has been implicated as a risk factor in the development and spreading of antibiotic resistance **(Gosh and LaPara 2007).** Increasing of antimicrobial resistance is a very important public health concern, and the emergence and spread of antimicrobial resistance is a complex problem driven by numerous interconnected factors. In-vitro antimicrobial sensitivity testing of veterinary pathogens provides valuable guidance to the veterinarian in the choice of appropriate drug treatment **(Radwan et al. 2016).** Moreover, it is very useful to detect the MDR isolates. Therefore, the appropriate antibiotic should be selected on the basis of its sensitivity that could be detected by laboratory examination. In the present work, *E. coli* isolates were tested for their susceptibility to 11 different antimicrobial drugs to detect the drug of choice for treatment as well as to detect MDR isolates for further analyses of the isolates. The results of in-vitro antimicrobial susceptibility tests for *E. coli* isolates were demonstrated in table (4). *E. coli* isolates showed high sensitivity to colistin sulphate only (70%). On the other hand, high resistances were recorded to all other antimicrobials including amoxicillin, cefotaxime sodium and florfenicol, apramycin, ciprofloxacin and gentamicin and streptomycin (as 90-97.5%) as well as enrofloxacin, trimethoprim-sulphamethoxazol and doxycycline (as 77.5-87.5%). Concerning the result of colistin sulphate susceptibility, it was supported by several previous reports in Egypt and all over the world. In Egypt, this result run hand to hand with that obtained by **El-Seedy et al. (2019)** who found that colistin had the sensitivity of 63.6%. Also, **Chen and Wang (1997**) in Taiwan recorded high sensitivities to colistin. Moreover, **Salehi and Bonab (2006)** and **Zakeri and Kashefi (2012)** in Iran recorded low resistance to colistin. Also **Messaï et al. (2013)** in Algeria found that 94.5% of *E. coli* isolates were sensitive to colistin. Moreover, **Solà-Ginés et al. (2015)** in Spain and **Dandachi et al. (2018)** in Lebanon recorded complete susceptibility of *E. coli* isolates to colistin. On the other hand, **Saberfar et al. (2008)** in Iran recorded resistance against colistin sulphate in 99% of isolates. Regarding the increasing incidences of antibiotic-resistance of *E. coli* isolates in such study; these findings were coincided with those recorded by many authors in Egypt **(Abd El Tawab et al. 2014 and 2015; Radwan et al. 2014, 2016 and 2018; Awad et al. 2016; ElShazly et al. 2017; Amer et al. 2018; El-Seedy et al. 2019; Qurani 2019).** The same finding was reported worldwide including **AL Ghamdi et al. (1999)** in Saudi Arabia, **Adelaide et al. (2008)** in Kenya, **Chandran et al. (2008)** and **Sharada et al. (2009)** in India, **Aggad et al. (2010)** and **Messaï et al. (2013)** in Algeria **Bashar et al. (2011)** in Bangladesh, **Saidi et al. (2012)** in Zimbabwe, **Momtaz and Jamshidi (2013)** and **Rahimi (2013)** in Iran, **Solà-Ginés et al. (2015)** in Spain, **Dou et al. (2016)** and **Li et al. (2016)** in China, **Rahman et al. (2017)** in Bangladesh; **Dandachi et al. (2018)** in Lebanon; **Subedi et al. (2018)** in Nepal. Therefore, no single antimicrobial drug was effective by 100% against *E. coli* isolates, which might be due to the development of resistance due to the indiscriminate use of antibiotics **(Sharada et al. 2001).** Moreover, in the current study, MDR was detected in all *E. coli* isolates (100%). Such results agreed also with several previous reports in Egypt and all over the world. In Egypt, **Amer et al. (2018)** and **Qurani (2019)** found that all *E. coli* isolates were MDR. Meanwhile**, Radwan et al. (2014)** recorded MDR in 90.4% of the isolates. Also, **Adelaide et al. (2008)** in Kenya, **Chandran et al. (2008)** and **Sharada et al. (2009)** in India, **Bashar et al. (2011)** in Bangladesh, **Saidi et al. (2012)** in Zimbabwe, **Zakeri and Kashefi (2012) i**n Iran, **Messaï et al. (2013)** in Algeria and **Jahantigh and Dizaji (2015)** found that all *E. coli* isolates were MDR. Lower percentages of MDR were recorded all over the world by **Dou et al. (2016)** in China; 80.3%, **Rahman et al. (2017)** in Bangladesh; 76%, **Aggad et al. (2010)** in Algeria; 72% and **Rahimi (2013)** in Iran; 63.3%. Moreover, about half (54.6%) exhibited resistance to four or more antibiotics, as observed in previous work of **Yang et al. (2004), Zhao et al. (2005)** and **Ozawa et al. (2008).** Moreover, **Blanco et al. (1997a&b), Chen and Wang (1997)** and **Hammoudi and Aggad (2008)** found high levels of resistance to antibacterial drugs in pathogenic strains of *E. coli* isolated from chickens ensuring that multiple drug resistance was

common. The spread of MDR bacteria has been recognized as an increasing problem in both medical and veterinary fields, and movable DNA elements such as plasmids, integrons and transposons favor the proliferation of resistance genes in the bacteria **(Speer et al. 1992; Liebert et al. 1999).** Antimicrobial resistance of *E. coli* species is widespread and of concern to poultry veterinarians. This increasing resistance has received considerable attention in Egypt and all over the world. Plasmids are the major vector in the spreading of resistance genes through the bacterial population **(Radwan et al. 2016).** The R-plasmids have been extensively studied in view of the prevalence of MDR **(O'Brien et al. 1982).** Several virulence and resistance-associated genes were reported on plasmids of *E. coli* recovered from diseased poultry **(Kelly et al. 2009).** There is a wide variety of MDR *E. coli* and PCR can be used to detect antimicrobial resistance genes in *E. coli* isolates. In the current work, PCR was applied on 10 MDR *E. coli* isolates to detect the 3 resistance associated genes including plasmid-mediated quinolone resistance gene (qnrA), resistance to tetracycline (tetA) and resistance to aminoglycosides (*aac(6')*-Ib-cr). The results represented in tables (5& 6) and figs. (1-3) showed that 100% of the tested isolates harbored both qnrA and tetA genes meanwhile *aac(6')*-Ib-cr gene was not detected in any isolate. Many genes were detected for tetracycline resistance including tetA, tetB, tetC, tetD, and tetE genes **(Ng et al. 2001).** The efflux proteins have been the best studied of the Tet determinants including tetA, tetB, tetC, tetD, tetE, tetG, tetH, tetK, tetL and tetA(P) genes which have been identified. All of them were coded for energy-dependent membrane associated proteins which export tetracycline out of the cell reducing the intercellular concentration of tetracycline and thus leads to protect the bacterial ribosomes **(El-Seedy et al. 2019).** Regarding the obtained results of tetA which was detected in all tested isolates (100%), they were supported by those of **El-Seedy et al. (2019)** who found tetA (A) gene in all isolates. Moreover, **Qurani (2019)** found that 93.3% of *E. coli* isolates recovered from broiler chickens were positive for tetA (A). Also, **Guerra et al. (2006)** found that the most frequent resistance genes in *E. coli* were tetA (86%). Lower prevalence were recorded by **Guerra et al. (2003);** 66% for tetA and 42% for tetB, **Younis et al. (2017);** tetA gene as 60%, **Momtaz et al. (2012);** 52.6% for both of tetA and tetB genes. On the contrary, much lower prevalences were recorded by **Amer et al. (2018)** who tested 20 *E. coli* isolates and detected the resistance tetA, and tetB genes as 40 and 55% of isolates, respectively. Also**, Radwan et al. (2016**) found tetA gene in 35.7% of isolates while **Adelowo et al. (2014)** recorded the resistance genes tetA and tetB in 21% and 17% in *E. coli* isolates, respectively. Moreover, **Glenn et al. (2012)** detected this gene in *E. coli* isolates from broilers. Regarding the obtained results of qnrA which was detected in all tested isolates (100%), they were higher than those obtained by **Momtaz et al. (2012)** who investigated the distribution of resistance genes in *E. coli* isolates from slaughtered commercial chickens in Iran by PCR and recorded the presence of qnrA antibiotic-resistance gene in 36.8% of the *E. coli* isolates. Also, **El-Seedy et al. (2019**) found that 18 % of isolates harbored qnrA gene. On the other hand, the obtained results of aminoglycosides resistance encoding gene (*aac(6')*-Ib-cr); which is not detected in any tested isolate (0%), were the same of those obtained by **Radwan et al. (2016)** who couldn't detect aacC gene in any isolates. On contrary, **Coque et al. (2008)** recorded that majority of plasmids enclosed *aac(6')*-Ib-cr gene. Also, **Guerra et al. (2003)** recorded aadA1-like; for streptomycin resistance, and aac(3)-IV; for gentamicin resistance, in 61 and 60% of isolates, respectively. While, **Guerra et al. (2006)** found that the most frequent resistance genes in *E. coli* were aac3-IV and aac3-II (50% for each) and aadA1- like (66%). Also, **Amer et al. (2018)** tested 20 *E. coli* isolates and detected resistance genes aac3-IV and aadA1 in 60 and 45% of isolates, respectively, while, **Abd El Tawab et al. (2015)** detected aadB resistance genes (gentamicin resistance gene) in 26% of *E. coli* isolates. Not all APECs are equally virulent. Highly pathogenic APECs generally cause primary infections while less pathogenic strains only cause poultry disease under severe stress conditions including other diseases or environmental stresses leading to compromised host immunity then secondary infections can occur **(Dho-Moulin and Fairbrother 1999)**. Virulence in APEC is caused by virulence genes present either in the chromosome or on the plasmids **(Ginns et al. 2000; Dozois et al. 2003).** Multiple varieties of virulenceassociated genes exist and are associated with colibacillosis. Plasmids have an important role in *E. coli* virulence **(Kovudzhiiski et al. 1982).** Several virulence-encoded genes were harbored on plasmids of APEC **(Kelly et al. 2009). Tivendale et al. (2004)** reported that highly virulent *E. coli* strains carried at least 4 virulence encoded genes on their largest plasmids. These large plasmids have an important role in chickens' tissue invasion via the air sac **(Yahia 2014).** The strains losing their plasmid revealed a low

virulence toward chickens **(Sekizaki et al. 1989).** The frequency of different APEC virulence-associated genes has been studied, but knowledge about the frequencies of combined virulence genes and patterns of virulence gene accumulation in APEC strains is limited, which decreases the understanding the effect of APEC on pathogenicity **(Dziva and Stevens 2008).** Various studies have highlighted the ability of using some virulence-associated genes for identifying APEC strains. They have attempted to detect a common scheme for identification allowing better identification of APEC strains than serotyping. These methods are mainly based on genotyping using PCR methods for the detection of virulence genes carried on colicin V (ColV) plasmids **(Yahia 2014).** The genotyping methods allow more identification of APEC isolates with higher reliability than the classical serotyping methods used in veterinary labs **(Schouler et al. 2012).** More than 90% of the total APEC examined possessed iss, iutA, hlyF, and ompT indicating that the Egyptian APECs, resemble their United States counterparts, harbor plasmid pathogenicity islands (PAIs**) (Hussein et al. 2013).** In the current study, PCR was applied on 10 MDR *E. coli* isolates to detect 5 virulencea ssociated genes including iron transport encoding gene (iutA), avian haemolysin gene (hly), enterotoxin encoding genes including heat-stable enterotoxin (sta) and heat-labile enterotoxin (lt) and entero-aggregative toxin encoding gene (arginine succinyl transferase A; astA). The results represented in tables (5& 6) and figs. (4-8) showed that all the tested isolates (100%) harbored both iutA and astA genes meanwhile no isolates (0%) harbored hly, sta, and lt genes. A putative avian haemolysin gene (hlyF) and an aerobactin siderophore receptor gene (iutA); contributed to iron uptake, were studied by **Williams and Warner (1980)** and **Morales et al. (2004).** Also, **Ngeleka et al. (1996)** investigated for the presence of DNA sequences related to aerobactin synthesis (iuc) and transport (iut) as well as hemolysin operon hly. Moreover, **Johnson et al. (2008)** described and named a minimum number of genes could be used to identify an APEC strain of which iutA and hlyF genes were included. Also, **Radwan et al. (2014)** screened 40 *E. coli* strains by a pentaplex PCR recovered from broilers with colibacillosis for the presence of some virulence-associated genes including iutA and hlyF. APEC strains survive and grow in environments with low iron availability, mainly inside the host, due to the expression of iron acquisition systems; such as aerobactin, **(Dho and Lafont 1984)** which are concerned with virulence in chickens **(Vandekerchove 2004).** Aerobactin is a siderophore produced mainly by most APEC while absent in most commensal *E. coli* **(Dozois et al. 1992** and **Delicato et al. 2003).** The gene encoding aerobactin receptor is called iutA (iron uptake system). The iutA protein is a multifunctional receptor involved in the binding and internalization of the bacteriocin Cloacin DF13 and the bacteriophage 74 **(De Graaf et al. 1969).** The virulence-associated gene iutA was more significantly detected in colibacillosis-derived *E. coli* isolates than faecal isolates recovered from healthy chickens (**Delicato et al. 2003).** Regarding the obtained results of iutA which was detected in all tested isolates (100%), they were supported by those of **Trampel et al. (2007)** and **Abd El Tawab et al. (2014)** who detected iutA virulence associated gene in all examined *E. coli* isolates. Also, **Ngeleka et al. (1996)** found that all *E. coli* isolates expressed 2-5 iron-regulated outer membrane proteins and possessed DNA sequences homologous to iuc/iut. Meanwhile, **Hussein et al. (2013)** reported that more than 90% of the total APEC examined contained iutA and other genes including iss, iroN, hylF and ompT indicating that the Egyptian strains of APEC resembled their counterparts from the United States in harboring plasmid pathogenicity islands (PAIs). Lower prevalences were reported by many authors such as **Moon et al. (2006)** and **Won et al. (2009)** who recorded iutA gene in 50% of the examined *E. coli* isolates while Rocha et al. (2008) recorded iutA genes as 45.9%. Many lower prevalences were recorded by **Radwan et al. (2014)** who recorded that 5% of the examined *E. coli* isolates harbored iutA gene by only isolates. Also, **Dai et al. (2010)** analyzed APEC isolates by PCR and found that some strains harbored iutA gene. Regarding the obtained results of astA which was detected in all tested isolates (100%), they were supported by those of **Kwon et al. (2008)** who studied by PCR 120 *E. coli* isolates from chickens and hatchery for detection of many genes to evaluate the epidemiological prevalence of APEC. The astA gene was presented as 56%. On contrary, **Paixão et al. (2016)** found that astA gene was less prevalent in APEC isolates. Also, Moon et al. (2006) and **Won et al. (2009)** recorded astA gene as 17% in *E. coli* strains. Moreover, **Paixão et al. (2016)** checked *E. coli* by using multiplex-PCR APEC to detect presence of 11 virulence genes and reported that astA gene was less prevalent in APEC isolates. On the other hand, the obtained results of haemolysin operon (hly) which was not detected in any tested isolate were the same of those obtained by Ngeleka et al. (1996) who didn't found hly gene in the examined

E. coli isolates. Meanwhile, **Dai et al. (2010)** analyzed by PCR APEC isolates and found that some strains were negative for hlyA gene. On the other hand, high prevalences of hly gene were recorded by **Jeong et al. (2012)** who studied 101APEC isolates from diseased chickens and characterized the virulence genes including hlyF and recorded that hlyF gene was highly prevalent as 87.1%. Also, **Hussein et al. (2013)** reported that more than 90% of the total APEC examined contained hylF. Moreover, **Radwan et al. (2014**) recorded a high prevalence of hlyF genes (80%) in the tested isolates.

The obtained results of enterotoxin encoding genes; including heat-stable enterotoxin (sta) and heat-labile enterotoxin (lt) which were not detected in any tested isolate, were the same of those obtained by **Jeong et al. (2012)** who studied 101 APEC isolates from diseased chickens and characterized the virulence genes concerning enterotoxins including st and lt and recorded that all isolates were negative for all enterotoxin encoding genes.

5. Conclusion

Colibacillosis is one of the most important diseases of chickens, resulting in significant economic losses as well as high morbidity and mortality. Presence of multidrug resistance pathogens occurred due to the misuse of the antibiotics and is considered a great problem. The in-vitro antimicrobial susceptibility testing revealed that *E. coli* isolates showed high sensitivity to colistin sulphate only meanwhile high resistances were recorded against other antimicrobials. All *E. coli* isolates were MDR. PCR results showed that all the tested isolates harbored qnrA, tetA, iutA and astA genes meanwhile *aac(6')*-Ib-cr, hly, sta and lt genes were not detected in any isolate.

6. Authors contribution

All authors contributed equally in the planning of the study, drafting the manuscript. All of them approved the final version of the article.

7. Conflict of interest

The authors declared there is no conflict of interest.

8. References

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