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Research Article

# Evaluation of Updated Phylogenetic Group Analysis Among Extended-Spectrum β-Lactamase Producing and Multidrug-Resistant *Escherichia coli* Isolated from Colisepticemia in Poultry

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## Abstract

*Escherichia coli* is reported as the most common organism in humans and animals and has been identified as a critical priority bacterium due to antibiotic resistance by World Health Organization (WHO). Multidrug-resistant (MDR) and Extended-Spectrum β-Lactamase (ESBL)-producing *E. coli* strains have emerged as a significant global health challenge worldwide due to the difficulty in treating related infections. Poultry has been recognized as an important reservoir of *E. coli* and may play a crucial role in transmitting these strains to humans. The objective of this study was to determine the prevalence of ESBL-producing and MDR *E. coli* isolated from poultry and to investigate their association with different phylogenetic groups. The current study was conducted on a collection of 100 *E. coli* isolates from colibacillosis in poultry. Antimicrobial susceptibility testing, ESBL production, and the prevalence of ESBL-mediated genes (*blaTEM*, *blaSHV*, *blaOXA*, and *blaCTX-M*) were assessed. Phylogenetic groups were analyzed using both the Clermont 2013 and 2019 updated methods. The highest resistance rates were observed against tetracycline (88%), trimethoprim/sulfamethoxazole (86%), and chloramphenicol (70%). The frequencies of ESBL production and MDR were 41% and 70%, respectively. The *blaTEM* gene was the most prevalent among isolates, with a frequency of 48%. Phylogenetic group analysis assigned *E. coli* isolates to B1 (23%), D (22%), A (10%), G (11%), F (8%), B2 (5%), and C (4%). Implementing antimicrobial stewardship is crucial because the circulation of ESBL-producing *E. coli* and MDR isolates poses a significant threat to both human and veterinary medicine. Furthermore, our results revealed a notable prevalence of phylogroup G in poultry, which is the first report of this finding in Iran.

***Keywords*:** *Escherichia coli*, ESBL, Colisepticemia, multi-drug resistance, phylogroup analysis, poultry

## Introduction

Nowadays, with increasing bird populations and high stocking densities, the development and implementation of robust biosecurity guidelines to minimize mortalities are vitally important in commercial poultry production systems. However, even in highly managed systems, infectious diseases can still occur (Swelum et al. 2021). Infections caused by harmful bacteria result in significant economic losses due to increased mortalities, disrupted flock uniformity, and impaired weight gain (Mehdi et al. 2018). Among various bacterial pathogens, *Escherichia coli*, a Gram-negative bacterium, is reported as one of the most common causative agents of disease syndromes such as omphalitis, enteritis, cellulitis, and septicemia in poultry (Thøfner et al. 2021; Koutsianos et al. 2022).

In-feed antibiotic use has been a common practice in poultry production to prevent diseases and infections. However, driven by the rising global demand for protein-rich foods, the poultry industry has experienced significant growth. This has led to the overuse of antibiotics, contributing to the emergence of antimicrobial-resistant bacteria, a critical global health concern (Muhammad et al. 2020; Tian et al. 2021). *Escherichia coli* has been listed as a critical priority pathogen by the World Health Organization (WHO) due to its increasing antibiotic resistance. (Fuga et al. 2022).

A common mechanism for bacteria to acquire resistance is the production of β-lactamase enzymes, including extended-spectrum β-lactamases (ESBLs). 1 These enzymes inactivate β-lactam antibiotics, such as penicillins, cephalosporins, cephamycins, monobactams, and carbapenems, rendering them ineffective against the bacteria (Economou et al.

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2023). ESBL-producing *E. coli* has emerged as the most frequent carrier of ESBLs in farm animals, particularly in poultry (Becker et al. 2021). This is supported by previous investigations that reported the prevalence of ESBL-producing *E. coli* in a varying range of 32% to 70% (Klimienė et al. 2018; Aworh et al. 2020; Misumi et al. 2023). ESBL-producing *E. coli* exhibits resistance to third- and fourth-generation cephalosporins and aztreonam, which are among the last-line treatment options for infections. Furthermore, these strains are frequently associated with multidrug-resistant (MDR) phenotypes, demonstrating resistance to antibiotics from other classes (Ramatla et al. 2023).

Several typing methods based on polymerase chain reaction (PCR) are commonly used for tracking outbreaks and conducting epidemiological investigations. Clermont et al. divided *E. coli* strains into eight phylogroups (A, B1, B2, C, D, E, F, and G) using a multiplex PCR-based method (Clermont et al. 2013; Clermont et al. 2019). Different phylogroups exhibit distinct ecological niches, biochemical traits, antibiotic resistance profiles, and other phenotypic characteristics. For example, Walk et al. found that environmental *E. coli* strains frequently belong to the B1 phylogroup (Walk et al. 2007). Johnson et al. observed that *E. coli* strains from the B2 and D phylogroups are often more pathogenic than strains from other phylogroups (Johnson et al. 2001). This is supported by a previous investigation in Mashhad by Tohmaz et al., which reported a notable potential for members of the D phylogroup to develop antimicrobial resistance in poultry (Tohmaz et al. 2022). However, Wang et al. observed that *E. coli* isolates from colibacillosis exhibiting heightened resistance belonged to the F phylogroup; indeed, more than half of these isolates were resistant to cephalosporin classes, and 32% were ESBL-producing (Wang et al. 2021). Phylogroup G is the most recently described phylogroup, positioned between phylogroups F and B2. Clermont et al. have suggested that strains belonging to phylogroup G may be virulent due to the presence of virulence factors such as *ironN*, *fyuA*, *sitA*, and *iutC/iutA* (Clermont et al. 2019).

Given the ubiquitous nature of *Escherichia coli* in environments, farm animals, and humans, the emergence of ESBL-producing *E. coli* strains poses a significant threat due to the development and dissemination of antimicrobial resistance. Poultry, as a reservoir of resistant *E. coli*, can play a crucial role in the transmission of these strains to humans through the consumption of poultry products, including eggs and meat. Therefore, this study aims to determine the prevalence of ESBL-producing and multidrug-resistant (MDR) *E. coli* isolates from poultry in Semnan province and investigate their association with different phylogenetic groups.

## Materials and Methods

### Sample collection

The present study was conducted on 100 non-duplicate *E. coli* isolates previously isolated from post-mortem specimens (liver and heart blood) collected from 15 broiler farms located in Semnan province. These isolates have been preserved at -70°C in the Microbiology Laboratory of the Veterinary Faculty of the Ferdowsi University of Mashhad. All isolates were revived by culturing in Brain Heart Infusion (BHI) broth. Following overnight incubation at 37°C, the isolates were subcultured onto Eosin Methylene Blue (EMB) and MacConkey agar. Pure colonies were presumptively identified as *E. coli* based on colony morphology and standard biochemical tests (e.g., Gram staining, oxidase/catalase, IMViC, triple sugar iron agar). Subsequently, *E. coli* isolates were confirmed by amplification of a specific region of the 16S rRNA gene (Aflakian et al. 2023).

### Antimicrobial susceptibility testing

The susceptibility of isolates has been determined using the Kerby-Bauer method as recommended (CLSI, 2023). Nine antibiotics were tested, comprising cefazoline (CZO, 30µg), ceftazidime (CAZ, 30µg), ceftriaxone (CRO, 30µg), cefotaxime (CTX, 30µg), amoxicillin/clavulanic acid (AMC, 30/10µg), aztreonam (AZT, 30µg), chloramphenicol (CHL, 30µg), tetracycline (TET, 30µg), and trimethoprim/sulfamethoxazole (SXT, 1.25/23.75µg). *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 278 were tested as quality controls.

### Evaluation of ESBL production

Isolates susceptible to at least one of the following antibiotics: ceftriaxone, cefotaxime, or aztreonam were screened for ESBL production using the double-disk synergy test (DDST). A bacterial suspension was prepared from fresh cultures to a 0.5 McFarland standard (1-1.5 $\times$ $10^8$ cfu/ml). A combined amoxicillin/clavulanic acid (30/10 µg) disk was placed at the center of a Mueller-Hinton agar plate. Discs containing ceftriaxone, cefotaxime, and aztreonam were then placed around the combined disk at a distance of 20 mm. Plates were incubated at 35 ± 2°C for 16-18 hours. ESBL production was considered positive if an increase in the zone of inhibition was observed around any of the cephalosporin discs towards the

amoxicillin/clavulanic acid disk (Zomorodi et al. 2020). *Klebsiella pneumoniae* ATCC 700603 was used as a positive control.

**Molecular detection of resistance genes**

The boiling method was conducted as explained previously for genomic DNA extraction (Zomorodi et al. 2022). A multiplex polymerase chain reaction (m-PCR) was performed to detect four resistance genes in all *E. coli* isolates. These genes were blaTEM, *bla*CTX-M, *bla*SHV, and *bla*OXA. Each PCR reaction was prepared in a 25 µl final volume consisting of 12.5 µL of PCR 2× Master Mix (Amplicon, Denmark), 1 µL of each primer (2 µM), 2 µL of template DNA, and up to the final volume (25 µL) added nuclease-free water. The sequence of primers used for detection was as follows: TEM-F (5'-CATTTCCGTGTCGCCCTTATTC-3') and TEM-R (5'-CGTTCATCCATAGTTGCCTGAC-3') for *bla*TEM, CTX-M-F (5' TTAGGAARTGTGCCGCTGYA-3') and CTX-M-R (5'- CGATATCGTTGGTGGTRCCAT-3') for $bla_{\text{CTX-M}}$, SHV-F (5'-AGCCGCTTGAGCAAATTAAAC-3') and SHV-R (5'-ATCCCGCAGATAAATCACCAC-3') for *bla*SHV, OXA-F (5'-GGCACCAGATTCAACTTTCAAG-3') and OXA-R (5'-GACCCCAAGTTTCCTGTAAGTG-3') for *bla*OXA. PCR conditions include of initial denaturation at 94 ℃ for 3 minutes, followed by 35 cycles of denaturation at 94 ℃ for 30 seconds, annealing at 57 ℃ for 30 seconds, extension at 72 ℃ for 40 seconds, and final extension at 72 ℃ for 5 minutes. The expected sizes of the amplified fragments were 800 bp, 713 bp, and 564 bp, respectively (Dallenne et al. 2010).

**Phylogroup determination**

*E. coli* isolates were assigned to phylogroups A, B1, B2, C, D, E, F, and G using a combination of quadraplex PCR and additional PCR methods as described by Clermont et al. (Clermont et al. 2013). In quadraplex-PCR, four genes (*arpA*, *chuA*, *yjaA*, and *TspE4.C2*) were amplified. The presence or absence of these genes was used to assign isolates to different phylogroups as follows: A (*arpA*+, *chuA*-, *yjaA*±, *TspE4.C2*-), B1 (*arpA*+, *chuA*-, *yjaA*-, *TspE4.C2*+), B2 (*arpA*-, *chuA*+, *yjaA*±, *TspE4.C2*+) or (*arpA*-, *chuA*+, *yjaA*+, *TspE4.C2*±), C (*arpA*+, *chuA*-, *yjaA*+, *TspE4.C2*-), D (*arpA*+, *chuA*+, *yjaA*-, *TspE4.C2*±), and E (*arpA*+, *chuA*+, *yjaA*±, *TspE4.C2*-) or (*arpA*+, *chuA*+, *yjaA*-, *TspE4.C2*±), F (*arpA*-, *chuA*+, *yjaA*-, *TspE4.C2*-), G (*arpA*-, *chuA*+, *yjaA*-, *TspE4.C2*±). However, additional PCR assays were performed using specific primers to confirm the assignment of isolates to phylogroups C, E, F, and G (Clermont et al. 2013; Clermont et al. 2019) (Table 1). The *trpA* gene was included as an internal control in all PCR reactions.

**Statistical analysis**

Data were analyzed using IBM SPSS Statistics version 22.0. Descriptive statistics, including relative frequencies, were calculated. Fisher's exact test was used to assess statistical significance, with a significance level of $p \leq 0.05$.

## Results

**Antimicrobial susceptibility testing and ESBL detection**

The highest resistance was against tetracycline (88%), followed by trimethoprim/sulfamethoxazole (86%), and chloramphenicol (70%). Conversely, the lowest resistance was detected against ceftriaxone (8%), aztreonam (13%), and cefotaxime (15%) (Figure 1). According to the MDR definition, isolates resistant to at least one agent in ≥ 3 different classes were considered MDR. Among the isolates studied, 70% were classified as MDR. Additionally, 41% of isolates were identified as ESBL producers. A significantly higher prevalence of MDR isolates was observed among ESBL-producing *E. coli* isolates compared to non-ESBL producer isolates (87.8% vs. 57.6%, $p < 0.001$). Furthermore, significant differences in resistance to cefazolin, ceftazidime, aztreonam, ceftriaxone, and chloramphenicol were observed among ESBL-producing *E. coli* isolates ($p \leq 0.005$) (Table 2). According to the antimicrobial resistance profile, MDR isolates were classified into 21 distinct resistance profiles. The most prevalent profile was resistance to TET-SXT-CZO (observed in 19/70 isolates, 27.1%), followed by resistance to TET-SXT-AMC (14/70 isolates, 20%) and resistance to TET-SXT-AMC-CZO (9/70 isolates, 12.8%) (Table 3).

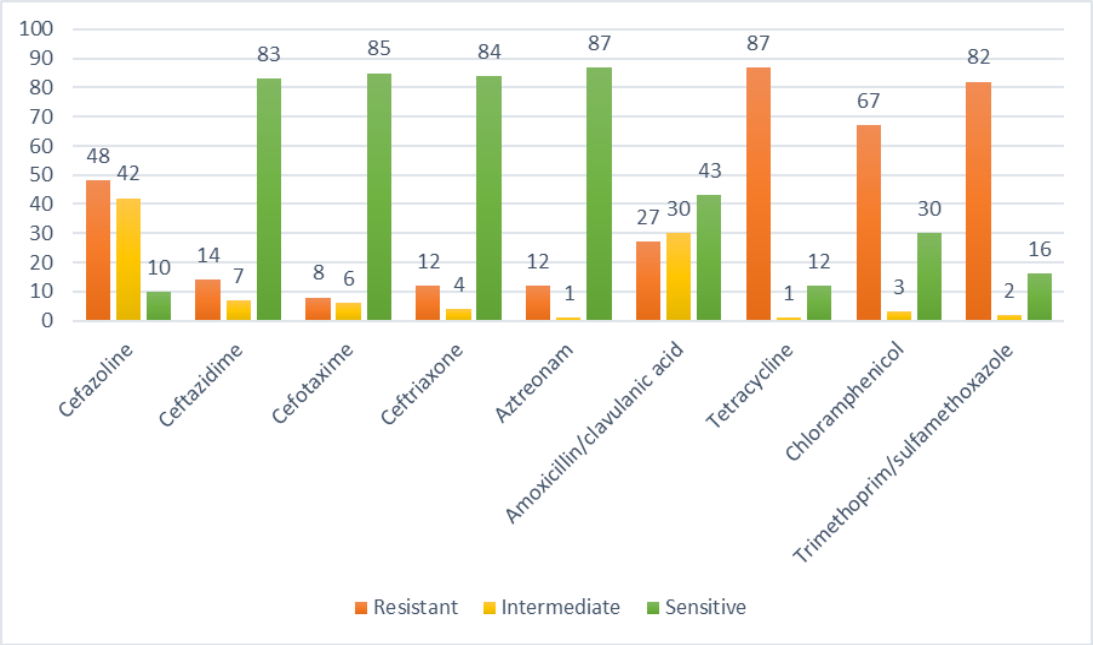



**Figure 1.** Antimicrobial susceptibility patterns of *E. coli i*solates from poultry Collibacillosis (N=100).

**Table 1.** The oligonucleotide sequence information of phylotyping PCR assays based on Clermont 2013 and 2019

| PCR name | Target gene | Primer sequence (5'-3') | Annealing (°C) | Product size (bp) | References |
|---|---|---|---|---|---|
| **Q-PCR** | *arpA* | F- AACGCTATTCGCCAGCTTGC<br>R- TCTCCCCATACCGTACGCTA | 59 °C | 400 | (Clermont et al. 2013) |
| | *chuA* | F-ATGGTACCGGACGAACCAAC<br>R-TGCCGCCAGTACCAAAGACA | | 288 | |
| | *yjaA* | F- CAAACGTGAAGTGTCAGGAG<br>R- AATGCGTTCCTCAACCTGTG | | 211 | |
| | *TspE4.C2* | F- CACTATTCGTAAGGTCATCC<br>R- AGTTTATCGCTGCGGGTCGC | | 152 | |
| **C-PCR** | *trpAgpC* | F- AGTTTTATGCCCAGTGCGAG<br>R- TCTGCGCCGGTCACGCCC | | 219 | |
| **E-PCR** | *arpAgE* | F- GATTCCATCTTGTCAAAATATGCC<br>R- GAAAAGAAAAAGAATTCCCAAGAG | 57 °C | 301 | (Clermont et al. 2019) |
| **F-PCR** | *cfaB* | F- CTAACGTTGATGCTGCTCTG<br>R- TGCTAACTACGCCACGGTAG | 59 °C | 384 | |
| **G-PCR** | *ybgD* | F- TATGCGGCTGATGAAGGATC<br>R- GTTGACTAAGCGCAGGTCGA | | 177 | |
| **Control** | *trpA* | F- CGGCGATAAAGACATCTTCAC<br>R- GCAACGCGGCCTGGCGGAAG | | 489 | |

**Molecular detection of resistance genes**

The prevalence of *bla*$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{OXA}$ was 48%, 3%, and 2%, respectiveley. Also, there were no *bla*$_{SHV}$ contanied isolates. Both of the *bla*$_{OXA}$ positive isolates co-harbored *bla*$_{TEM}$. Also, two out of three *bla*$_{CTX-M}$ containing isolates were positive for *bla*$_{TEM}$.

**Phylogroup analysis**

Most of the isolates belonged to B1 and D phylogroups, 23% and 22%, respectively. Generally, 30% of isolates were belonged to D or E phylogroups which 8% of them were placed in E phylogroup due to detecting phylogroup E specefic primer (ArpAgpE). Furthermore, isolates belonging to A or C phylogroups (14%) were discriminated using a phylogroup C-specific primer (trpAgpC). Of these, 10% and 4% were categorized as phylogroup A and C, respectively. The frequency of the recently updated phylogroup G was 11%, which was discriminated among isolates belonging to phylogroups B2 and F using specific primers for G (ybgD) and C (cfaB) (Figure 2A-C). However, 13%

of isolates could not be assigned to any phylogroup (Table 3).

Additionally, no significant differences were observed in the distribution of MDR and ESBL-producing *E. coli* isolates among the different phylogroups ($p > 0.05$). Table 4 presents the frequency of antimicrobial resistance, MDR, and ESBL-producing *E. coli* isolates separately for each phylogroup.

## Discussion

While the overuse of antimicrobials poses a serious threat to global health, the use of antibiotics in farm animals can contribute to reducing mortality rates. It is estimated that antibiotic usage in farm animals, particularly poultry, in middle-income countries will increase from 63,151 tons in 2010 to approximately 105,500 tons by 2030. (De Mesquita Souza Saraiva et al. 2022).

The WHO has identified a number of antimicrobials, including third- and fourth-generation cephalosporins, as critically important for both human and veterinary medicine (Organization 2021).

**Table 2.** The prevalence of antimicrobial resistance among ESBL and non-ESBL *E. coli* isolates from poultry (N=100).

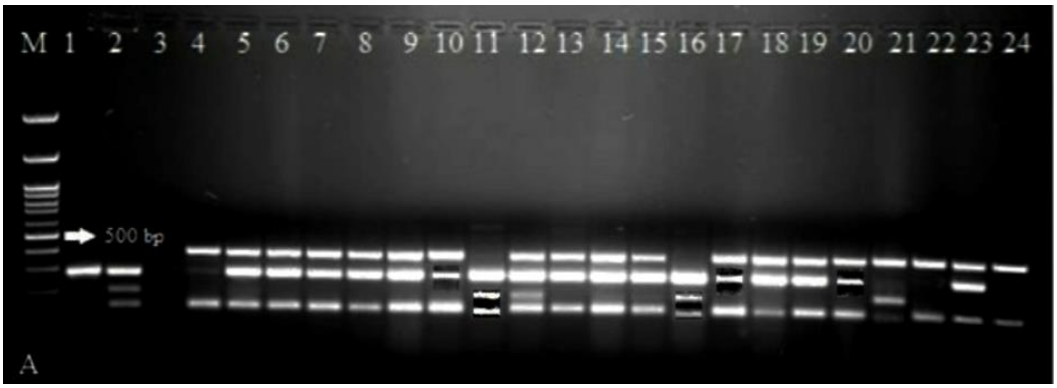
| Antibiotics | Resistance (%) | ESBL producer (n = 41) | Non-ESBL producer (n=59) | *p* value |
|---|---|---|---|---|
| **CZO** | 48 | 28 (68.3%) | 20 (33.9%) | **0.002** |
| **CAZ** | 14 | 12 (29.3%) | 2 (3.4%) | **0.001** |
| **CTX** | 8 | 8 (100%) | 0 | NC |
| **CRO** | 12 | 8 (19.5%) | 4 (6.8%) | **0.048** |
| **AZT** | 12 | 9 (22%) | 3 (5.1%) | **0.034** |
| **AMC** | 27 | 13 (31.7%) | 14 (23.7%) | 0.657 |
| **SXT** | 82 | 34 (82.9%) | 48 (81.4%) | 0.173 |
| **TET** | 87 | 36 (87.8%) | 51 (86.4%) | 0.157 |
| **CHL** | 67 | 34 (82.9%) | 33 (55.9%) | **0.017** |
| **MDR** | 70 | 36 (87.8%) | 34 (57.6%) | **0.001** |

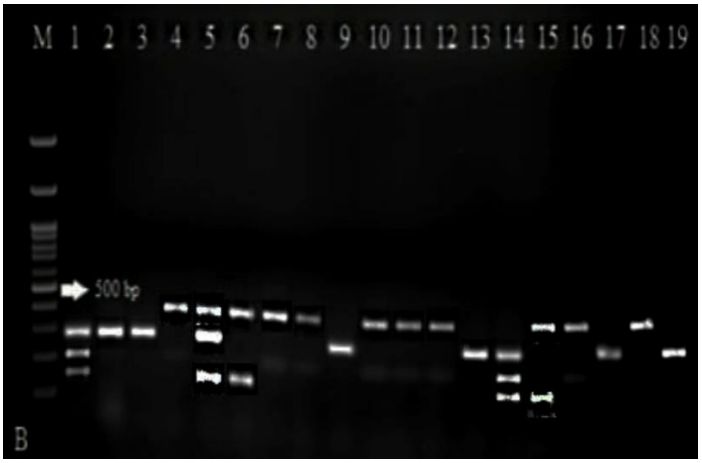
Abbreviation: CZO, cefazolin; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; AZT, aztreonam; AMC, amoxicillin/clavulanic acid; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; CHL, chloramphenicol; MDR, multi-drug resistance; NC, not calculable.

**Table 3.** Antimicrobial resistance profile of MDR *E. coli* isolates from poultry (N=70).

| | |
|---|---|
| TET-SXT-CZO | 19 |
| TET-SXT-AMC | 14 |
| TET-CHL-SXT | 3 |
| TET-AMC-CZO | 1 |
| TET-SXT-AMC-CZO | 9 |
| TET-SXT-AZT-CZO | 2 |
| TET-CHL-SXT-CZO | 2 |
| TET-CTX-SXT-CZO | 2 |
| TET-CHL-SXT-AZT | 1 |
| TET-CTX-SXT-AMC-CZO | 2 |
| TET-CHL-SXT-AMC-CZO | 2 |
| TET-CHL-SXT-AMC-CAZ | 1 |
| TET-CHL-SXT-AZT-CAZ-CZO | 2 |
| CHL-SXT-AMC-AZT-CAZ-CZO | 2 |
| TET-CHL-SXT-CRO-CAZ-CZO | 1 |
| TET-CTX-SXT-AMC-CAZ-CZO | 1 |
| TET-CTX-SXT-CRO-AZT-CAZ-CZO | 2 |
| TET-CTX-SXT-CRO-AMC-CAZ-CZO | 1 |
| TET-CTX-CRO-AMC-AZT-CAZ-CZO | 1 |
| TET-CHL-SXT-CRO-AMC-AZT-CZO | 1 |
| TET-CHL-SXT-CRO-AZT-CAZ-CZO | 1 |

Abbreviation: CZO, cefazolin; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; AZT, aztreonam; AMC, amoxicillin/clavulanic acid; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; CHL, chloramphenicol.



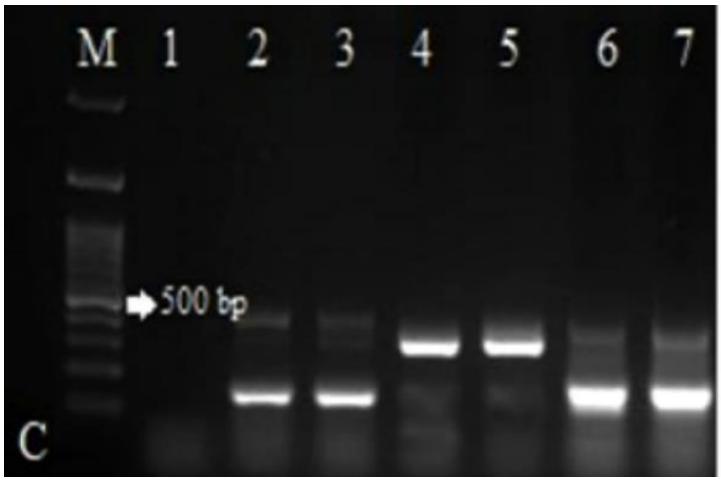




**Figure 2. A)** Line M: 100 bp DNA Ladder; Line 1: phylogroup F (arpA-, chuA+, yjaA-, TspE4.C2-); Lines 2, 11, and 16: phylogroup B2 (arpA-, chuA+, yjaA+, TspE4.C2+); Lines 4-10, 13-15, 17, 19, 20, and 23: phylogroups D or E (arpA+, chuA+, yjaA-, TspE4.C2+). **B)** Line M: 100 bp DNA Ladder; Line 1 and 14: phylogroup B2 (arpA-, chuA+, yjaA+, TspE4.C2+); Lines 2, 3, 9, 13,17 and 19: phylogroup F (arpA-, chuA+, yjaA-, TspE4.C2-); Lines 4, 7, 8, 10-12, 16, 18: phylogroups A or C (arpA+, chuA-, yjaA-, TspE4.C2-); Line 15: phylogroup B1 (arpA+, chuA-, yjaA-, TspE4.C2+); Line 5: phylogroups D or E (arpA+, chuA+, yjaA-, TspE4.C2+). **C)** Line M: 100 bp DNA Ladder; Line 1: control negative; Lines 2, 3, 6, and 7 phylogroup G (ybgD +; 177bp); Lines 4, 5: phylogroup F (cfaB +; 384bp).

**Table 4.** Frequency of antimicrobial resistance, MDR, and ESBL producing *E. coli* isolates among each phylogroups.

| Phylogroups (%) | CZO (N=48) | CAZ (N=14) | CTX (N=8) | CRO (N=12) | AZT (N=12) | AMC (N=27) | SXT (N=82) | TET (N=87) | CHL (N=67) | MDR (N=70) | ESBL (N=41) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A (10) | 5 | 0 | 0 | 0 | 1 | 2 | 8 | 6 | 8 | 8 | 5 |
| B1 (23) | 11 | 1 | 0 | 1 | 0 | 7 | 16 | 21 | 15 | 15 | 9 |
| B2 (5) | 2 | 0 | 1 | 0 | 0 | 1 | 4 | 4 | 3 | 3 | 3 |
| C (4) | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 3 | 3 | 0 |
| D (22) | 8 | 2 | 2 | 4 | 2 | 2 | 19 | 20 | 12 | 14 | 7 |
| E (8) | 3 | 2 | 1 | 2 | 1 | 4 | 7 | 7 | 4 | 5 | 4 |
| F (4) | 3 | 1 | 2 | 1 | 1 | 2 | 3 | 4 | 3 | 3 | 2 |
| G (11) | 9 | 7 | 1 | 4 | 6 | 5 | 11 | 10 | 9 | 11 | 6 |
| Unknown (13) | 7 | 1 | 1 | 0 | 1 | 4 | 10 | 11 | 10 | 8 | 5 |

In the present investigation, susceptibility of *E. coli* isolates from poultry to the tested third-generation cephalosporins ranged from 83% to 85%. This frequency is slightly lower than that reported in previous studies, which ranged from 90% to 95%. (Assoumy et al. 2021; Aworh et al. 2021). However, the prevalence of ESBL-producing *E. coli* isolates was 49%, which is in good agreement with a previous survey conducted in turkeys (Baran et al. 2020). These findings were lower than those reported by other studies, such as 62.5% by Ilyas et al. in Pakistan and 75% by Badr et al. in Egypt (Ilyas et al. 2021; Badr et al. 2022). Although some studies have reported a low prevalence of ESBL-producing *E. coli* isolates in poultry (6.8% and 6.5%) (Kim et al. 2021; Tan et al. 2023), it seems the use of third-generation cephalosporins requires more caution. ESBL-producing *E. coli* strains can turn commensal and antimicrobial-susceptible strains to pathogens and resistant phenotypes. Indeed, ESBL-producing *E. coli* is considered a reservoir for transferring ESBL genes through horizontal gene transfer phenomena among different strains or even species (Mitman et al. 2022).

In addition, ESBL-producing *E. coli* isolates frequently exhibit resistance to multiple antibiotics, which is a significant factor in the development of MDR phenotypes in this study, 70 isolates (70%) were classified as MDR, and a significant proportion of these (36/70, 87.8%) were ESBL producers. Prior studies (Aworh et al. 2020; Mgaya et al. 2021) have also demonstrated this significant association. The highest resistance rates were observed for tetracycline (87% of samples) and trimethoprim/sulfamethoxazole (82% of samples). Notably, 19/70 (27.1%) of the MDR isolates exhibited resistance to cefazolin, trimethoprim/sulfamethoxazole, and tetracycline. The high rate of tetracycline and trimethoprim/sulfamethoxazole resistance is in line with what was found before, which was that tetracycline resistance was present in 97.1% of cases (Assoumy et al. 2021), trimethoprim/sulfamethoxazole resistance was present 91.4% of the time, and 74.2% of cases (Elmi et al. 2021). These high resistance values can be attributed, in part, to the widespread use of these antibiotics in poultry farming worldwide. Tetracycline, in particular, alongside penicillins and aminoglycosides, has been extensively used in sub-therapeutic concentrations as a growth promoter in livestock and poultry, contributing to the development of antimicrobial resistance (Rahman et al. 2022).

According to the phylogenetic analysis, the most prevalent isolates (23%) belonged to phylogroup B1. This finding is consistent with previous investigations that reported phylogroup B1 as the most common among *E. coli* isolates from poultry (Coura et al. 2017; Aworh et al. 2021). Several studies have indicated that *E. coli* isolates from phylogroups A and B1, which are closely related, are generally not associated with high virulence potential based on genotyping analysis (Deku et al. 2022; Murase et al. 2022). Furthermore, previous studies have demonstrated that MDR *E. coli* isolates are frequently associated with phylogroup B2 (Yang et al. 2018; Gatya Al-Mayahie et al. 2022). While our findings revealed that the majority of MDR *E. coli* isolates belonged to phylogroups A and B1 (a total of 23/70, 32.8%), these results are supported by similar investigations that reported the predominance of MDR *E. coli* isolates from poultry in phylogroups A and B1 (Olowe et al. 2019; Bhowmik et al. 2023). In addition, 14 out of 41 ESBL-producing *E. coli* isolates from poultry were assigned to phylogroups A and B1. Notably, this finding aligns with other research that has demonstrated the predominance of ESBL-producing *E. coli* isolates from diarrheic cases in phylogroups A and B1 (Jafari et al. 2020). These comparisons are concerning because commensal *E. coli* isolates have the potential to acquire ESBL-producing and MDR phenotypes, posing a significant threat to global health. Further investigations in diverse

geographical areas are necessary to more comprehensively understand this phenomenon.

Our results identified phylogroup D as the second most prevalent phylogroup, representing 22% of the isolates. Previous studies have reported that phylogroups D and B2 are frequently associated with pathogenic and virulent *E. coli* strains (Aswal et al. 2023). This is supported by Aguirre-Sánchez et al., who found that isolates belonging to phylogroup D were frequently associated with clinical samples (Aguirre-Sánchez et al. 2022). These findings suggest that poultry may play a role in the transmission and circulation of virulent *E. coli* strains. However, virulence genotyping and pathotyping were not evaluated in the present study. Phylogroup G has been designated as an intermediate between phylogroups F and B2 based on recent whole-genome sequencing analyses (Clermont et al. 2019). Interestingly, 11 isolates were assigned to phylogroup G; all of these were MDR, and six were ESBL producers. While previous surveys of *E. coli* isolates from animals and humans have reported lower prevalence of phylogroup G (Marin et al. 2022; Lagerstrom et al. 2023), its true frequency remains to be fully elucidated. Variations in phylogroup prevalence across studies may be influenced by host factors, environmental conditions, and methodological differences in *E. coli* characterization. This study primarily focused on the prevalence of ESBL-producing and MDR *E. coli* isolates and their phylogenetic distribution. Further investigations, including the characterization of virulence factors and pathotypes, would likely provide more comprehensive insights.

## Conclusion

This study revealed a high prevalence of ESBL-producing and multidrug-resistant (MDR) *E. coli* isolates among *E. coli* strains isolated from poultry. The *bla*TEM gene was the most prevalent among the ESBL-mediated genes detected. Applying antimicrobial stewardship is critical because the circulation of ESBL-producing *E. coli* and MDR isolates threatens medicine and veterinary. The high prevalence of ESBL-producing and MDR *E. coli* isolates within the B1 phylogroup, which is frequently associated with environmental isolates, raises concerns about potential public health implications. Our findings contribute to a better understanding of the significant presence of D and G phylogroup isolates in poultry. These results suggest that further molecular epidemiological investigations are warranted to assess the poultry industry as a potential major reservoir for pathogenic *E. coli*.

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**Conflict of Interests**

The authors declare no conflict of interest.

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