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# CHARACTERIZATION OF MULTIDRUG-RESISTANT *E. COLI* ISOLATES RECOVERED FROM BROILERS WITH SPECIAL REFERENCE TO QUINOLONE RESISTANCE

By

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## ABSTRACT

The misuse of antibacterial therapeutics in the veterinary fields may result in the emergence and spread of multidrug resistant (MDR) pathogens. This became a worldwide concern due to not only its drawbacks on the animal production economy but also the possible transmission of such strains to humans leading to untreatable diseases. In the present study, 40 *E. coli* isolates, recovered from broiler flocks in Egypt from 2019 to 2021, were serotyped and tested against different antibacterial therapeutic agents. Meanwhile, selected MDR strains were tested for the existence of two quinolone resistance genes,namely *qnr*A and *qep*A.*Onr*A gene that is responsible for protecting bacterial DNA gyrase and type IV topoisomerase from quinolones while *qep*A is responsible for extruding quinolones from the bacterial cell. Serotyping of the isolates revealed that, the isolates belonged to different 18 *E. coli* serotypes. Out of the 40 isolates, the most common serotypes were O91: K- (9, 22.5%), O78:K80 (5, 12.5%), and O125:K70 (4, 10%).Other detected serotypes were O159: K- (3, 7.5%), O86: K (3, 7.5%). Serotypes O166, O26:K60 and O126 were represented by 2 isolates of each (2, 5%). The rest of the isolates were serotyped as O103: K-, O128, O142, and O144:K90, O155, O158: K-, O27, O28, O55:H7, and O6, one isolate of each (2.5%). Antibiogram conducted against 14 antibacterial drugs revealed that of the 40 isolates, all of them were resistant to ampicillin and amoxicillin /clavulanic acid (100%). On the other hand, 39 isolates were sensitive to fosfomycin (97.5%), 34 isolates were sensitive for both norfloxacin and levofloxacin (85%) while 29 isolates showed intermediate sensitivity for ciprofloxacin (72.5%). Detection of

quinolone resistance genes for Selected *E. coli* isolates recovered in the present study were tested by PCR assays to detect the *qnr*A and *qep*A. Out of 17 tested isolates all of them were harboring *qnr*A gene while only 5 were positive for the *qep*A gene (29.4%).

**Keywords:**

Multidrug- Resistance - *E.coli* -Broilers -Quinolone - *Qnr*A - *Qep*A.

## INTRODUCTION

*Escherichia coli* (*E. coli*) is a Gram-negative bacterium of the family *Enterobacteriaceae* that commonly inhabits the intestines of many warm-blooded organisms and comprises both non-pathogenic and pathogenic strains. In poultry flocks, diseases caused by extra-intestinal associated *E. coli* represent significant cause of economic losses. Avian pathogenic *E. coli* (APEC) strains have been implicated in diseases of birds including broilers, layers, turkeys and geese. Resulting in clinical syndromes that include cellulitis, omphalitis, colibacillosis, airsacculitis and salpingitis **(Barbieri *et al.*, 2015; Dias da Silveira *et al.*, 2002; Maluta *et al.*, 2016; Nakamura *et al.*, 1997; Nolan *et al.*, 2013).**

Colibacillosis caused by APEC is a devastating disease of poultry that results in multi-million-dollar losses annually to the poultry industry all over the world. Clinical manifestations associated with APEC infection include colisepticemia, cellulitis, air sac disease, peritonitis, salpingitis, omphalitis and osteomyelitis **(Newman *et al.*, 2018).**

Infection associated with APEC is often viewed as secondary to other predisposing factors such as compromised immune system or mucosal and skin barriers that can no longer hinder the pathogen **(Nolan *et al.*, 2013).** However, it has been demonstrated that APEC can also be a primary pathogen **(Nolan *et al.*, 2013; Collingwood *et al.*, 2014).** In addition, vertical transmission of APEC has been reported from infected hens to progeny **(Nolan *et al.*, 2015).**

Under field conditions, commercial broiler farms with inadequate biosecurity measures and with the presence of colibacillosis in early age birds have associated with more severe (infectious bronchitis virus) IBV infections in late ages **(Ariaans *et al.*, 2008).**

*E. coli* is classified into serotypes or serogroups based on somatic (O), capsular (K), fimbrial (F), and flagellar (H) antigens. Strains of the *E. coli* species are currently divided into 183Ogroups and 53 H-types **(Joensen *et al.*, 2015).** The various pathotypes of *E. coli* tend to be clonal groups that are characterized by shared O and H antigens that define serogroups (O antigen only) or serotypes (O and H antigens) **(Kaper *et al.*, 2004).**

Several specific O-serotypes of APEC have been frequently confirmed to be closely associated with serious diseases in human and animals. Notably, certain APEC belonging to the serotypes O1, O2, O18, and O78 have been considered the most common pathogenic strains that is accounted for more than 80% of the APEC isolates **(Ewers *et al.*, 2007, Me hat *et al.*, 2021).** Several APEC virulence factors are recognized such as adhesions, toxins, iron acquisition mechanisms, invasions, and plasmids **(Nolan *et al.*, 2013, 2015, 2020).** Moreover, the organism has multiple flagella used for motility and is covered with pili, which are important for both adherence to the host cell and the transfer of genetic material between organisms by way of plasmids. A big concern is the presence of antibiotic resistant ExPEC isolates. Existence of multiple drug resistant bacterial pathogens has been one of the ten threats to global health for 2019 as reported by the World Health Organization. Antibiotic resistance genes, such as extended-spectrum-lactamases (ESBLs) - encoding genes have been reported in extraintestinal pathogenic *E. coli* (ExPEC) isolates **(Putouts *et al.*, 2012).**

Antimicrobial resistance and virulence in APEC is well known and related to mobile genetic elements such as plasmids and transposons **(Johnson *et al.*, 2002, 2010a, 2005, 2006a, 2006b; Johnson, Kariyawasam *et al.*, 2006).** The misuse of antimicrobials in food animal production has led to various adverse effects and there is also growing evidence linking livestock antimicrobial consumption to antimicrobial resistance in humans **(Aarestrup *et al.*, 2008; Silbergeld *et al.*, 2008).** As a result, there is an increasing public health hazard regarding the zoonotic transmission of resistance to critically important antimicrobial classes **(Overdevest *et al.*, 2011. Ewers *et al.*, 2012).** As resistance to the third-generation cephalosporins and fluoroquinolones grows, increasing attention is being placed on extended-spectrum b-lactamase (ESBL) genes, AmpC-type b-Lactamase (ACBL) genes, and plasmid-mediated quinolone resistance (PMQR) genes. To identify genes responsible for ESBL production, reference laboratories use molecular analyses, primarily polymerase chain reaction **(Wittum *et al.*, 2012).** Multiple mechanisms are involved in resistance to fluoroquinolones (FQ) in Enterobacteriaceae. Mutations in chromosomal genes encoding for DNA gyrase and topoisomerase IV could be one mechanism **(Ferrero *et al.*, 1994, Kumagai *et al.*, 1996).** In addition, plasmid-mediated quinolone resistance (PMQR) has also been reported, including a

*Qnr*-mediated inhibition of quinolone binding to DNA **(Tran and Jacoby, 2002; Poirel *et al.*, 2005b)** and a *Qep*A encoded efflux pump **(Yamane *et al.*, 2007; Rodriguez-Martinez *et al.*, 2016; Karp *et al.*, 2018).**

The purpose of the present study was to characterize antimicrobial-resistant *E. coli* isolates recovered from lesions of colibacillosis in broilers from 2019 to 2021. Quinolone resistance was investigated in the MDR *E. coli* isolates using PCR assays.

**Materials and methods:**

***E. coil* isolates.**

Fourty APEC isolates were recovered from lesions of colibacillosis in chickens of different ages collected from broiler flocks between 2019 and 2021. Samples were represented by lungs, air sacs, brains, peritonea, hearts and livers. APEC isolates were recovered and identified biochemically **(Rodriguez-Siek *et al.*, 2005; Lee *et al.*, 2008).** The isolates were serotyped using specific antisera against the *E. coli* somatic (O) antigens with the kit supplied by Sifin Antisera Co. (Germany). As descried by the manufacturer, slide agglutination method was carried out by suspending colonies of fresh pure culture in a drop of normal saline followed by mixing with a drop of antisera. Isolates were tested against the polyvalent 1-3 safin antisera and followed by testing the negative isolates with the monovalent O antisera.

**Antimicrobial susceptibility testing:**

Antimicrobial susceptibility of *E. coli* isolates was determined by the modified Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute method (CLSI, 2021). Isolates were tested against 14 different antimicrobial agents. The tested agents were ampicillin (AMP, 10 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), doxycycline (DO, 30 µg), gentamycin (CN, 10 µg), naldixic acid (NA, 30 µg), norfloxacin (NOR, 10 µg), streptomycin (S, 10 µg), tetracyclin (TE, 30 µg), levofloxacin (LEV 5 µg), ceftriaxone (CRO, 30µg), fosomycin (FF, 50 µg), lomefloxacin (LOM, 10 µg), amoxicillin-clavulanic acid (AMC, 20 µg amoxicillin+10 µg clavulanic acid).

*E. coli* was defined as a multidrug-resistant isolate when it was found non-susceptible to at least one agent in three or more different classes of antimicrobial agents, excluding the broad-spectrum penicillins without a β-lactamase inhibitor **(Aarestrup *et al.*, 2008).**

**Extraction of bacterial genomic DNA and PCR:**

The genomic DNA of 17 *E. coli* isolates was extracted by boiling from overnight nutrient broth cultures. Briefly, the bacterial culture was centrifuged at 15,000 × g for 15 min. The supernatant

was eliminated, and the pellet was suspended in molecular biology grade water (Eppendorf, Hamburg, Germany) and centrifuged at 15,000 × g for 10 min. The supernatant was eliminated, and the pellet was suspended in 40 µl of molecular biology grade water, subjected to boiling at 100°C in a water bath for 10 min, cooled on ice, centrifuged at 15,000 × g for 10 s and stored at-20°C. Aliquots of 2 µl of DNA were used as templates for PCR **(Maria *et al.*, 2008).**

PCR assays were carried out using two-primer sets specific for the bacterial *qep*A and *qnr*A genes. Primer sequences, expected PCR products and references are depicted in (Table 1).

**Table (1):** Primer sequences specific for *Qep*A and *Qnr*A genes and the expected PCR products.

| Gene | Sequence (5'-3') | Amplified product | Annealing temperature | Reference |
|---|---|---|---|---|
| ***Qep*A** | **F: ATTTCTCACGCCAGGATTTG** | **516 bp** | **53˚C, 40 sec.** | **(Robicsek *et al.*, (2006)** |
| | **R: GATCGGCAAAGGTTAGGTCA** | | | |
| ***Qnr*A** | **F: CGTGTTGCTGGAGTTCTTC** | **403 bp** | **50˚C, 40 sec.** | **(Cattoir *et al.*, (2008)** |
| | **R: CTGCAGGTACTGCGTCATG** | | | |

**F: forward R: reverse**

## RESULTS

**Serotypes of *E. coli* isolates from chickens:**

As shown in (Table 2), serotyping of 40 *E. coli* isolates recovered from chickens indicates that serotype O91: K- showed the highest incidence among (22.5 %), followed by serotypes O78:K80 (12.5%), and serotypes O125:K70 (10%) and O159 (7.5%). The lowest incidences were represented by serotypes O103: K-, O12728, O142, and O144:K90, O155, O158: K-, O27, O28, and O55:H7 and O6 (2.5% each).

**Table (2 ):** Serotyping of 40 *E. coli* isolates recovered from diseased chickens.

| Serotype | | Number of isolates | % | Serotypes | | Number of isolates | % |
|---|---|---|---|---|---|---|---|
| 1 | O103:K- | 1 | 2.5% | 10 | O166 | 2 | 5.0% |
| 2 | O125:K70 | 4 | 10.0% | 11 | O26:K60 | 2 | 5.0% |
| 3 | O126 | 2 | 5.0% | 12 | O27 | 1 | 2.5% |
| 4 | O128 | 1 | 2.5% | 13 | O28 | 1 | 2.5% |
| 5 | O142 | 1 | 2.5% | 14 | O55:H7 | 1 | 2.5% |
| 6 | O144:K90 | 1 | 2.5% | 15 | O6 | 1 | 2.5% |
| 7 | O155 | 1 | 2.5% | 16 | O78:K80 | 5 | 12.5% |
| 8 | O158:K- | 1 | 2.5% | 17 | O86:K- | 3 | 7.5% |
| 9 | O159 | 3 | 7.5% | 18 | O91:K- | 9 | 22.5% |

**Antibiogram of *E. coli* isolates:**

As shown in (Table 3), Fig.(1), results of the antibiogram of 40 *E. coli* isolates against 14 antimicrobial drugs revealed that all isolates were resistant to ampicillin and amoxicillin/ Clavulanic acid, 21 isolates were resistant to tetracycline (52.5%) while 39 (97.5%) isolates were sensitive to fosfomycin. On the other hand, 34 isolates were sensitive for norfloxacin and levofloxacin (85%) and 29 isolates were sensitive for Ciprofloxacin (72.5%).

**Table (3):** The antibiogram of *E. coli* isolates recovered from diseased chickens.

| Antimicrobial agent | | Number, percentage and profile of isolates | | | | | |
|---|---|---|---|---|---|---|---|
| | | R | | I | | S | |
| | | No. | % | No. | % | No. | % |
| Nalidixic acid | NA | 19 | 47.5 | 0 | 0 | 21 | 52.5 |
| Lomefloxacin | LOM | 11 | 27.5 | 8 | 20 | 21 | 52.5 |
| Ciprofloxacin | CF | 6 | 15 | 29 | 72.5 | 5 | 12.5 |
| Norfloxacin | NOR | 3 | 7.5 | 3 | 7.5 | 34 | 85 |
| Levofloxacin | LEV | 4 | 10 | 2 | 5 | 34 | 85 |
| Ceftriaxone | CRO | 7 | 17.5 | 8 | 2 | 25 | 62.5 |
| Fosfomycin | FF | 0 | 0 | 1 | 2.5 | 39 | 97.5 |
| Streptomycin | S | 18 | 45 | 2 | 5 | 20 | 50 |
| Gentamycin | G | 5 | 12.5 | 5 | 12.5 | 30 | 75 |
| Chloramphenicol | C | 13 | 32.5 | 11 | 27.5 | 16 | 40 |
| Tetracycline | TE | 21 | 52.5 | 3 | 7.5 | 16 | 40 |
| Doxycycline | DO | 4 | 10 | 4 | 10 | 32 | 80 |
| Amoxicillin/Clavulanic acid | AMC | 40 | 100 | 0 | 0 | 0 | 0 |
| Ampicillin | AM | 40 | 100 | 0 | 0 | 0 | 0 |
| R= Resistant | | I=Intermediate | | S=Sensitive | | | |

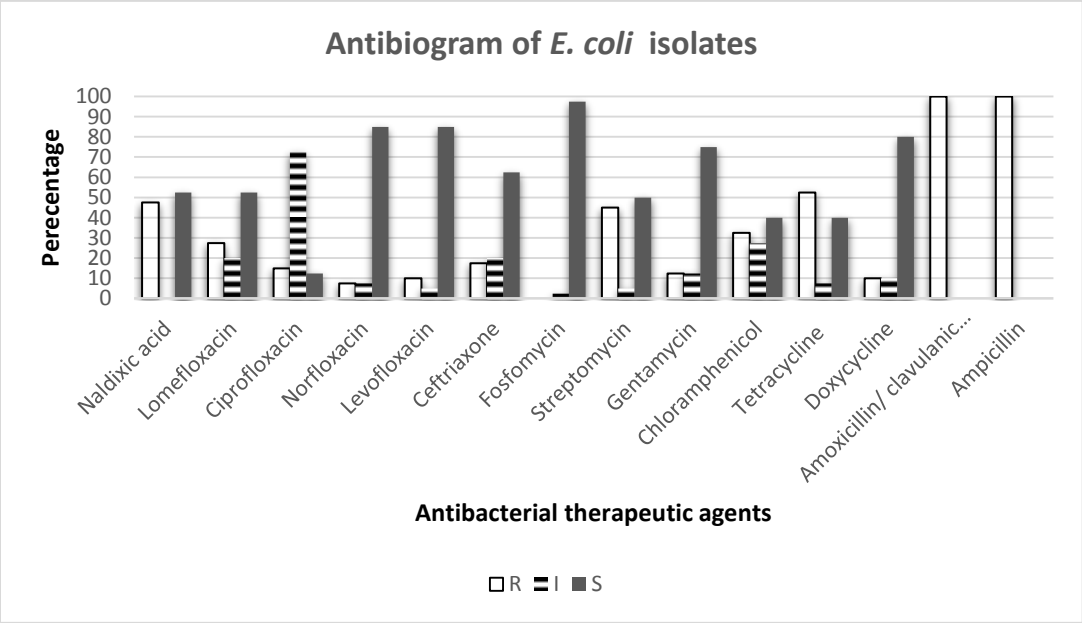


**Fig. (1):** The antibiogram of 40 *E. coli* isolates of chicken origins expressed as percentages of resistance, intermediate susceptibility and susceptibility.

**Antimicrobial resistance profile of *E. coil* isolates:**

Out of 40 *E. coil* isolates studied 13 (32.5%) were resistant to two antimicrobial agents (AMC-AM). The remaining isolates were resistant to at least three antimicrobial agents and the frequency of MDR *E.coli* isolates were 67.5% (n = 27). One of these isolates was resistant to 12 antimicrobial agents, whereas one isolate was resistant to 10 antimicrobial agents and 3 isolates showed resistance to 8 tested antimicrobials (table 4). The resistance profile (NA- S- C- TE- AMC- AM) was shared by 3 isolates 7.5%) isolates out of 40 have the same resistance profile, 2 (5%) isolates have the resistance profile (NA-LOM-S-TE-AMC- AM) and 2 (5%) isolates have the resistance profile (NA-LOM-S-C-TE-AMC-AM).

**Table (4):** Antimicrobial resistance profile of 40 chicken pathogenic *E. coli* isolates**.**

| Antimicrobial resistance profile | No. of isolates | % of isolates | No. of antibiotics |
|---|---|---|---|
| **AMC-AM** | **13** | **32.5** | **2** |
| **C- AMC-AM** | **1** | **2.5** | **3** |
| **S- AMC- AM** | **1** | **2.5** | **3** |
| **TE-AMC-AM** | **1** | **2.5** | **3** |
| **CRO-AMC-AM** | **1** | **2.5** | **3** |
| **NA-S-AMC-AM** | **1** | **2.5** | **4** |
| **CRO-S-AMC-AM** | **1** | **2.5** | **4** |
| **NA-C-TE-AMC-AM** | **1** | **2.5** | **5** |
| **CRO-CN-TE-AMC-AM** | **1** | **2.5** | **5** |
| **CRO-TE-DO-AMC-AM** | **1** | **2.5** | **5** |
| **NA- S- C- TE- AMC- AM** | **3** | **7.5** | **6** |
| **NA-CIP-S- TE- AMC- AM** | **1** | **2.5** | **6** |
| **NA-LOM-S-TE-AMC- AM** | **2** | **5** | **6** |
| **NA-CRO-S-TE- AMC- AM** | **1** | **5** | **6** |
| **NA-S-C-TE- DO- AMC- AM** | **1** | **2.5** | **7** |
| **NA-LOM-S-C-TE-AMC-AM** | **2** | **5** | **7** |
| **NA-CRO-C-TE-DO-AMC-AM** | **1** | **2.5** | **7** |
| **NA-LOM-CIP-S-TE-AMC-AM** | **1** | **2.5** | **7** |
| **NA-LOM-CIP-NOR-LEV-AMC-AM** | **1** | **2.5** | **7** |
| **NA- LOM- S- CN- C- TE- AMC- AM** | **1** | **2.5** | **8** |
| **NA-LOM-CIP-NOR-LEV-TE-AMC-AM** | **1** | **2.5** | **8** |
| **NA LOM - S - CN - C - TE - AMC – AM** | **1** | **2.5** | **8** |
| **LOM-CIP-LEV-CRO-S-CN-C-TE-AMC-AM** | **1** | **2.5** | **10** |
| **NA-LOM-CIP-NOR-LEV-S-CN-C-TE-DO-AMC-AM** | **1** | **2.5** | **12** |

**PCR detection of *qnr*A and *qep*A genes in *E. coli* isolates of chicken origin:**

PCR on *E. coli* DNA using *qnr*A gene-specific primers resulted in amplification of the expected product size (516 bp)as evidenced by the electrophoresis Fig**.**(2). All the tested isolates

(No. =17) harbored the gene. Isolate serotypes were O26:K60, O91: K, O159, O86: K-, O159, O142, O86: K-, O91: K-, O125:K70, O28, O55:H7, O78:K80, O78:K80, O78:K80, O91: K-, O91: K-, O78:K80). On the other hand, only 6 out of 17 (29.4%) tested isolates harbored the *qep*A gene Fig. (3) is representing the serotypes (O91: K-, O159, O91: K-, O86:K-, O86:K-). Table (5) is a collective one showing the antibiotic resistance profiles related to the PCR results of 17 *E. coli* isolates.

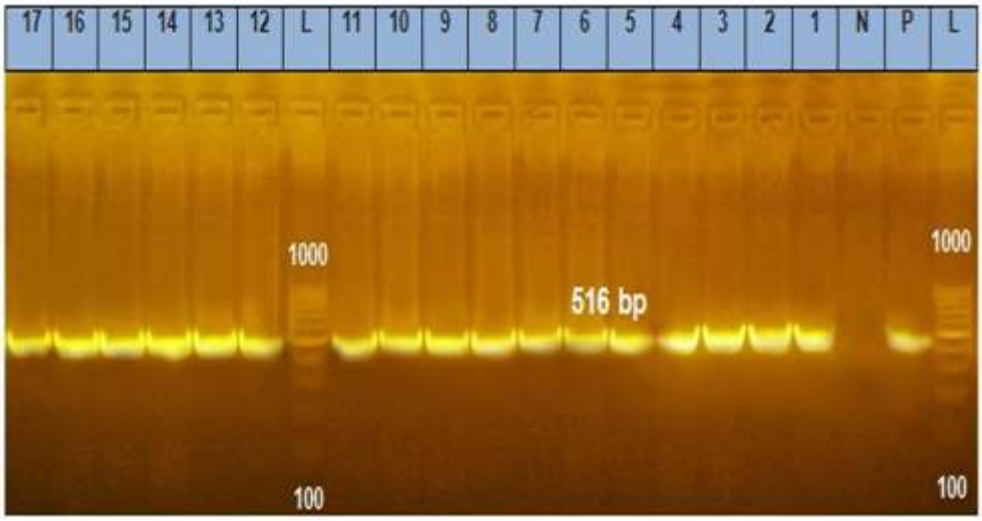


**Fig. (2):** Agarose gel electrophoresis showing PCR with amplification of 516 bp fragments specific for ***qnr*A** gene. Lane L: ladder, Lane N: Negative control (*S. Enteritidis* ATCC 13076), Lane P: Positive control (*E. coli* NCIMB 50034).

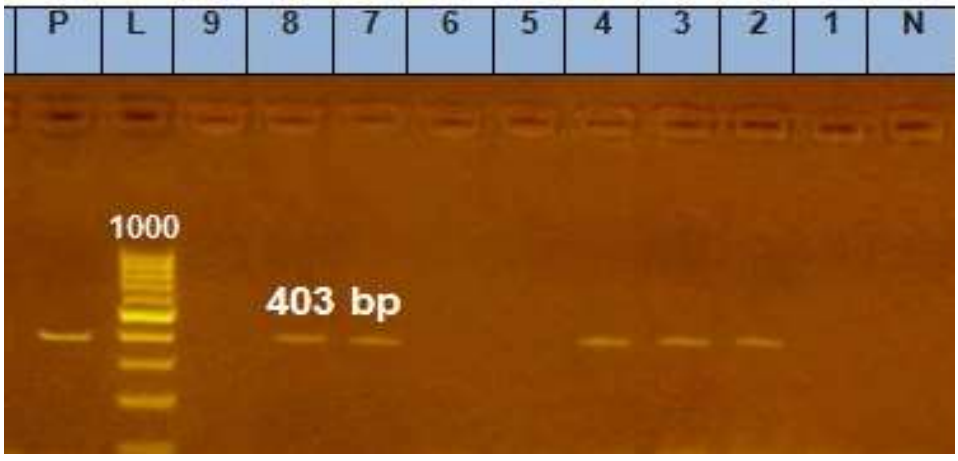


**Fig. (3):** Agarose gel electrophoresis showing PCR with amplification of 403 bp fragments specific for ***qep*A** gene Lane L: ladder, Lane N: Negative control (*S. Enteritidis* ATCC 13076), Lane P: Positive control (*E. coli* NCIMB 50034).

**Table (5):** Antibiogram of 17 *E. coli* isolates in relation to the PCR amplification of *qnr*A and

| Sample Code | Serotype | NA | LOM | CIP | NOR | LEV | QnrA | QepA |
|---|---|---|---|---|---|---|---|---|
| **3** | **O91:K-** | **R** | **I** | **R** | **S** | **S** | **+** | **+** |
| **4** | **O159** | **S** | **S** | **I** | **S** | **S** | **+** | **+** |
| **6** | **O86:K-** | **R** | **R** | **R** | **R** | **R** | **+** | **+** |
| **15** | **O86:K-** | **R** | **R** | **R** | **R** | **R** | **+** | **+** |
| **20** | **O91:K-** | **R** | **R** | **R** | **I** | **I** | **+** | **+** |
| **2** | **O26:K60** | **S** | **S** | **S** | **S** | **S** | **+** | **-** |
| **7** | **O159** | **S** | **S** | **S** | **S** | **S** | **+** | **-** |
| **8** | **O142** | **R** | **R** | **R** | **R** | **R** | **+** | **-** |
| **21** | **O125:K70** | **R** | **I** | **I** | **S** | **S** | **+** | **-** |
| **25** | **O28** | **S** | **S** | **S** | **S** | **S** | **+** | **-** |
| **28** | **O55:H7** | **R** | **R** | **I** | **S** | **S** | **+** | **-** |
| **30** | **O78:K80** | **S** | **S** | **I** | **S** | **S** | **+** | **-** |
| **33** | **O78:K80** | **S** | **S** | **I** | **S** | **S** | **+** | **-** |
| **37** | **O78:K80** | **S** | **S** | **S** | **S** | **S** | **+** | **-** |
| **38** | **O91:K-** | **R** | **R** | **I** | **S** | **S** | **+** | **-** |
| **39** | **O91:K-** | **S** | **S** | **I** | **S** | **S** | **+** | **-** |
| **40** | **O78:K80** | **S** | **R** | **R** | **I** | **R** | **+** | **-** |
| **Total** | | | | | | | **100%** | **29.4%** |

*qnp*A genes results

**I: Intermediate sensitivity S: sensitive R: resistant**

## DISCUSSION

Most *Escherichia coli* strains are commensals. However, in humans and warm-blooded animals, certain strains are pathogenic. One of the significant forms of pathogenic *E. coli* is extraintestinal pathogenic *Escherichia coli* (ExPEC) which may cause systemic infection in humans and animals **(Manges *et al.*, 2019)**.

Avian pathogenic *E. coli* (APEC), a sub pathotype of *ExPEC*, is the causative agent of colibacillosis in poultry, a disease that can cause significant economic losses due to high morbidity and mortality, medication costs, and condemnation of carcasses **(Kim *et al.*, 2020).** Colibacillosis, caused by APEC, is a devastating disease of poultry that results in multi-million-dollar losses annually to the poultry industry. Disease syndromes associated with APEC include colisepticemia, cellulitis, air sac disease, peritonitis, salpingitis, omphalitis, and osteomyelitis.

In the current study, many APEC isolates were recovered from chicken samples. Serotyping of the isolates indicated that they were dominated by O91: K- (22.5%) followed by O78:K80 (12.5%), O125:K70 (10%), O159: K- (, 7.5%), O86: K- (7.5%) serotypes. The rest of isolates were serotyped as O166, O26:K60 O126 O103: K- (, O128, O142, O144:K90, O155, O158: K-, O27, O28, O55:H7, and O6 (2.5% each).

**Badr *et al.* (2021)** conducted a study on multidrug-resistant *Escherichia coli* infections in broiler chickens, on 70 diseased flocks in Egypt and their serotype results were nearly similar to ours. Antimicrobial sensitivity testing of 40 *E. coli* isolates obtained in this study were carried out against 14 antibacterial drugs.Results revealed that all isolates showed resistance to ampicillin and amoxicillin/clavulanic acid while 52% of the isolates were resistant to tetracycline. On the other hand the highest sensitivity was for fosfomycin (97.5%) followed by norfloxacin (85%) and levofloxacin 34 (85%). Ciprofloxacin sensitivity was recorded in 29 isolates (72.5%). The antibiogram behavior recorded in this study is almost consistent with the findings of **(Ievy *et al.*, 2020)** concerning resistance to ampicillin and tetracycline.

**Abed, (2021**) reported that, the highest resistance of *E. coli* isolates was recorded against amoxicillin (97.5%) followed by Cefotaxime sodium and florfenicol (95% for each). **Thomrongsuwannakij *et al*. (2022)** recorded resistance rates of chicken *E. coli* isolates as 98.89% to ampicillin and 88.9% to tetracycline. Concerning quinolone resistance, *E. coli* isolates that were sensitive and containing the resistant *qnrA* gene at the same time appeared to harbor pseudogenes, which are defined as inactive but stable components of the genome derived by the mutation of an ancestral active gene. This is likely to be an underestimate of the true prevalence of pseudogenes in the tested *E. coli* populations. This represents a serious limitation of an assay depends on the detection of phenotype-genotype discrepancies with the intent to discover pseudogenes **(George *et al*., 2015)**. Most studies on the mechanisms of antibiotic resistance address the development of resistance as the consequence of a genetic, inheritable change, which can be a mutation or the acquisition of a resistance gene. These are off/on directional situations in which bacteria are either susceptible or resistant and reversion from resistance to susceptibility is not a frequent event. Several works have shown that this is a part of the resistance landscape and that there are situations in which resistance is not driven by a genetic change. Transient, reversible resistance can be achieved by different mechanisms, which are linked to the physiological state of bacteria and to the inputs that

microorganisms receive when confronted with different habitats and stressors. The study of the mechanisms of phenotypic resistance is of relevance to understanding the reasons for therapeutic failures for bacteria that are classified as susceptible using classical testing methods, but also to increase the susceptibility to antibiotics of bacterial pathogens **(Munita1 and Arias, 2016).** Regarding the resistance-associated genes, results revealed that all the PCR tested isolates (n=17) harbored *qnrA* gene (100%) which comes in agreement with the reports of **Abed *et al.* (2021).**

*E. coli* isolates with resistance to quinolones and lacking the gene *qep*A gene can be attributed to mutations in the promoter and attenuator sequences of chromosomal genes. Generally, the development of antibiotic resistance in bacteria is usually associated with genetic changes, either to the acquisition of resistance genes or to mutations in elements relevant to the activity of the antibiotic. However, in some situations resistance can be achieved without any genetic alteration; this is called phenotypic resistance. Non-inherited resistance is associated with specific processes such as growth in biofilms, a stationary growth phase, or persistence. These situations might occur during infection, but they are not usually considered in the classical susceptibility tests at clinical microbiology laboratories **(Fernando and Jose, 2013).**

In conclusion, the results obtained in this study indicate that, the *E. coli* infection in poultry has been dramatically increased with severe economic losses worldwide and increased resistance rates in some antibiotics. This rising percentage of antibiotics resistance reflects the negative feedback of antibiotic abuse in poultry farms. In this study, the tested isolates showed variation in their resistance rates to the evaluated antimicrobial agents taking special reference to quinolone resistance. Misuse of antibiotics as a general treatment or growth promoter may increase the generation of antibiotic resistance genes in bacteria that can infect poultry farms, resulting in high treatment costs due to a lack of antibiotics that can treat multidrug-resistant bacteria, as well as affecting human health. Moreover, virulence genes *qepA* and *qnrA* were detected at high percentages in broiler *E. coli* isolates. These findings emphasize the importance of using antibiotics judiciously at the right dose and before administrating antibiotics on diseased broiler poultry farms, antibiotic sensitivity tests must be performed and take into consideration the withdrawal period of the used antibiotics to protect human health.

Our recommendations are to decrease the misusage of antibiotics as general treatment or

growth promoters by putting a national strategy for controlling Anti-Microbial Resistance (AMR), decreasing diseases following biosecurity and biosafety measures in poultry farms.

**Conflicts of interest:**

All authors included in this paper announce that there is no any conflict of interest.

## REFERENCES

**Aarestrup, F. M., H. C. Wegener, and P. Collignon (2008):** Resistance in bacteria of the food chain: Epidemiology and control strategies. Expert Rev. Anti-infect. There, 6:733 -750.

**Ahmed H. Abed (2021):** Molecular characterization of antimicrobial resistant *Escherichia coli* isolated from broiler chickens, Journal of Veterinary Medical Research, 27 (2): 128 -142

**Ariaans MP, Matthijs MGR, and Van Haarlem .D*, et al.*, (2008):** The role of phagocytic cells in enhanced susceptibility of broilers to colibacillosis after infectious bronchitis virus infection. Vet Immunol Immunopathol.; 123(3-4):240 -250.

**Badr H, Nabil NM, Tawakol MM. (2021):** Effects of the prebiotic lactoferrin on multidrug-resistant *Escherichia coli* infections in broiler chickens. Vet World, 14 (8):2197-2205. doi: 10.14202/vetworld.2021.2197-2205.Epub2021Aug25.PMID: 34566339;PMCID: PMC8448632.

**Barbieri NL, De Oliveira AL, Tejkowski TM, Pavanelo DB, Matter LB, Pinheiro SR, Vaz TM, Nolan LK, Logue CM, and De Brito BG, Horn F. (2015):** Molecular characterization and clonal relationships among *Escherichia coli* strain isolated from broiler chickens with colisepticemia. Foodborne Pathogens and Disease, 12 (1):74-83.

**Cattoir V.; Poirel L. and Nordmann P. (2008):** Plasmid-mediated quinolone resistance pumps *QepA*2 in an *Escherichia coli* isolate from France. Antimicrobial Agents Chemotherapy, 52:3801-3804.

**CLSI/NCCIS (2021):** Performance Standards for Antimicrobial Disk Susceptibility Tests, CLSI, Wayne, PA, USA, 2013.

**Collingwood C, Kemmett K, and Williams N, Wigley P. (2014):** Is the concept of Avian Pathogenic *Escherichia coli* as a single pathotype fundamentally flawed Frontiers in Veterinary Science, 1:5.

**Dias da Silveira W, Ferreira A, Brocchi M, Maria de Hollanda L, Pestana de Castro AF, Tatsumi Yamada A, Lancellotti M.(2002):** Biological characteristics and pathogenicity of avian *Escherichia coli* strains. Veterinary Microbiology, 85 (1):47-53.

**Ewers, C., A. Bethe, T. Semmler, S. Guenther, and L. H. Wieler (2012):** Extended-spectrum β-lactamase-producing and Amp producing *Escherichia coli* from livestock and companion

animals, and their putative impact on public health: A global perspective. Clin. Microbiol. Infect. , 18:646-655.

**Ewers, G. Li, H. Wilking *et al.*, (2007):** "Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia Coli* , International Journal of Medical Microbiology. Vol. 297, no. 3, pp. 163-176.

**Fernando Corona and Jose L. Martinez (2013):** Phenotypic Resistance to Antibiotics, National Library of Medicine.

**Ferrero L., Cameron B., Manse B., Lagneaux D., Crouzet J., and Famechon A., Blanche F. (1994):** Cloning and primary structure of Staphylococcus aureus DNA topoisomerase IV: a primary target of fluoroquinolones. Molecular Microbiology, 13, 641–653.

**George A. Jacoby, Jacob Strahilevitz and David C. Hooper (2015):** Plasmid-mediated quinolone resistance, National Library of Medicine.

**Ievy S, Islam MS, Sobur MA, *et al,* (2020):**Molecular Detection of Avian Pathogenic *Escherichia coli* (APEC) for the First Time in Layer Farms in Bangladesh and their Antibiotic Resistance Patterns. Microorganisms. 2020; 8 (7):1021. Published 2020 Jul 9.

**J. W. Mehat, A. H. M. Van Vliet, and R. M. La Ragione (2021):** "Avian pathogenic *Escherichia coli* (APEC) pathotype are comprised of multiple distinct, independent genotypes," Avian Pathology, Vol. 50, no. 5, pp. 402-416.

**Johnson TJ, Giddings CW, Horne SM, *et al.*, (2002):** Location of increased serum survival gene and selected virulence traits on a conjugative R plasmid in an avian Escherichia *coli* isolate. Avian Dis. 2002; 46 (2):342-352.
I: 10.1637/00052086(2002)046[0342: LOISSG] 2.0.CO; 2

**Johnson TJ, Siek KE, Johnson SJ, Nolan LK. (2005):** DNA sequence and comparative genomics of pAPEC-O2-R, an avian pathogenic *Escherichia coli* transmissible R plasmid. Antimicrobial Agents and Chemotherapy 49 (11):4681- 4688.

**Johnson TJ, Siek KE, Johnson SJ, Nolan LK. (2006a):** DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli* strains. Journal of Bacteriology 188 (2):745-758

**Johnson TJ, Johnson SJ, Nolan LK. (2006):** Complete DNA sequence of a ColBM plasmid from avian pathogenic *Escherichia coli* suggests that it evolved from closely related ColV Virulence plasmids. Journal of Bacteriology 188 (16):5975-5983.

**Johnson TJ, Wannemeuhler YM, Scaccianoce JA, Johnson SJ, Nolan LK. (2006):** Complete DNA sequence, comparative genomics, and prevalence of an IncHI2 plasmid occurring

among extra intestinal pathogenic *Escherichia coli* isolates. Antimicrobial Agents and Chemotherapy 50 (11):3929-3933.

**Johnson TJ, Thorsness JL, Anderson CP, Lynne AM, Foley SL, Han J, Fricke WF, McDermott PF, White DG, Khatri M, Stell AL, Flores C, Singer RS. (2010):** Horizontal gene transfer of a ColV plasmid has resulted in a dominant avian clonal type of *Salmonella* enterica serovar Kentucky. PLOS ONE 5 (12):e15524.

**Johnson TJ, Jordan D, Kariyawasam S, Stell AL, Bell NP, Wannemuehler YM, Alcaron CF, Li G, Tivendale KA, Logue CM, and Nolan LK. (2010):** Sequence analysis and characterization of a transferrable hybrid plasmid encoding multidrug resistance and enabling zoonotic potential for extraintestinal *Escherichia coli*. Infection and Immunity 78 (5):1931-1942

**Joensen, K. G., Tetzschner, A. M., Iguchi, A., Aarestrup, F. M., and Scheutz, F. (2015):** Rapid and easy in silico serotyping of *Escherichia coli* isolates by use of whole-genome sequencing data. J. Clin. Microbiol. 53, 2410 -2426.

**Jose M. Munita, and Arias, C. A. (2016):** Mechanisms of Antibiotic Resistance. Microbiology spectrum, 4 (2), 10.1128/microbiolspec.VMBF-0016-2015.

**Kaper J.B.,Nataro J.P.,Mobley H. L.(2004):** Pathogenic *Escherichia coli*. Nat.Rev. Microbiol, 2: 123 -140.

**Kariyawasam S, Johnson TJ, Debroy C, Nolan LK. (2006):** Occurrence of Pathogenicity Island I (APEC-O1) genes among *Escherichia coli* implicated in avian colibacillosis. Avian Diseases 50 (3):405-410.

**Karp, B. E., Campbell, D., Chen, J. C., Folster, J. P., and Friedman, C. R. (2018):** Plasmid-mediated quinolone resistance in human non-typhoidal *Salmonella* infections: an emerging public health problem in the United States. Zoonoses Public Health 65, 838-849.

**Kumagai Y., Kato J.I., Hoshino K., Akasaka T., Sato K., Ikeda H. (1996):** Quinolone-resistant mutants of *Escherichia coli* DNA topoisomerase IV parC gene. Antimicrobial Agents and Chemotherapy, 40, 710–714.

**Kim, Y.B., Yoon, M.Y., Ha, J.S., Seo, K.W., Noh, E.B., Son, S.H. and Lee, Y.J. (2020):** Molecular characterization of avian pathogenic Escherichia coli from broiler chickens with colibacillosis. Poultry Science, 99, 1088-1095.

**M. D. Lee, K. L. Nolan, D. Zavala, *et al.*, (2008):** "Editorial board for the American Association of avian pathologists," A Laboratory Manual for the Isolation and Identification of Avian Pathogen Louis, Blackwell Publishing, Hoboken, NJ, USA, 5th edition.

**Manges, A. R., Geum, H. M., Guo, A., Edens, T. J., Fibke, C. D., and Pitout, J. D. D. (2019):** Global Extraintestinal Pathogenic Escherichia coli (ExPEC) Lineages. Clin. Microbiol. Rev. 32, e00135-18. doi: 10.1128/CMR.00135-18**.**

**Maluta RP, Nicholson B, Logue CM, Nolan LK, Rojas TC, and Dias da Silveira W. (2016):** Complete genomic sequence of an avian pathogenic *Escherichia coli* strain of serotype O7: HNT. Genome Announcements 4 (1):123.

**Maria Isabel Queipo-Ortun ,Juan De Dios Colmenero, Manuel Macias, Maria Jose Bravo,4 and Pilar Morata (2008):** Preparation of Bacterial DNA Template by Boiling and Effect of Immunoglobulin G as an Inhibitor in Real-Time PCR for Serum Samples from Patients with Brucellosis.

**Nakamura K, Mase M, Tanimura N, Yamaguchi S, Nakazawa M, Yuasa N. (1997):** Swollen head syndrome in broiler chickens in Japan: its pathology, microbiology, and biochemistry. Avian Pathology 2 (1):139 -154.

**Newman DM, Barbieri NL, de Oliveira AL, Willis D, and Nolan LK, Logue CM. (2018):** Characterizing avian pathogenic *Escherichia coli* (APEC) from colibacillosis cases. PeerJ. 2021; 9:e11025. Published 2021 Mar 4. doi:10.7717/peerj.11025.

**Nolan LK, Barnes HJ, Abdul-Aziz T, Logue CM, Vaillancourt J-P. (2015):** Colibacillosis. In: Brugere-Picoux J, Vaillancourt J-P, Shivaprasasd HL, Venne D, Bouzouaia M, and Association francaise pour l'avancement des sciences (AFAS), ed. Manual of Poultry Diseases. China: Toppan Printing Leefung, 301-315.

**Nolan LK, Barnes HJ, Vaillancourt J-P, Abdul-Aziz T, Logue CM. (2013):** Colibacillosis. Hoboken: Wiley-Blackwell.

**Nolan LK, Vaillancourt J-P, Barbieri N, Logue CM. (2020):** Colibacillosis. In: Swayne DE, Boulianne M, Logue CM, McDougald LR, Nair VL, Suarez DL, eds. Diseases of Poultry. 14th Edition. Hoboken: John Wiley and Sons, Inc, 770–830.

**Overdevest, I., I. Willemsen, M. Rijnsburger, A. Eustace, L. Xu, P. Hawkey, M. Heck, P. Savelkoul, C. Vandenbroucke-Grauls, K. Zwaluw, X. Huijsdens, and J. Kluytmans (2011):** Extended-spectrum B-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. Emerg. Infect. Dis., 17:1216 -1222.

**Pitout, J.D. (2012):** Extraintestinal Pathogenic *Escherichia coli*: A Combination of Virulence with Antibiotic Resistance. Front. Microbiol, 3, 9.

**Poirel L., Liard A., Rodriguez-Martinez J.M.,Nordmann P. (2005):** Vibrionaceae as a possible source of Qnrlike quin olone resistance determinants. The Journal of Antimicrobial Chemotherapy, 56, 1118-1121.

**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 KE, Giddings CW, Doetkott C, Johnson TJ, Fakhr MK, and Nolan LK. (2005):** Comparison of Escherichia coli isolates implicated in human urinary tract infection and avian colibacillosis. Microbiology (Reading); 151(Pt 6):2097-2110. doi:10.1099/mic.0.27499-0.

**Rodriguez-Martinez, J. M., Machuca, J., Cano, M. E., Calvo, J., Martinez- Martinez, L., and Pascual, A. (2016):** Plasmid-mediated quinolone resistance: two decades on. Drug Resist. Update, 29, 13-29.

**Silbergeld, E. K., M. Davis, J. H. Leibler, and A. E. Peterson (2008):** One reservoir: Redefining the community origins of antimicrobial-resistant infections. Med. Clin. North Am. 92: 1391–1407.

**Thomrongsuwannakij T, Narinthorn R, Mahawan T, Blackall PJ. (2022):** Molecular and phenotypic characterization of avian pathogenic *Escherichia coli* isolated from commercial broilers and native chickens. Poult Sci. 2022, 101(1):101527.

**Tran J.H., Jacoby G.A. (2002):** Mechanism of plasmid mediated quinolone resistance. Proceedings of the National Academy of Sciences of the United States of America, 99, 5638-5642.

**Wittum, T. E., Mollenkopf, D. F.,and Erdman, M. M. (2012):** Detection of *Salmonella* enterica isolates producing CTX-M Cephalosporin's in U.S. livestock populations. Appl. Environ. Microbiol, 78, 7487–7491.

**Yamane K., Wachino J.I., Suzuki S., Kimura K., Shibata N., Kato H., Shibayama K., Konda T., Arakawa Y. (2007):** New plasmid-mediated fluoroquinolone efflux pump, *QepA*, found in an *Escherichia coli* clinical isolate. Antimicrobial Agents and Chemotherapy, (51), 3354 -3360.