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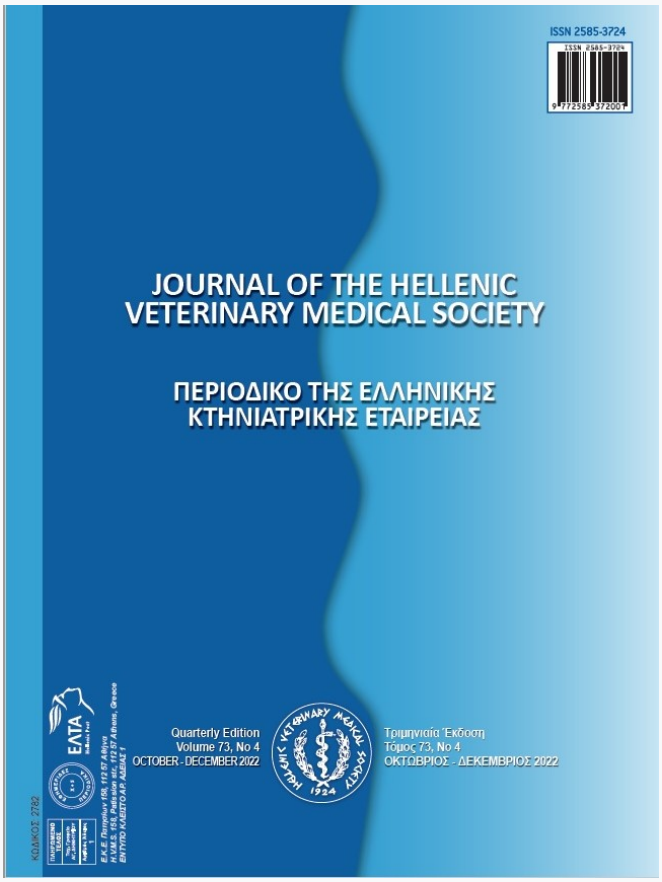

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Beta-lactam and Fluoroquinolone Resistant Extraintestinal *Escherichia coli* from Broiler Chickens and Ducks: Public Health Threat

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ABSTRACT: *Escherichia coli* (*E. coli*) is a zoonotic bacterial pathogen commonly found in human, birds and animals. The widespread use of broad-spectrum beta-lactams for poultry treatment has resulted in the production of extended spectrum β-lactamases (ESBLs) in *E. coli*. In this study, we analyzed the antimicrobial resistance pattern of *E. coli* isolates from broiler chickens and ducks with clinical colibacillosis, and investigated the presence of beta-lactam (bla_{TEM}, bla_{SHV}, bla_{ampC} and bla_{CTX-M}) and fluoroquinolone (*qnr*S and *qep*A) resistance genes using PCR.*E. coli* isolates showed a higher resistance against beta-lactams: ceftriaxone (100%), penicillin G (97.5%), cephalexin (92.5%), ampicillin (77.5%), amoxicillin/clavulanic acid (62.5%), with considerable resistance for ciprofloxacin (52.5%), nalidixic acid (45%) and levofolxacin (45%) from fluoroquinolone group. Isolates were susceptible to cefotaxime (65%) and norfolxacin (50%). All tested isolates carried bla_{TEM}, bla_{SHV} and bla_{ampC} resistance genes, and this was associated with the phenotypic resistance too. Although some isolates showed phenotypic intermediate resistance to fluoroquinolones, or resistance to only one agent from this class, the *qnr*S (83.3%) has been detected with high frequency. The bla_{CTX-M} (27.87%) and *qep*A (16.7%) showed the lowest occurrence and had been detected in isolates from broiler chickens only. The occurrence of these resistant *E. coli* serotypes in broiler chickens and ducks pose a potential threat to poultry industry, and constitutes a public health risk to human consumers, slaughterhouse and poultry packing plant workers due to dissemination of beta-lactam and fluoroquinolone resistant *E. coli* serotypes.

Keywords: Broiler chickens; Ducks;*Escherichia coli* (*E. coli*); Antimicrobial Resistance; Resistance genes.

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INTRODUCTION

E. coli is a zoonotic bacterial pathogen commonly present in human, animals and birds. Although poultry harbors a large number of normal microflora in their intestine, including *E. coli*, specific strains like avian pathogenic *E. coli* (APEC) can spread to several internal organs of the bird causing avian colibacillosis (Oh et al., 2011). APEC strains belongs to extraintestinal pathogenic *E. coli* (ExPEC), that were reported to carry similar virulence attributes as human ExPEC, and poultry and their products are considered a possible source and reservoir forExPEC dissemination in the community(Bergeron et al., 2012).

Antimicrobial resistance is recognized universally to be one of the most serious threats for public health. Resistance to β-lactams, antibacterial compounds that are massively prescribed in human and veterinary practices, is developing quickly where additional β-lactamases are being identified on a daily basis such as the recent advent of extended spectrum β-lactamases (ESBLs)(Ur Rahman et al., 2018). A large proportion of ESBL-producing bacteria obtained from clinical isolates harboring bla_{SHV}, bla_{TEM}, and bla_{CTX-M}, are known to produce hospital and community-acquired infections (Pitout et al., 2005).

Fluoroquinolones are another useful type of synthetic broad-spectrum bactericidal agents that are enormously used in poultry production, however, the extreme and careless usage of fluoroquinolones results in development of resistance in bacteria(Hopkins et al., 2005). The plasmid mediated quinolone resistance is encoded by the *qnr* genes (Tran and Jacoby, 2002), and the efflux pump that is mediated by *qep*A genes (Yamane et al., 2007). Plasmids harboring *qnr* genes, can also mediate resistance of extended-spectrum beta-lactamases like CTX-M, SHV and TEM type (Jacoby et al., 2006).

These multi-drug resistant characteristics can be transferred to humans via food chain, posing a significant threat to human health. Numerous investigations supported the idea that antimicrobial resistance may arise without selective pressure, emphasizing the critical role of antibiotic resistance genes in the emergence of multi-drug resistant bacteria (Bean et al., 2009). The present study aimed to provide an update on the antimicrobial resistance profile of *E. coli* isolates obtained from clinically affected broiler chickens and ducks with colibacillosis, and investigate the presence of ESBL and PMQR genes and the associated public health implications.

MATERIALS AND METHODS

Samples

A total of 40 pre-identified *E. coli* isolates20 from broiler chickens (8 serotyped and 12 serologically untyped) and 20 from ducks (7 serotyped and 13 serologically untyped) were used. Isolates were originated from internal organs (liver, heart blood and lung) of birds with colibacillosis, which had been collected from various poultry farms (10 broiler chicken farms and 7 duck farms) at Ismailia Governorate, Egypt.

Antimicrobial susceptibility testing

Isolates were tested against 11 antibacterial agents (Oxoid Hampshire, UK) belonged to two antimicrobial classes typically named; beta-lactams: penicillin G (P) (10 µg), ampicillin (AMP) (10 µg), amoxicillin/clavulanic acid (AMC) (30 µg), ceftriaxone (CRO) (30 µg), cephalexin (CL) (30 µg), cefotaxime (CTX) (30 µg) and ceftazidime (CAZ) (30 µg);and fluoroquinolones: ciprofloxacin (CIP) (5 µg), norfloxacin (NOR) (10 µg), levofloxacin (LEV) (5 µg) and nalidixic acid (NA) (30 µg). On Muller Hinton agar (Oxoid Hampshire, UK), antibiogram was obtained using disc diffusion method as previously described (Bauer et al., 1996). Briefly, 4-5 colonies, from pre-enriched preserved identified isolates, were inoculated in 5ml Muller Hinton broth (Oxoid Hampshire, UK) and incubated for 4-5hrs until turbidity was observed. The inoculated broth was then adjusted to a density equivalent to 0.5 McFarland standard. Sterile swabs were used to streak the bacterial suspension in different directions to wet the entire surface of labeled Muller Hinton agar plates and left for 30 minutes. Antibiotic discs were then applied on the surface of the streaked plates using sterile forceps and the antibiotic dispenser. After overnight incubation at 37ºC, the inhibition zone was measured for each antibiotic using a caliber. The size of inhibition zone was interpreted according to the prescribed guidelines of Clinical and Laboratory Standards Institute (CLSI, 2021).

Molecular detection of antibiotic resistance genes.

Detection of *E. coli* beta-lactam (bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{ampC}), and fluoroquinolone (*qnr*S and *qep*A) resistance genes were carried out using PCR according to the previously published protocols (Table 1). DNA was extracted from *E. coli* using QIAamp DNA Mini kit (Qiagen, GmbH, Germany/ Catalogue No.51304) according to the manufacturer's recommendations. The PCR reaction volume (25µl) consisted of6µl of genomic DNA, 12.5µl of 2x Master

Mix and 20 pmol of each primer (Biobasic, Canada), and the final volume completed to 25µl by DNAse RNAse free water. Positive control and negative control (DNAse free water) were included in all reactions.

Statistical analysis

Data were handled using Microsoft Office Excel, 2013 and statistical analysis was performed using SPSS software for windows version 22.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp). All data were tabulated and statistically analyzed using Chi-square test at P-value ≤0.05.

Table 1. Target genes, primer sequences, cycling conditions, specific amplicon size of *E. coli* beta-lactams (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$ and *bla*$_{ampC}$), and fluoroquinolones (*qnr*S and *qep*A) resistance genes

Target Genes	Primer sequence(5'-3')	PCR cycling condition	Length of amplified product	Reference
***bla*$_{TEM}$**	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	Initial denaturation 94°C/5 min. 35 cycles: Denaturation at 94°C/30 s Annealing at 54°C/40 s Extension at 72°C/45 s	516 bp	(Colom et al., 2003)
***bla*$_{SHV}$**	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	Initial denaturation 94°C/5 min. 35 cycles: Denaturation at 94°C/30 s Annealing at 54°C/40 s Extension at 72°C/40 s	392 bp	
***bla*$_{CTX-M}$**	ATGTGCAGYACCAGTAARGTKATGGC TGGGTRAARTARGTSACCAGAAYCAGCGG	Initial denaturation 94°C/5 min. 35 cycles: Denaturation at 94°C/30 s Annealing at 54°C/40 s Extension at 72°C/45 s	593 bp	(Archambault et al., 2006)
***bla*$_{ampC}$**	TTCTATCAAMACTGGCARCC CCYTTTTATGTACCCAYGA	Initial denaturation 94°C/5 min. 35 cycles: Denaturation at 94°C/30 s Annealing at 55°C/40 s Extension at 72°C/45 s	550 bp	(Srinivasan et al., 2005)
***qnr*S**	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	Initial denaturation 94°C/5 min. 35 cycles: Denaturation at 94°C/30 s Annealing at 55°C/40 s Extension at 72°C/40 s	417 bp	(Robicsek et al., 2006)
***qep*A**	CGTGTTGCTGGAGTTCTTC CTGCAGGTACTGCGTCATG	Initial denaturation 94°C/5 min. 35 cycles: Denaturation at 94°C/30 s Annealing at 50°C/40 s Extension at 72°C/40 s	403 bp	(Cattoir et al., 2008)

Plasmid-mediated beta-lactamase resistance genes: *bla*$_{TEM,}$ *bla*$_{SHV,}$ *bla*$_{CTX-M,}$ and *bla*$_{ampC}$. Quinolone resistance genes: *qnr*S and *qep*A.

RESULTS

Antimicrobial susceptibility testing.

Antimicrobial susceptibility testing of the forty *E. coli* isolates revealed that overall there was a very high resistance to beta-lactams: ceftriaxone (100%), penicillin G (97.5%), cephalexin (92.5%), ampicillin (77.5%) and amoxicillin/clavulanic acid (62.5%), while there was a considerable resistance to ceftazidime (52.5%), ciprofloxacin (52.5%) and levofloxacin (45%). On the other hand, isolates were susceptible to cefotaxime (65%) from β-lactam group and norfloxacin (50%) from fluoroquinolones (Table 2 and Fig.1). Statistically, there was a significant difference in the resistance of the tested isolates to various antimicrobial agents ($P< 0.05$). For each species, isolates from ducks showed a marked lower resistance to fluoroquinolones than chicken broilers, however, both of them showed a considerable susceptibility to cefotaxime despite the higher resistance to other β–lactam antimicrobial agents (Fig. 2).

There were 11 different resistance patterns among the 20 *E. coli* isolates from broiler chickens. Only one pattern was repeated ten times which was as the following: penicillin- ampicillin- amoxicillin\clavulanic acid- ceftriaxone- cephalexin- cirpofloxacin- nalidixic acid. For duck isolates, 14 different patterns were observed and one pattern was repeated 7 times which was: penicillin- ampicillin- amoxicillin\clavulanic acid- ceftriaxone- cephalexin (Fig. 3).

Table 2. Antimicrobial resistance of *E. coli* isolates from broiler chickens and ducks

Antibiotic class	Tested antibiotic	Interpretation					
		Resistant		Intermediate		Sensitive	
		No.	%	No.	%	No.	%
Beta-lactams	Penicillin G	39	97.5	1	2.5	-	-
	Ampicillin	31	77.5	1	2.5	8	20
	Amoxicillin/clavulanic acid	25	62.5	3	7.5	12	30
	Ceftriaxone	40	100	-	-	-	-
	Cephalexin	37	92.5	3	7.5	-	-
	Cefotaxime	14	35	-	-	26	65
	Ceftazidime	21	52.5	4	10	15	37.5
Fluoroquinolones	Ciprofloxacin	21	52.5	4	10	15	37.5
	Norfloxacin	13	32.5	7	17.5	20	50
	Nalidixic acid	18	45	10	25	12	30
	Levofloxacin	18	45	6	15	16	40
P value		<0.0001		<0.0001		<0.0001	

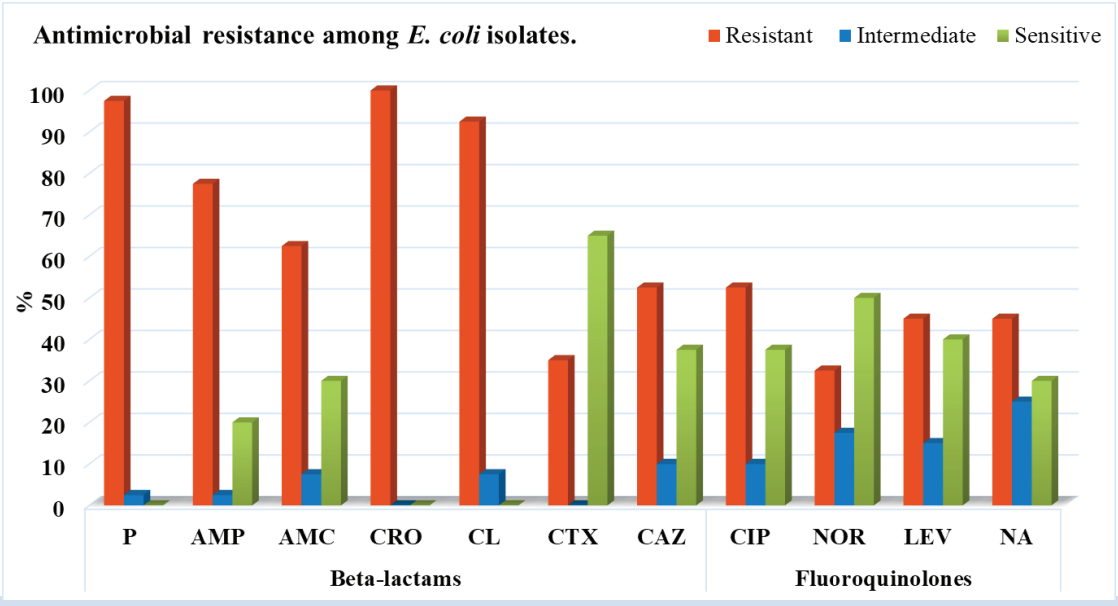

Fig. 1. Overall antimicrobial resistance among E. coli isolates from broiler chickens and ducks

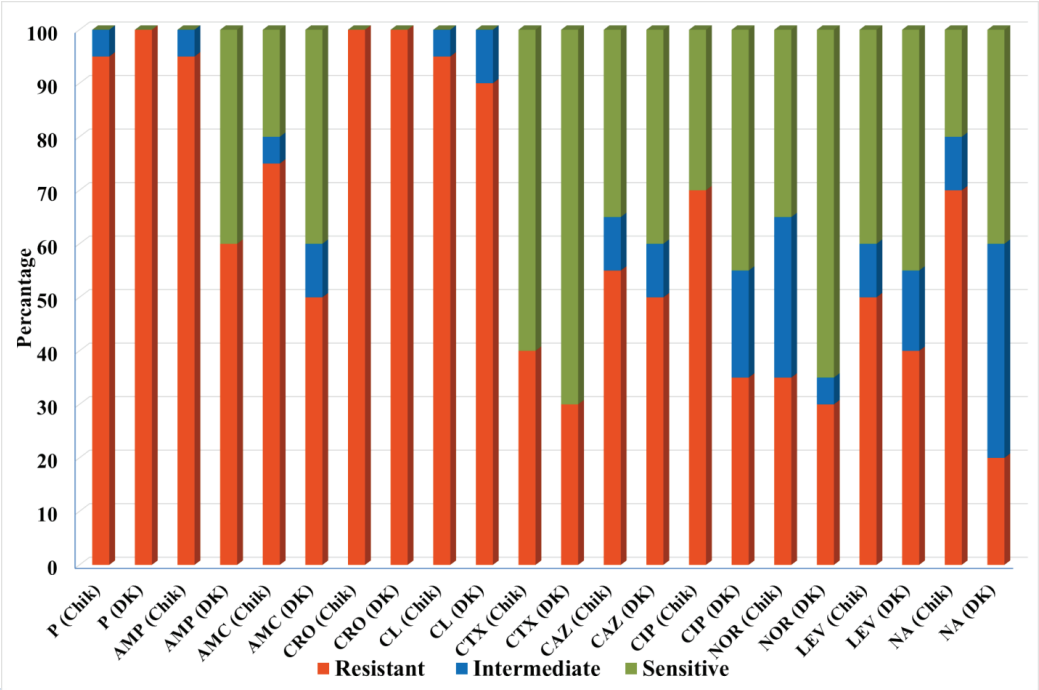

Fig. 2. Antimicrobial sensitivity testing of *E. coli* isolates from broiler chickens and ducks

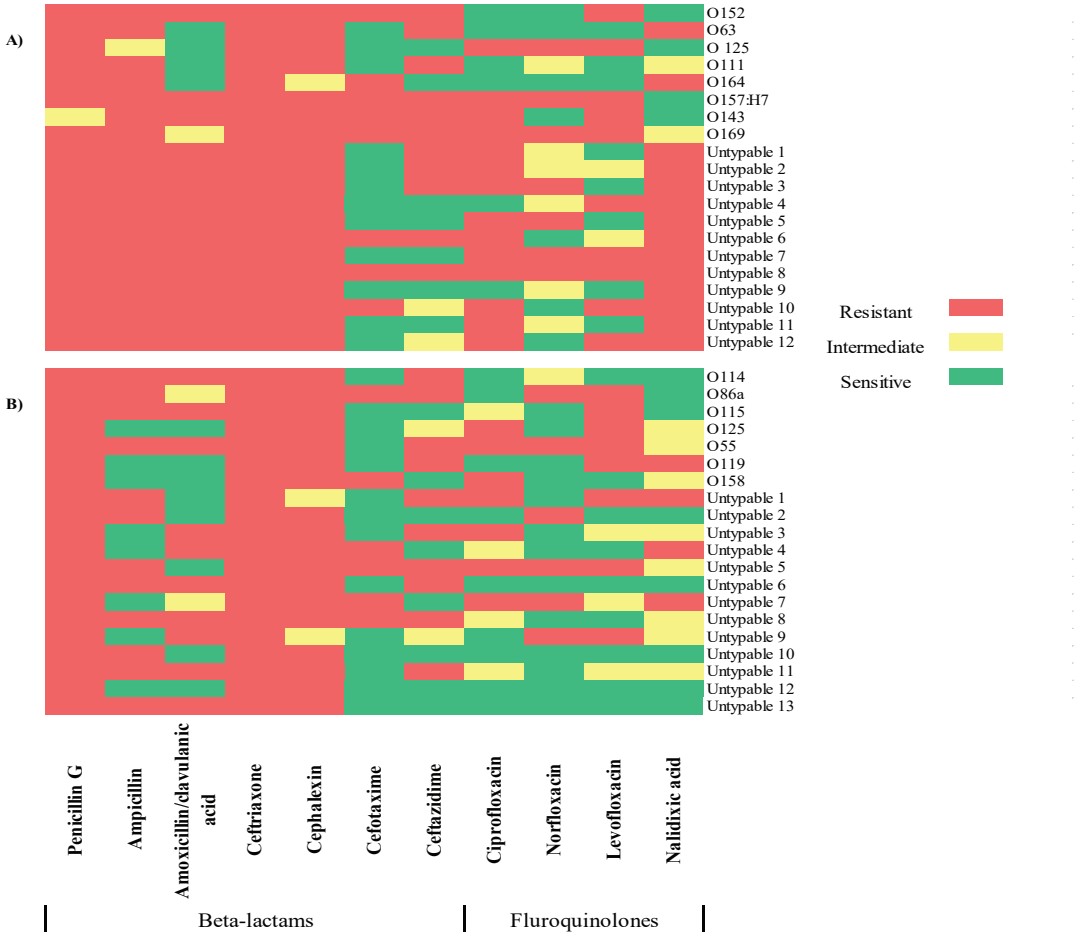

Fig. 3. Heat map showing individual antimicrobial resistance pattern of *E. coli* isolates from broiler chickens (A) and ducks (B). Red blocks represents resistance, yellow blocks represents intermediate, and green blocks represents sensitivity to the antimicrobial agents

Detection ofbeta-lactam and fluoroquinolone resistance genes in *E. coli* isolates.

Screening for the presence of genes encoding beta-lactams (bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{ampC}) and fluoroquinolone resistance (*qnr*S and *qep*A) was carried out using conventional PCR for all serotyped and three untyped isolates. Other isolates, unfortunately, couldn't be revived due to the second COVID-19 lock down.Overall, the bla_{TEM}, bla_{SHV} and bla_{ampC} genes were detected in all isolates (100% for each), however, bla_{CTX-M} gene was detected in five isolates (27.8%). For fluoroquinolone resistance genes, *qnr*S was detected in 15 serotypes (83.3%), while *qep*A was detected in only three serotypes (16.7%) (Table 3). The bla_{CTX-M} and *qep*A were detected in samples from broiler chickens only (Table 4). Statistically, there was a significant difference in the prevalence of beta-lactam and fluoroquinolone resistance genes among the tested serotypes ($P< 0.05$).

Relationship between in-vitro beta-lactam and fluoroquinolone phenotypic drug-resistance and the existence of their resistance genes.

E. coli isolates that were phenotypically resistant to beta-lactam antibiotics, successfully amplified the corresponding beta-lactam resistance genes, however, for fluoroquinolones (83.3%) of the tested isolates amplified the *qnr*S gene but only three (16.7%) isolates amplified *qep*A gene (Table 4).

Table 3. Antibiotic resistance genes in *E. coli* serotypes from broiler chickens and ducks

Target genes		No.	%	*P* value
beta-lactam resistance genes	bla_{TEM}	18	100	0.03
	bla_{SHV}	18	100	
	bla_{CTX-M}	5	27.8	
	bla_{ampC}	18	100	
Fluoroquinolone resistance genes	*qnr*S	15	83.3	0.03
	*qep*A	3	16.7	

Table 4. Relationship between beta-lactam and fluoroquinolone phenotypic drug-resistance pattern and the occurrence of resistance genes

Isolate source	*E. coli* serotype	Sample type	Phenotypic resistance	Resistance genes
Broiler chickens	O152	Liver	**R** (P, AMP, AMC, CRO, CL, CTX, and CAZ, LEV)	bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{ampC}
	O63	Liver	**R** (P, AMP, CRO, CL, CAZ, and NA)	bla_{TEM}, bla_{SHV}, and bla_{ampC}
	O125	Heart blood	**R** (P, CRO, CL,CIP, NOR, and LEV), **I** (AMP)	bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{ampC}
	O111	Liver	**R** (P, AMP, CRO, CL, and CAZ), **I** (NOR and NA)	bla_{TEM}, bla_{SHV}, $bla_{CTX-M,}$ bla_{ampC}, *qnr*S and *qep*A
	O164	Liver	**R** (P, AMP, CRO, CTX, and NA), **I** (CL)	bla_{TEM}, bla_{SHV}, $bla_{CTX-M,}$ bla_{ampC}, *qnr*S and *qep*A
	O157:H7	Liver	**R** (P, AMP, AMC, CRO, CL, CTX, CAZ, CIP, NOR, and LEV)	$bla_{TEM,}$ $bla_{SHV,}$ bla_{ampC}, and *qnr*S
	O143	Liver	**R** (AMP, AMC, CRO, CL, CTX, CAZ, CIP, and LEV), **I** (P)	$bla_{TEM,}$ $bla_{SHV,}$ $bla_{CTX-M,}$ bla_{ampC} and *qnr*S
	O169	Liver	**R** (P, AMP, CRO, CL, CTX, CAZ, CIP, NOR, and LEV), **I** (NA and AMC)	$bla_{TEM,}$ $bla_{SHV,}$ bla_{ampC}, *qnr*S and *qep*A
	Untyped No. 1	Liver	**R** (P, AMP, AMC, CRO, CL, CAZ, CIP, and NA), **I** (NOR)	$bla_{TEM,}$ $bla_{SHV,}$ $bla_{ampC}$and*qnr*S
	Untyped No. 2	Liver	**R** (P, AMP, AMC, CRO, CL, CAZ, CIP, and NA), **I** (NOR and LEV)	$bla_{TEM,}$ $bla_{SHV,}$ $bla_{ampC}$and*qnr*S
	Untyped No. 3	Liver	**R** (P, AMP, AMC, CRO, CL, CAZ, CIP, NOR, and NA)	$bla_{TEM,}$ $bla_{SHV,}$ $bla_{ampC}$and*qnr*S

	O114	Liver	**R** (P, AMP, AMC, CRO, CL, CAZ), **I** (NOR)	$bla_{TEM,}$ $bla_{SHV,}$ bla_{ampC} and *qnr*S
	O86a	Liver	**R** (P, AMP, CRO, CL, NOR, CTX, CAZ, NOR, and LEV), **I** (AMC)	$bla_{TEM,}$ $bla_{SHV,}$ bla_{ampC} and *qnr*S
	O115	Liver	**R** (P, AMP, AMC, CRO, CL, and LEV), **I** (CIP)	$bla_{TEM,}$ $bla_{SHV,}$ bla_{ampC} and *qnr*S
Ducks	O125	Liver	**R** (P, CRO, CL, CIP, and LEV), **I** (NA)	$bla_{TEM,}$ $bla_{SHV,}$ bla_{ampC} and *qnr*S
	O55	Liver	**R** (P, AMP, AMC, CRO, CL, CAZ, CIP, NOR, and LEV), **I** (NA)	$bla_{TEM,}$ $bla_{SHV,}$ bla_{ampC} and *qnr*S
	O119	Heart blood	**R** (P, CRO, CL, CAZ, LEV, and NA)	$bla_{TEM,}$ $bla_{SHV,}$ bla_{ampC} and *qnr*S
	O158	Heart blood	**R** (P, CRO, CL, CTX, CIP), **I** (NA)	$bla_{TEM,}$ $bla_{SHV,}$ bla_{ampC} and *qnr*S

R: resistant,I: intermediate, P: Penicillin, AMP: Ampicillin, AMC: Amoxicillin/Clavulanic Acid, CRO: Ceftriaxone, CL: Cephalexin, CAZ: Ceftazidime, CIP: Ciprofloxacin, NOR: Norfloxacin, LEV: Levofloxacin, NA: Nalidixic acid.

DISCUSSION

Avian colibacillosis is an extraintestinal infection that gives rise to diverse infections in various organs that may end in death of the bird. This condition leads to high economic losses in commercial poultry industry sector, especially in intensive breeding, due to excessive morbidities, mortalities, slaughterhouse condemnation, and the diminished productivity of diseased birds (Dho-Moulin and Fairbrother, 1999). Previous studies proved the phenotypic and genotypic similarities between avian pathogenic *E. coli*, urinary tract infection in humans (Johnson et al., 2008) and newborn meningitis (Tivendale et al., 2010). This data support the zoonotic potential of birds as a reservoir for pathogenic *E. coli* and necessitates continuous surveillance among avian species.

In the present study, overall *E. coli* isolates showed a very high level of resistance against beta-lactam antibiotics, namely: ceftriaxone (100%), penicillin G (97.5%), cephalexin (92.5%), ampicillin (77.5%) and amoxicillin/clavulanic acid (62.5); however, a relatively lower resistance was observed towards fluoroquinolones;nalidixic acid (45%), levofolxacin (45%) and norfloxacin (32.5%) (Table 2, Fig. 1, 2). These findings were similar to results reported from other Egyptian studies where high resistance against penicillin G (95%), ampicillin (95%) and amoxicillin/clavulanic acid (75%) was observed in *E. coli* isolates associated with high mortality in poultry flocks (Eid and Erfan, 2013). Also, a lower resistance to norfloxacin (36.9%) of *E. coli* from broiler chickens was reported in another study (Younis et al., 2017). However, in Algeria, high resistance of *E. coli* isolates from broiler chickens with colibacillosis against cephalexin and ceftriaxone (95%) was noted (Halfaoui et al., 2017). Also, in Nepal, *E. coli* isolates obtained from commercial poultry were resistant to ciprofloxacin (40%) (Khanal, 2017).

The increased antibiotic use in the poultry sector, due to increased market demand, may have had a role in the development and spread of multi-drug resistant (MDR) *E. coli* which might pose a substantial public health issue (Laxminarayan and Chaudhury, 2016). Since the release of third-generation cephalosporins, a considerable number of ESBL and *Amp*C-lactamase producers in gram-negative bacteria, notably in Enterobacteriaceae such as *E. coli*, have been reported (Seo et al., 2018). This finding clearly appeared in our data, where a very high level of resistance against such agents have been reported in *E. coli* isolates from both broiler chickens and duck (Table 2, Fig. 1, 2 and 3). Third-generation cephalosporins are crucial in human medicine as they are used for the treatment of various conditions such as streptococcal endocarditis, sexually transmitted diseases, pseudomonas pneumonia, and gram negative meningitis and osteomyelitis owing to their ability to cross blood brain barrier (Arumugham and Cascella, 2021).However, if the resistance become widely distributed in humans, it may be responsible for treatment failures.

All tested strains in the present study, except four isolates from ducks, were resistant to at least one agent from each class of the tested antimicrobials. Interestingly, the O157:H7 strain isolated from broiler chicken liver was found resistant to all beta-lactam antibiotics and fluoroquinolones except the nalidixic acid (Fig. 3). Although resistance of *E. coli* to two or more antibiotic classes is observed frequently in both human and veterinary medicine (Sáenz et al., 2004), it represents a potential public health concern

by influencing the effective therapeutic choices, as well as, increasing the possibility for dissemination of infection in the population (Smith et al., 2007). This widely disseminated resistance might be due to frequent use of antibiotics in drinking water and feed in poultry production for preventive and treatment purposes(Guenther et al., 2011).

ESBL or AmpC-lactamase are very important resistant genes in *E. coli* as they make the bacteria resistant to cephalosporins, the third-generation antimicrobials, which has designated by the World Health Organization as "critically important in human medicine" due to their great importance in treating bacterial infections caused by *Campylobacter*, *Salmonella*, and *E. coli*(WHO, 2015). In the current study, beta-lactam resistance genes, bla_{TEM}, bla_{SHV} and bla_{ampC}, had been verified in all of the tested isolates (100%) (Table 3). This indicates that it was responsible for the beta-lactam phenotypic resistance pattern of the isolates. Similarly, high occurrence (100%) of bla_{TEM} gene in *E. coli* isolates was previously demonstrated (Abd El Tawab et al., 2015).Also, another study confirmed the presence of plasmid-mediated *amp*C genes in all extended-spectrum cephalosporin-resistant *E. coli* isolates from poultry and human clinical samples in Romania (Maciuca et al., 2015). However, variable occurrences were reported in other studies from different sources; bla_{TEM} gene (25.4%) in *E. coli* isolates from poultry feces in Algeria and France (Chabou et al., 2018), and bla_{SHV} gene (67.2%) in *E. coli* isolates from broiler chicken meat in Italy (Ghodousi et al., 2015). The present work demonstrated that $bla_{\text{CTX-M}}$ gene had a lower occurrence (27.7%) and has been detected in isolates from chicken only. This was in agreement with previous studies on *E. coli* isolates from poultry elsewhere (Maciuca et al., 2015; Bhave, 2019).

The careless use of beta-lactams not only resulted in the development of resistance against beta-lactams, but also to non-beta-lactams in *E. coli* since plasmid-mediated beta-lactamases are the main element for multidrug resistance genes (Bortolaia et al., 2010). Among the 18 β-lactamase producing *E. coli* molecularly tested isolates, *qnrS* had been detected in 15 of them (83.3%) (Table 3). Similarly, a high occurrence of *qnr*S gene (72.22%) has been confirmed in *E. coli* isolates from healthy broiler chickens (Mahmud et al., 2018). The simultaneous occurrence of β-lactamase and fluoroquinolone resistance genes, in the present study, suggests an association between the presences of these two types of resistance genes. This was in agreement with a previous study (Seo and Lee, 2019). The presence of such resistance genes are of especial interest to other microbial community in the birds and the surrounding environment. In a previous study, *qnrS* plasmids were found to be transferable among *E. coli* isolates (Briales et al., 2012). These findings suggest the dissemination and the extensive horizontal plasmid transfer of β-lactamase and PMQR genes, which might assist in spreading of these genes to humans via food chain. Interestingly, although the high occurrence of fluoroquinolone *qnr*S resistance gene (15/18, 83.3%) in the present study, some of these isolates showed only an intermediate phenotypic resistance to fluoroquinolones (Table 4, Fig. 3). Thus necessitates taking into consideration the intermediate phenotypic resistance during antimicrobial sensitivity testing.

The occurrence of *qep*A gene was the lowest (3/18, 16.7%) and had been detected in isolates from chicken only. In another Egyptian study, increased existence of *qep*A (64%)was reported in *E. coli* isolates implicated in high mortality in poultry flocks (Eid and Erfan, 2013).However, a very low occurrence of *qep*A gene (1.3%)was reported in *E. coli* isolates from different sources in China (Chen et al., 2012), while none of the APEC strains carried *qep*A gene in Japan (Kawanishi et al., 2013).According to previous reports, the incidence of plasmid-mediated quinolone resistance (PMQR) genes in chicken varies between 2.0 and 3.2 percent in Korea and Europe(Kuo H.-C., 2009; Kim et al., 2013).The frequent and inappropriate use of fluoroquinolones and third-generation cephalosporins has resulted in PMQR-producing *E. coli* with high liability for fluoroquinolone resistance, as well as the advent of ESBL and AmpC-lactamase-producing *E. coli* (Seo and Lee, 2019).It's critical to emphasize that handling of infected birds with these resistant strains may pose a potential zoonotic hazard to poultry slaughterhouse workers and packing plants, as well as, to the surrounding environment and human consumers.

CONCLUSION

In conclusion, this study proved that the occurrence of bla_{TEM}, bla_{SHV}, bla_{ampC} and *qnr*S genes among *E. coli* isolates is compatible with the important role for these resistance attributes in the development of beta-lactams and fluoroquinolones resistance. Special consideration should be giv-

en to isolates and serotypes that show intermediate phenotypic resistance pattern as they may carry resistance genes similarly as the phenotypically resistant isolates. Broiler chickens and ducks may be an important source for PMQR and ESBL-producing *E. coli* that may pose a public health threat to slaughterhouse and poultry packing processing plant workers and human consumers.

CONFLICT OF INTEREST

The authors have no competing interests.

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