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Original Research Article

Antimicrobial patterns of Avian Pathogenic *Escherichia coli* isolated from broiler chickens

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ABSTRACT

E. coli infections in avian species are an economic threat to the poultry industry worldwide. The spread of MDR bacteria has been recognized as an increasing problem in the veterinary and medical fields. The current study aimed to investigate the phenotypic and genotypic antimicrobial patterns of avian Pathogenic *E. coli* isolated from broiler chickens. Results of *in-vitro* antimicrobial susceptibility testing showed that *E. coli* isolates were more sensitive to imipenem only (72.4%). On the other hand, they were highly resistant to most of used antimicrobials including ciprofloxacin (95.9%), amikacin (94.9%), cefotaxime sodium (92.9%), gentamicin (89.9%), cefotriaxone (89.9%), topramycin (87.8%), sulphamethoxazole/trimethoprime (85.7%), ceftazidim (84.7%). Also, they were resistant to cefoprazone (79.9%), doxycycline (72.4%) and amoxycillin/clavulinic acid (69.4%). All the tested isolates of *E. coli* (100%) were multi drug resistant (MDR). PCR was applied on 15 MDR *E. coli* isolates to determine 4 genes responsible for antibiotic resistance included *amp*C, bla_{CTX}, bla_{SHV} and *tet*A (A). The results revealed that that *amp*C and bla_{CTX} genes were the most prevalent found in all isolates (100%) while *tet*A (A) and bla_{SHV} genes were harbored in 14 isolates (93.3%).

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Introduction

Avian Pathogenic *Escherichia coli* (*APEC*) causing avian colibacillosis which is an infectious disease of birds and considered one of the principal causes of morbidity and mortality and resulting in heavy economic losses for the poultry industry **(Ewers *et al.*, 2004 and Paixao *et al.*, 2016)**.

Colibacillosis in poultry is characterized by septicemia in its acute form, with considerable death rates, while sub-acute forms are often characterized by pericarditis, airsacculitis, and perihepatitis with characteristic fibrous lesions **(Hancock *et al.*, 2008; Huja *et al.*, 2015** and **Younis *et al.*, 2017)**.

Primary infection is most commonly via the respiratory tract and is usually secondary to a mycoplasma or viral infection **(Ramirez *et al.*, 2009)**. The disease inducing potential of these isolates has been explained by the natural event of specific virulence factors. Many virulence factors have been associated with *APEC* strains, although their role in the pathogenesis is not well known **(Mellata *et al*., 2003)**.

Although antimicrobials are valuable tools to treat clinical disease and to maintain healthy and productive birds, 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)**. Food animals and their production environments are reservoirs of both resistant bacteria and resistance genes that could be transferred to humans either by direct contact between animals and humans or indirectly via the food production chain **(WHO, 2011)**; or as a result of the spread of animal waste on land **(Heuer and Smalla, 2007)**. Therefore, the appropriate antibiotic should better be selected on the basis of its sensitivity which could be detected by laboratory examination.

The purpose of this study was to investigate the phenotypic and genotypic antimicrobial patterns of avian Pathogenic *E. coli* isolated from broiler chickens.

MATERIAL AND METHODS

2.1. The tested *E. coli* isolates.

Ninety eight *E. coli* isolates were recovered from 297 broiler chickens of different ages (3-5 weeks) suffering from respiratory manifestations from different farms in El-Fayoum Governorate. 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, TSI, indole production, citrate utilization and motility test. Moreover, the appropriate API kit (API20E, Oxoid) was used for complete identification according to the manufacturer's instruction.

2.2. Antimicrobial susceptibility testing.

All *E. coli* isolates were tested for their antimicrobial susceptibility to 12 different antimicrobial discs. The antibiotics used were amoxycillin/clavulinic acid (30µg), ciprofloxacin (5 µg), gentamicin (10 µg), topramycin (10 µg), cefotaxime sodium (30µg), amikacin (30 µg), doxycycline HCl (30µg), cefoprazone (75 µg), imipenem (10 µg), cefotriaxone (5 µg), ceftazidim (30 µg), sulphamethoxazole/trimethoprime (25µg) (Oxoid, Basing Stoke, UK). Antimicrobial susceptibility testing was performed using disc diffusion method on Muller Hinton agar according to the 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) **(Chandran *et al*., 2008)**.

2.3. Polymerase chain reaction.

PCR was applied on 15 MDR *E. coli* isolates for detection of 4 resistance genes including *amp*C, *tet*A(A) , bla_{SHV} and bla_{CTX} genes **(Table 1)**.

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

Gene		Primer Sequence5'-3'	Amplified product	Reference
***amp*C**	*F*	*TTCTATCAAMACTGGCARCC*	550 bp	**Srinivasan *et al*. (2005)**
	R	*CCYTTTTATGTACCCAYGA*		
***tet*A(A)**	*F*	*GGTTCACTCGAACGACGTCA*	576 bp	**Randall *et al.* (2004)**
	R	*CTGTCCGACAAGTTGCATGA*		
***bla*$_{SHV}$**	*F*	*AGGATTGACTGCCTTTTTG*	392 bp	**Colom *et al*. (2003)**
	R	*ATTTGCTGATTTCGCTCG*		
***bla*$_{CTX}$**	*F*	*ATG TGC AGY ACC AGT AAR GTK ATG GC*	593 bp	**Archambault *et al*. (2006)**
	R	*TGG GTR AAR TAR GTS ACC AGA AYC AGC GG*		

RESULTS

3.1. Antimicrobial susceptibility testing. Results of *in-vitro* sensitivity tests showed that *E. coli* isolates were more sensitive to imipenem only (72.4%). On the other hand, they were highly resistant to most of used antimicrobials including ciprofloxacin (95.9%), amikacin (94.9%), cefotaxime sodium (92.9%), gentamicin (89.9%), cefotriaxone (89.9%), topramycin (87.8%), sulphamethoxazole/trimethoprime (85.7%), ceftazidim (84.7%). Meanwhile they were resistant to cefoprazone (79.9%), doxycycline HCl (72.4%) and amoxycillin/clavulinic acid (69.4%) **(Table 2)**. All the tested isolates of *E. coli* showed multi drug resistance (MDR) pattern (100%) **(Fig. 1)**.

Table (2): Results of antimicrobial sensitivity testing of *E. coli* recovered from broiler chickens (*n*=98).

Antibacterial agents	Disc content (µg/disc)	Sensitive		Resistant	
		No.	%	No.	%
Sulfamethoxazole/trimethoprim (SXT)	25 µg	14	14.3	84	85.7
Imipenem (IPM)	10µg	71	72.4	27	27.6
Amoxycillin/clavulanic acid (AMC)	30 µg	30	30.6	68	69.4
Cefoprazone (CFP)	75 µg	20	20.4	78	79.6
Doxycycline (DO)	30 µg	27	27.6	71	72.4
Ceftazidim (CAZ)	30 µg	15	15.3	83	84.7
Amikacin (AK)	30 µg	5	5.1	93	94.9
Cefotaxime sodium (CTX)	30 µg	7	7.1	91	92.9
Gentamycin (CN)	10 µg	10	10.2	88	89.9
Topramycin (TOB)	100 µg	12	12.2	86	87.8
Cefotriaxone (CRO)	5 µg	10	10.2	88	89.9
Ciprofloxacin (CIP)	5 µg	4	4.1	94	95.9

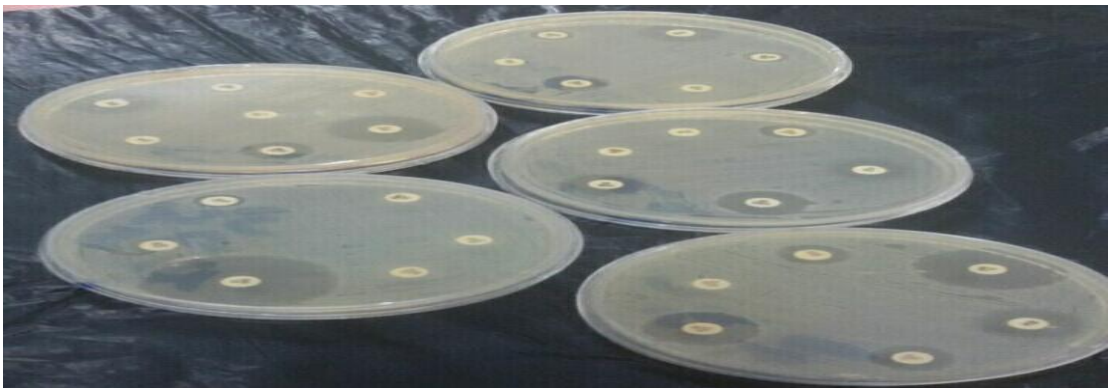

Fig. (1): Antimicrobial sensitivity testing of MDR *E. coli* recovered from broiler chickens.

3.2. Polymerase chain reaction. The results of PCR of 15 MDR *E. coli* isolates revealed that *amp*C and bla_{CTX} genes were the most prevalent found in all isolates (100%) followed by *tet*A(A) and bla_{SHV} genes which were harbored in 14 (93.3) (**Table 3& 4 and Figs. 2 a, b, c& d)**.

Table (3): Distribution of resistance genes in MDR 15 *E. coli* isolates.

Isolate No.	*amp*C	*tet*A(A)	bla_{SHV}	bla_{CTX}
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	+	+	+
8	+	+	+	+
9	+	+	+	+
10	+	+	-	+
11	+	-	+	+
12	+	+	+	+
13	+	+	+	+
14	+	+	+	+
15	+	+	+	+

Table (4): Prevalence of resistance associated genes among the examined *E. coli*.

Tested gene	*E. coli* isolates (*n*=15)			
	Positive		Positive	
	No.	%	No.	%
***amp*C**	15	100	0	0
***tet*A(A)**	14	93.3	1	6.7
***bla*$_{SHV}$**	14	93.3	1	6.7
***bla*$_{CTX}$**	15	100	0	0

% was calculated according to Number (n.) of examined isolates.

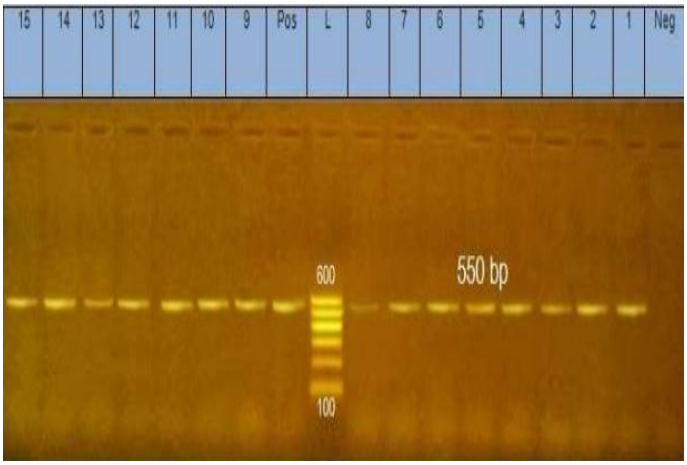

Fig. (2a): Detection of *amp*C gene in *E. coli* isolates at 550bp. All 15 *E. coli* isolates were positive for *amp*C gene. Lane L: 100-600bp DNA Ladder.
Neg.: Negative control. Pos.: Positive control.

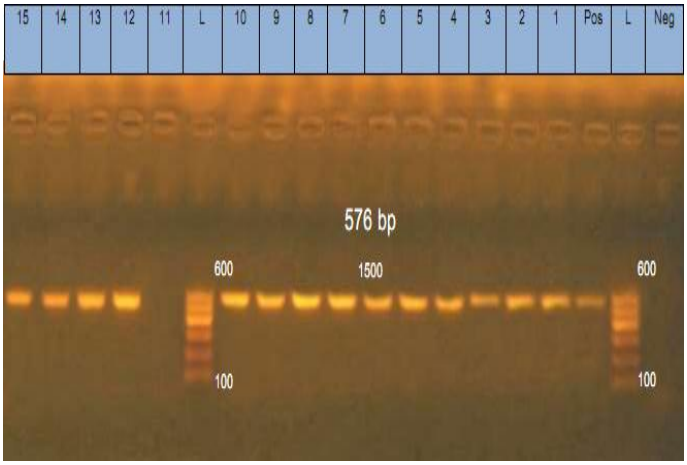

Fig. (2a): Detection of *tet*A(A)gene in *E. coli* isolates at 576bp. 14 *E. coli* isolates were positive for *tet*A(A) gene. Lane L: 100-600bp DNA Ladder.
Neg.: Negative control. Pos.: Positive control.

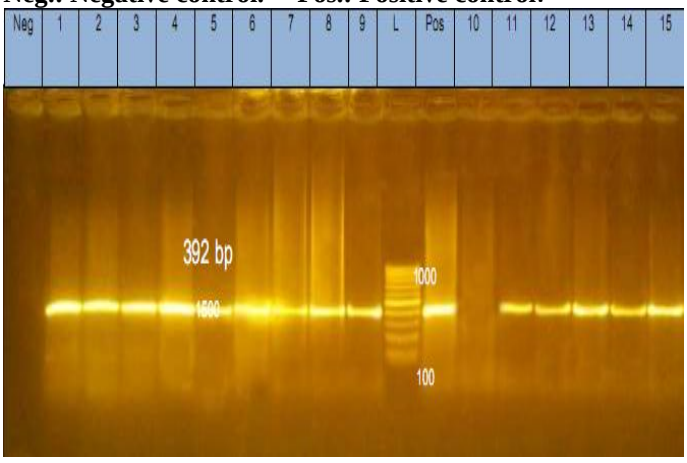

Fig. (2c): Detection of *bla*$_{SHV}$ gene in *E. coli* isolates at 392bp. 14 *E. coli* isolates were positive for *bla*$_{SHV}$ gene. Lane L: 100-1000bp DNA Ladder.
Neg.: Negative control. Pos.: Positive control.

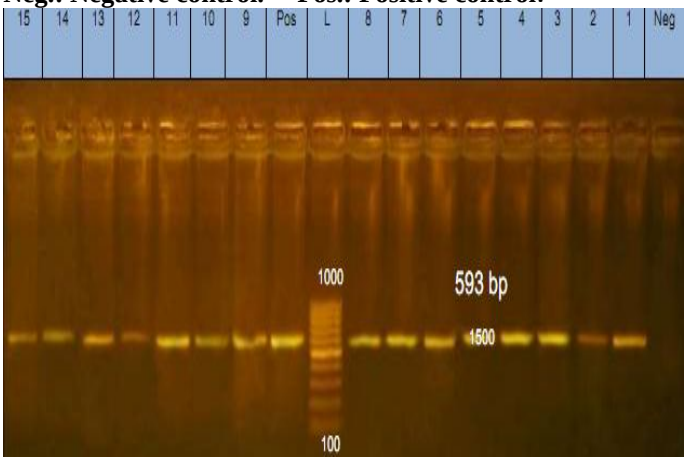

Fig. (2d): Detection of *bla*$_{CTX}$ gene in *E. coli* isolates at 593bp. All 15 *E. coli* isolates were positive for *bla*$_{CTX}$ gene. Lane L: 100-1000bp DNA Ladder.
Neg.: Negative control. Pos.: Positive control.

DISCUSSION

E. coli infections in avian species are an economic threat to the poultry industry worldwide **(Antao *et al.*, 2008)**. *APEC* isolates are associated with extraintestinal disease in chickens, turkeys, and other avian species. Colisepticaemia is the most severe manifestation of colibacillosis in poultry, which is characterized by the presence of pericarditis, perihepatitis, air sacculitis, and salpingitis. Primary infection is most commonly via the respiratory tract and is usually secondary to a mycoplasma or viral infection **(Ramirez *et al.*, 2009)**.

Antimicrobial therapy is one of the primary control for reducing both the incidence and mortality associated with avian colibacillosis therefore reducing their enormous losses in the poultry industry **(Blanco *et al.*, 1997)**. However, resistance to existing antimicrobials is widespread and of concern to poultry veterinarians **(Peighambari *et al.*, 1995)**. *In-vitro* antimicrobial susceptibility testing of veterinary pathogens can provide valuable guidance to the veterinarian in the choice of appropriate chemotherapy **(Blanco *et al.*, 1997)**. Moreover, it is very useful to detect the multidrug resistant isolates.

In the present work, all the recovered *E. coli* isolates (*n*=98) were subjected to *in-vitro* antimicrobial sensitivity tests against 12 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 antibiogram of *E. coli* isolates showed that sensitivity was observed against imipenem only (72.4%). On the contrary, high resistances were observed against most of antimicrobials used especially ciprofloxacin (95.9%), amikacin (94.9%), cefotaxime sodium (92.9%), gentamicin (89.9%), cefotriaxone (89.9%), topramycin (87.8%), sulphamethoxazole/trimethoprime (85.7%), ceftazidim (84.7%). Also, they were resistant to cefoprazone (79.9%), doxycycline HCl (72.4%) and amoxycillin/clavulinic acid (69.4%). Multidrug resistance was detected in all *E. coli* isolates (100%). These results agreed with several previous reports **(Peighambari *et al.*, 1995; Gomis *et al.*, 2001; Fard *et al.*, 2007; Hammoudi and Agaad, 2008 and Radwan *et al.*, 2014)** which have indicated increasing incidences of antibiotic-resistant *E. coli* strains isolated from chickens to several of the antibiotics frequently used in the poultry industry. Also, **Sharada *et al.* (2001)** found that no single antimicrobial drug was effective by 100% against *E. coli* isolates, which might be due to development of resistance due to indiscriminate use of antibiotics. Concerning MDR *E. coli*, the current results were supported those obtained by **Radwan *et al.* (2014)** who reported that MDR was detected in 90.4% of *E. coli* isolates. Moreover, **Blanco *et al.* (1997) ; 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.

On the other hand, the spread of MDR bacteria has been recognized as an increasing problem in the veterinary and medical fields, and mobile DNA elements, including plasmids, transposons, and integrons, facilitate the proliferation of resistance genes in bacteria **(Liebert *et al.*, 1999)**. Plasmids play an important role in virulence of *E. coli*

(Kovudzhiiski *et al.*, 1982). The R-plasmids have been extensively studied in view of the prevalence of MDR **(O'Brien *et al.*, 1984)**. Several resistance-associated genes were reported on plasmids of *E. coli* isolated from diseased poultry such as **plasmid-mediated among class C cephalosporinases (*amp*C) β-lactamases (e.g., cephamycinase [CMY] types), extended-spectrum β-lactamases (ESBLs) (e.g., *TEM*-, sulfhydryl variable [*SHV*]-, CTX-M-, oxacillin-types), and** resistance to tetracycline (e.g. *tet*A) resistance to trimethoprim (e.g. *dfrA*) **(Kelly *et al.*, 2009 and Pitout, 2012)**.

In the present work, PCR was applied on 15 MDR *E. coli* isolates to determine 4 genes responsible for antibiotic resistance included *amp*C, bla_{CTX}, bla_{SHV} and *tet*A (A). The results illustrated in **table (3& 4)** and **Figs. (2a, b, c& d) (Table 3& 4)** revealed that that *amp*C and bla_{CTX} genes were the most prevalent found in all isolates (100%) followed by *tet*A(A) and bla_{SHV} genes which were harbored in 14 (93.3%).

CONCLUSION

It was concluded that colibacillosis is one of the most important diseases of chickens, resulting in significant losses. The presence of multidrug resistance pathogens occurred due to the misuse of the antibiotics and is considered a great problem.

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