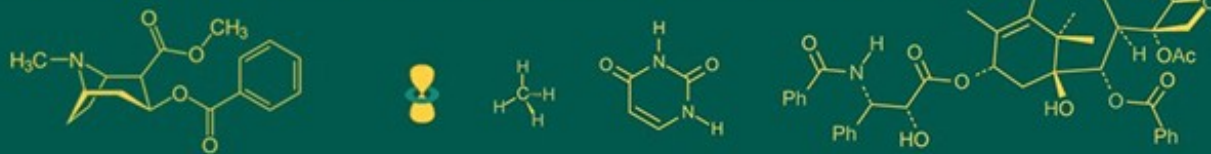


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 Indexing: Embase
 IJABR 2026; 10(9): 703-709
www.biochemjournal.com
 Received: 06-07-2026
 Accepted: 17-08-2026
 Published: 26-09-2026

VD Patel

M.V.Sc Scholar, Dept. of
 Veterinary Microbiology,
 College of Veterinary Science
 & Animal Husbandry,
 Kamdhenu University, Anand,
 Gujarat, India

RA Mathakiya

Associate Professor, Dept. of
 Veterinary Microbiology,
 College of Veterinary Science
 & Animal Husbandry,
 Kamdhenu University, Anand,
 Gujarat, India

BB Javia

Professor & Head, Dept. of
 Veterinary Microbiology,
 College of Veterinary Science
 & Animal Husbandry,
 Kamdhenu University, Anand,
 Gujarat, India

AC Patel

Associate Professor, Dept. of
 Veterinary Microbiology,
 College of Veterinary Science
 & Animal Husbandry,
 Kamdhenu University, Anand,
 Gujarat, India

Phenotypic and molecular characterization of antimicrobial resistance in *Escherichia coli* isolated from poultry cloacal samples

VD Patel¹, RA Mathakiya², BB Javia³, AC Patel⁴

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i9i.10155>

ABSTRACT

Antimicrobial resistance (AMR) in poultry-associated *Escherichia coli* (*E. coli*) poses a significant public health concern due to its potential transmission through the food chain. The present study was undertaken to isolate *E. coli* from poultry cloacal samples, detect extended-spectrum β -lactamase (ESBL) production phenotypically, and molecularly characterize antimicrobial resistance-associated genes in the recovered isolates. A total of 50 cloacal samples were collected from layer birds, of which 20 samples (40.00%) were confirmed as *E. coli* based on characteristic growth on MacConkey agar and eosin methylene blue (EMB) agar, standard biochemical tests, and molecular confirmation by polymerase chain reaction (PCR). Molecular identification was carried out by amplification of the 16S–23S rRNA intergenic region using species-specific primers Eco-223 (F) and Eco-455 (R). All confirmed isolates were subjected to antimicrobial susceptibility testing (AST) using the disk diffusion method. The highest resistance was observed against tetracycline (90.00%), followed by ciprofloxacin (70.00%) and levofloxacin (60.00%). In contrast, high levels of susceptibility were recorded for gentamicin (85.00%), amikacin (80.00%), ceftriaxone (80.00%), amoxicillin–clavulanic acid (75.00%), cefoperazone (75.00%), cefotaxime (65.00%), and cotrimoxazole (60.00%). Phenotypic screening identified ESBL production in one isolate (5.00%). Molecular characterization of antimicrobial resistance-associated genes by PCR revealed the presence of the AmpC β -lactamase-associated gene *bla_{CMY}* in 7 isolates (35.00%), *tetA* gene in 12 isolates (60.00%), *tetB* gene in 2 isolates (10.00%), class 1 integron-associated gene (*intI1*) in 6 isolates (30.00%), and *sul1* gene in 1 isolate (5.00%). The findings indicate substantial phenotypic antimicrobial resistance among the recovered *E. coli* isolates and the presence of molecularly characterize selected antimicrobial resistance-associated genes, including *tetA*, *tetB*, *sul1*, *bla_{CMY}* and class 1 integron-associated gene (*intI1*). These findings emphasize the importance of continuous AMR surveillance and judicious antimicrobial use in poultry production systems.

Keywords: *Escherichia coli*, Poultry, Antimicrobial resistance, ESBL, Resistance genes

Introduction

Antimicrobial Resistance (AMR) occurs when microorganisms evolve mechanisms that reduce the effectiveness of antimicrobial agents used to treat infectious diseases. It has emerged as one of the most significant global public health challenges of the twenty-first century. It is estimated that infections caused by antimicrobial-resistant pathogens could result in approximately 10 million deaths annually by 2050 if appropriate interventions are not implemented (O'Neill, 2016) [1]. Recognizing the urgency of this issue, the Quadripartite alliance comprising the Food and Agriculture Organization (FAO), the United Nations Environment Programme (UNEP), the World Health Organization (WHO), and the World Organisation for Animal Health (WOAH) has advocated for an integrated global surveillance system encompassing both human and animal health sectors under the One Health approach.

Among livestock production systems, the poultry industry has experienced remarkable growth worldwide owing to the increasing demand for affordable, high-quality animal protein. In India, poultry has become one of the fastest-growing sectors of animal husbandry. According to the Basic Animal Husbandry Statistics (2025) [2], poultry meat production reached 5.18 million tonnes during 2024-25, accounting for nearly 50% of the country's total meat production. Furthermore, poultry meat production increased by 3.10% compared with the previous year, reflecting the continuous expansion and improved productivity of the industry (BAHS, 2025) [2].

The rapid intensification of poultry production has been accompanied by increased use of antimicrobials for disease prevention, treatment, and, in some production systems, growth promotion (Gelband et al., 2015) [3]. However, the inappropriate use of antibiotics-including prophylactic administration without confirmed diagnosis, incorrect dosing, and failure to observe recommended withdrawal periods has contributed substan-

Corresponding Author:
VD Patel

M.V.Sc Scholar, Dept. of
 Veterinary Microbiology,
 College of Veterinary
 Science & Animal
 Husbandry, Kamdhenu
 University, Anand, Gujarat,
 India

tially to the emergence and spread of antimicrobial-resistant bacteria in poultry production (Rawat *et al.*, 2022; Tang *et al.*, 2022) [4, 5]. *Escherichia coli* (*E. coli*), belonging to the family *Enterobacteriaceae*, is a normal inhabitant of the gastrointestinal tract of poultry, animals, and humans, while also serving as an important foodborne pathogen (Levine, 1987) [6]. Based on their virulence characteristics and associated clinical manifestations, *E. coli* strains are categorized into several pathotypes, including enterohemorrhagic (EHEC), enterotoxigenic (ETEC), necrotoxicogenic (NTEC), enteroinvasive (EIEC), enteropathogenic (EPEC), attaching and effacing (AEEC), enteroaggregative (EAEC), avian pathogenic (APEEC), septicemic (SEPEC), and uropathogenic (UPEC) *E. coli* (Shahrani *et al.*, 2014) [7].

In poultry, *E. coli* is an important component of the normal intestinal microbiota and contributes to the maintenance of gut health and intestinal integrity. Although the majority of *E. coli* strains exist as harmless commensals, certain strains possess virulence factors that enable them to cause disease. Both pathogenic and non-pathogenic strains can successfully establish and persist within the avian gastrointestinal tract. However, factors such as environmental stress, poor management, immunosuppression, or concurrent infections may disrupt the normal microbial balance, allowing pathogenic *E. coli* to proliferate and cause disease (Dominick *et al.*, 1984) [8]. Additionally, antimicrobial-resistant *E. coli* can be transmitted from poultry to humans, potentially causing infections that are difficult or even impossible to treat (Hussain *et al.*, 2017; Stromberg *et al.*, 2017; Masachessi *et al.*, 2018; Mellata *et al.*, 2018; Meena *et al.*, 2021) [9, 10, 11]. Therefore, the present study aimed to characterize the phenotypic antimicrobial resistance patterns of *E. coli* isolated from cloacal samples of layer birds and to investigate selected antimicrobial resistance-associated genes, including *bla_{CMY}*, *tetA*, *tetB*, *sulI* and *intI1*, along with phenotypic ESBL production.

Materials and Methods

Collection of samples

A total of 50 cloacal swab samples were randomly collected from layer birds maintained at a poultry farm in Anand, Gujarat, India. Following collection, the swabs were placed in sterile transport containers and transported under cold-chain conditions to the Department of Veterinary Microbiology, College of Veterinary science and Animal Husbandry, Kamdhenu University, Anand, Gujarat for subsequent laboratory investigation.

Isolation and identification

Bacterial isolation was carried out in accordance with the procedures outlined by Barrow *et al.* (2004) [12] and Markey *et al.* (2013) [13]. Briefly, the collected cloacal swab specimens were inoculated onto MacConkey agar and incubated aerobically at 37 °C for 24 h. Lactose-fermenting colonies were selected and subsequently streaked onto Eosin Methylene Blue (EMB) agar for further differentiation. Colonies displaying the characteristic metallic-green appearance on EMB agar were considered presumptive *Escherichia coli*. The presumptive isolates were subjected to conventional biochemical characterization as part of the preliminary identification process. Following biochemical characterization and molecular confirmation, the con-

firmed *E. coli* isolates were maintained on Brain Heart Infusion (BHI) agar slants at 4 °C until further investigation.

Phenotypic antimicrobial susceptibility testing (AST)

The antimicrobial susceptibility of the isolates was evaluated using the Kirby–Bauer disk diffusion technique, following the method originally described by Bauer *et al.* (1966) [14]. Mueller–Hinton agar (HiMedia, India) was used for antimicrobial susceptibility testing (AST) of the *E. coli* isolates. A total of 12 antibiotic discs (HiMedia, India), representing different antimicrobial classes and commonly used in the poultry sector (Table 3), were tested using the standard Kirby–Bauer disc diffusion method described by Bauer *et al.* (1966) [14]. The results were interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI).

Phenotypic ESBL detection

Phenotypic detection of extended-spectrum β-lactamase (ESBL) production was carried out for all 20 confirmed *E. coli* isolates using the combination disc method. Cefotaxime (30 µg) and cefotaxime (30 µg) combined with clavulanic acid (10 µg) discs (HiMedia Laboratories Pvt. Ltd., India) were used for the test. The discs were placed on Mueller–Hinton agar inoculated with the test isolate and incubated at 37 °C for 18–24 h. After incubation, the zones of inhibition were measured in millimetres. An isolate was considered ESBL positive when an increase of ≥ 5 mm in the inhibition zone diameter was observed with the cefotaxime–clavulanic acid combination disc compared with the cefotaxime disc alone. The ESBL production status was determined for all 20 *E. coli* isolates based on this criterion.

Genomic DNA extraction

Genomic DNA from *E. coli* was extracted using the boiling colony method. Briefly, three to five colonies from an overnight culture were transferred into 200 µL of sterile distilled water and homogenized thoroughly by vortexing. The resulting suspension was heated at 95 °C for 15 min and then centrifuged at 14,000 rpm for 8 min. The clear supernatant containing the released DNA was carefully collected and stored at -20 °C until further molecular analysis (Salehi *et al.*, 2008) [15]. DNA concentration was determined by measuring absorbance at 260 nm using a NanoDrop spectrophotometer. An absorbance value of 1.0 at 260 nm was considered equivalent to 50 µg/mL of DNA. DNA purity was evaluated based on the A260/A280 absorbance ratio, with a value close to 1.8 considered indicative of good-quality, relatively pure DNA.

Molecular detection of *E. coli* isolates

Presumptive *E. coli* isolates identified by conventional culture and biochemical characterization were subjected to species-specific PCR for molecular confirmation. PCR amplification targeted the *E. coli*-specific Eco 223/Eco 455 primer pair, which generates a 232-bp amplicon. The primer sequences and expected amplicon size are provided in Table 1. PCR was performed in a final volume of 25 µL containing 12.5 µL of 2× Green PCR Master Mix (Genetaq), 1.0 µL each of forward and reverse primers (10 pmol/µL), 3.0 µL of template DNA, and 7.5 µL of nuclease-free water. Amplification was carried out using a Biometra TRIO thermal cycler (Analytik Jena GmbH + Co. KG, Germany) under the PCR conditions described below.

Table 1. Primer set used for Molecular Identification of *E. coli*

Targeted Organism/ Gene	Primer/Gene	Primer Sequence (5'-3')	Product Size (bp)	References
<i>E. coli</i>	Eco 223 (F)	ATCAACCGAGATTCCTCCAGT	232	Riffon <i>et al.</i> (2001)
	Eco 455 (R)	TCACTATCGGTCAGTCAGGAG		

Molecular detection of antimicrobial resistance genes in *E. coli* isolates

Genotypic antimicrobial resistance among the *E. coli* isolates was assessed by PCR amplification of selected antimicrobial resistance genes associated with resistance to tetracyclines, sulfonamides, and β-

lactam antibiotics. A total of 20 *E. coli* isolates were screened for the presence of *tetA*, *tetB*, *sulI* and *bla_{CMY}* genes using specific primers, as listed in Table (2). The *E. coli* isolates were screened for the presence of the integron gene, specifically *intI1*, as described in Table .

PCR amplification was carried out in a final reaction volume of 25 µL for each target gene. Each reaction contained 12 µL of 2× PCR Master Mix, 10 pmol each of the forward and reverse primers, 3 µL of template DNA, and nuclease-free water to obtain the final volume of 25 µL. The specific thermal cycling conditions and PCR parameters

used for each target gene are presented in Supplementary Table . The PCR products were separated by electrophoresis on 1.5–2% agarose gels containing ethidium bromide at a concentration of 0.5 µg/mL, and the amplified bands were visualized and documented using a gel documentation system.

Table 2. Primers used for the detection of *E. coli* specific gene, ARGs and integron gene

Sr. No.	Targeted Gene	Primer Sequence (5'-3')	Product Size (bp)	References
1	<i>blaCMY</i> (F)	TGGCCAGAACTGACAGGCAAA	462	Van <i>et al.</i> (2008)
	<i>blaCMY</i> (R)	TTTCTCCTGAACGTGGCTGGC		
2	<i>intI1</i> (F)	ACGAGCGCAAGGTTTCGG	565	Goudarzi and Eftekhari (2014)
	<i>intI1</i> (R)	GAAAGGTCTGGTCATACATG		
3	<i>sulI</i> (F)	TTCGGCATTCTGAATCTCAC	822	Shehata <i>et al.</i> (2016)
	<i>SulI</i> (R)	ATGATCTAACCCCTCGGTCTC		
4	<i>tetA</i> (F)	GTGAAACCCAACATACCCC	887	
	<i>tetA</i> (R)	GAAGGCAAGCAGGATGTAG		
5	<i>tetB</i> (F)	CCTTATCATGCCAGTCTTGC	773	
	<i>tetB</i> (R)	ACTGCCGTTTTTCGCC		

blaCMY: Beta-lactamase, *sulI*: Sulphonamide resistant dihydropteroate synthase, *intI1*: class 1 integron integrase, *tetA* & *tetB*: Tetracycline

Results

A total of 50 cloacal samples were collected from layer birds, of which 20 (40.00%) were confirmed as *Escherichia coli*. Identification was based on characteristic growth on MacConkey and eosin methylene blue (EMB) agar, biochemical characteristics, and molecular confirmation by PCR. Molecular identification was further confirmed by amplification of the 16S–23S rRNA intergenic region using the species-specific primers Eco-223 (F) and Eco-455 (R).

In vitro antimicrobial susceptibility testing of the 20 *E. coli* isolates revealed variable resistance to the antimicrobials tested. The highest resistance was observed against tetracycline (90.00%), followed by ciprofloxacin (70.00%), enrofloxacin (60.00%), and levofloxacin (60.00%). Moderate resistance was recorded against erythromycin (45.00%) and co-trimoxazole (40.00%). Comparatively lower resistance was observed against cefotaxime (25.00%), amikacin (20.00%), amoxicillin/clavulanic acid (15.00%), cefoperazone (15.00%), ceftriaxone (15.00%), and colistin sulphate (15.00%). None of the isolates showed resistance to gentamicin.

The MAR index was calculated for each *E. coli* isolate by dividing the number of antimicrobials to which the isolate exhibited resistance by the total number of antimicrobials tested ($n = 12$). The MAR index ranged from 0.17 to 0.75, with a mean value of 0.38.

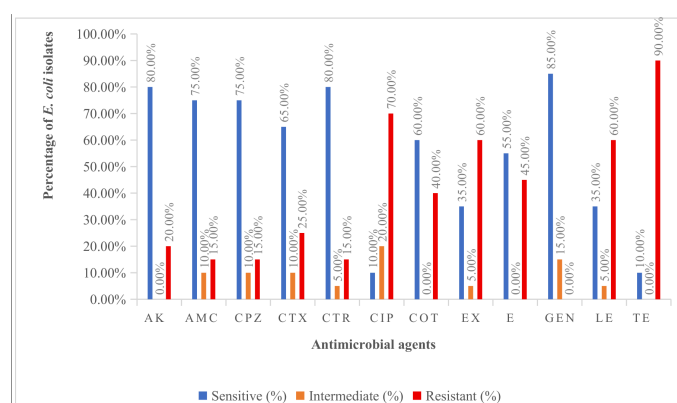


Figure 1. Antimicrobial susceptibility pattern of *E. coli* Isolates

(AK - Amikacin, AMC - Amoxycillin/clavulanic acid, CPZ - Cefoperazone, CTX - Cefotaxime, CTR - Ceftriaxone, CIP - Ciprofloxacin, CS - Colistin sulphate, COT - Co-Trimazole, EX - Enrofloxacin, E - Erythromycin, GEN - Gentamicin, LE - Levofloxacin, TE - Tetracycline)

Table 3. Antibiotic discs used for the evaluation of phenotypic antimicrobial susceptibility among *E. coli* isolates

Sr. No	Antibiotic class	Antibiotic used	Drug concentration (µg/disc)	Sensitivity Criteria (Zone Diameter in mm)		Resistance Criteria
1.	Aminoglycoside	Amikacin	30	≥ 20		≤ 16
		Gentamicin	10	≥ 18		≤ 14
2.	Penicillin with beta-lactamase inhibitor	Amoxicillin/clavulanic acid	20/10	≥ 18		≤ 13
3.	Third Generation Cephalosporin	Cefoperazone	10	≥ 21		≤ 15
		Cefotaxime	30	≥ 26		≤ 22
		Ceftriaxone	30	≥ 23		≤ 19
4.	Fluoroquinolone	Ciprofloxacin	5	≥ 26		≤ 21
		Enrofloxacin	10	≥ 40		≤ 30
		Levofloxacin	5	≥ 21		≤ 16
5.	Sulphonamide	Co-trimoxazole	23.75/1.25	≥ 16		≤ 10
6.	Macrolide	Erythromycin	15	≥ 13		≤ 12
7.	Tetracycline	Tetracycline	30	≥ 15		≤ 11

(continued on next page)

The highest MAR index (0.75) was observed in isolate no.3, which exhibited resistance to nine antimicrobials. Isolate no. 2 and 17 showed resistance to seven antimicrobials each, corresponding to a MAR index of 0.58. The lowest MAR index (0.17) was recorded for isolate no. 4, 9 and 16, each showing resistance to two antimicrobials.

Phenotypic ESBL testing identified one (5.00%) *E. coli* isolate as ESBL-positive, whereas the remaining 19 (95.00%) isolates were ESBL-negative

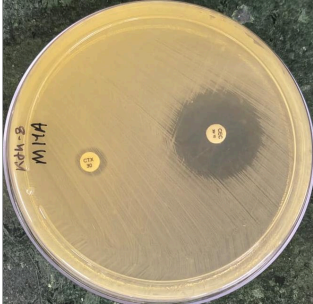


Figure 2. Phenotypic ESBL detection of *E. coli*

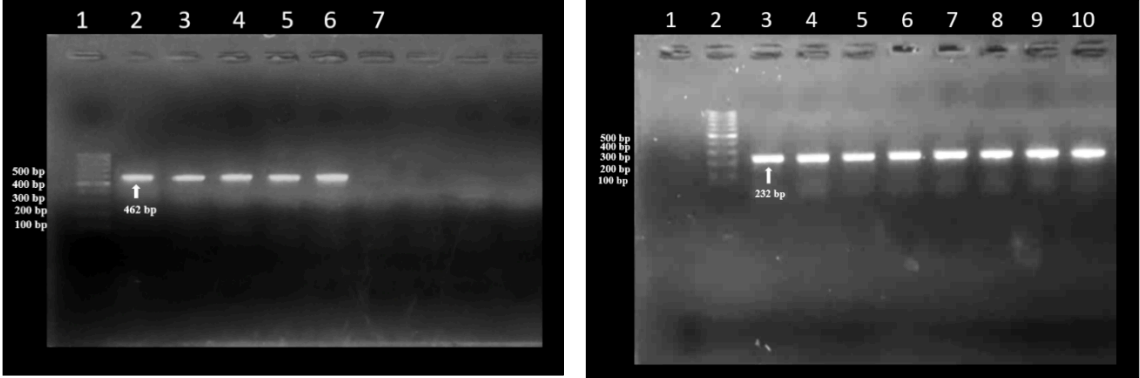


Figure 3. PCR amplification of *E. coli* isolates confirmation Fig. 4. PCR amplification of the *bla*_{CMY} resistance gene

Lane 1: NC; Lane 2: 100-bp DNA ladder; Lane 1: 100-bp DNA ladder; Lane 2: PC

Table 4. Multiple Antibiotic Resistance (MAR) index of 20 *Escherichia coli* isolates based on phenotypic antimicrobial susceptibility testing (Mean MAR index = 0.38)

Isolate No.	No. resistant antibiotics	MAR index	Isolate No.	No. resistant antibiotics	MAR index
1	3	0.25	11	6	0.50
2	7	0.58	12	5	0.42
3	9	0.75	13	5	0.42
4	2	0.17	14	4	0.33
5	4	0.33	15	3	0.25
6	5	0.42	16	2	0.17
7	6	0.50	17	7	0.58
8	6	0.50	18	6	0.50
9	2	0.17	19	3	0.25
10	4	0.33	20	3	0.25

Lane 3: PC; Lanes 4-10: positive samples Lanes 3-6 :Positive Samples, Lane 7: NC

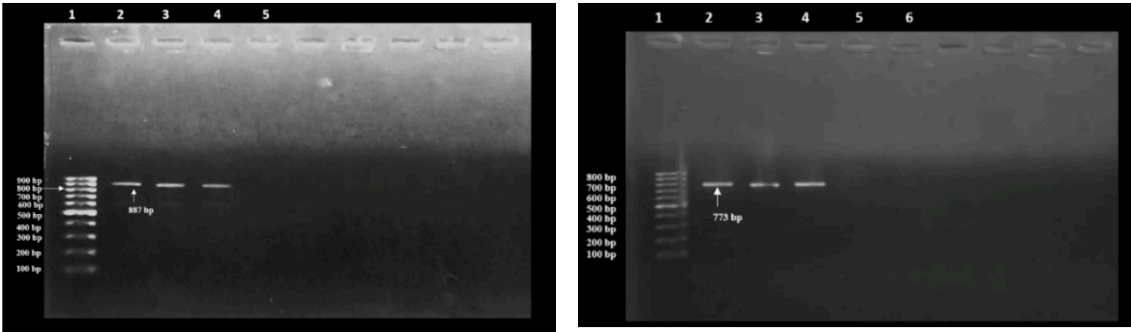


Figure 5. PCR amplification of the *tetA* resistance gene Fig. 6. PCR amplification of the *tetB* resistance gene

Lane 1: 100-bp DNA ladder; Lane 2: PC; Lane 1: 100-bp DNA ladder; Lane 2: PC; Lanes 3-4 :Positive Samples; Lane 5: NC Lanes 3-4 :Positive Samples; Lane 5: NC

Lane 1: 100-bp DNA ladder; Lane 2: PC; Lane 1: 100-bp DNA ladder; lane 2: PC

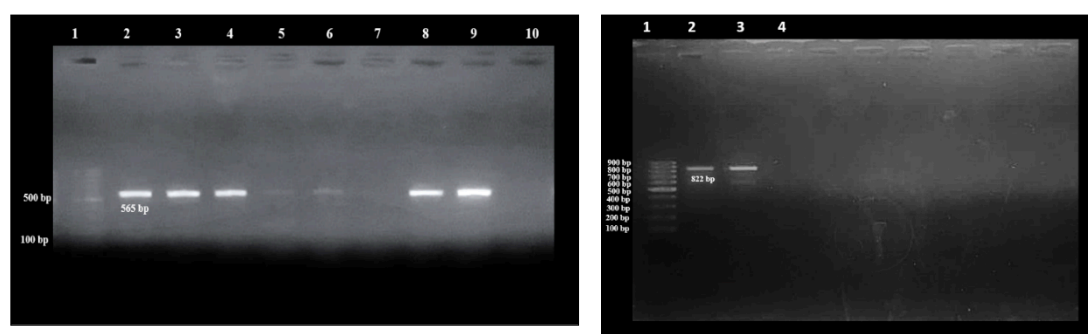


Figure 7. PCR amplification of the *Int1* gene **Fig. 8.** PCR amplification of the *Sull* resistance gene

Lanes 2-4 & 8,9: Positive samples; Lane 10: NC Lane 3: Positive sample; Lane 4: NC

(PC : Positive control; NC: Negative control; bp: Base pair)

Among the antimicrobial resistance-associated genes investigated, *tetA* was detected in 12 (60.00%) isolates, followed by *bla_{CMY}* in 7 (35.00%), *int1* in 6 (30.00%), *sull* in 1 (5.00%), and *tetB* in 2 (10.00%) isolates.

Discussion

The recovery of *E. coli* from 20 of 50 cloacal samples (40.00%) indicates the occurrence of *E. coli* among the layer birds examined in the present study. The isolates were confirmed using cultural, biochemical, and molecular approaches, with amplification of the 16S–23S rRNA intergenic region providing species-level confirmation. The combined use of phenotypic and molecular methods increased the reliability of isolate identification.

The antimicrobial susceptibility profile demonstrated substantial variation among the tested antimicrobials. The highest resistance was observed against tetracycline (90.00%), followed by ciprofloxacin (70.00%), enrofloxacin (60.00%), and levofloxacin (60.00%). The resistance rates observed against tetracycline and ciprofloxacin were comparable to those reported by Hussain *et al.* (2017) [9], who documented resistance rates of 84.00% and 70.00%, respectively, among poultry-associated *E. coli* isolates in India. Similarly, Patel *et al.* (2024) [16] reported a comparable ciprofloxacin resistance rate of 66.66% among avian pathogenic *E. coli* isolated from broiler chickens in India. In contrast, Kaushik *et al.* (2018) [17] reported a considerably lower ciprofloxacin resistance rate of 6.5%, indicating substantial geographical and epidemiological variation in fluoroquinolone resistance among *E. coli* populations. The high resistance to tetracycline and fluoroquinolones observed in the present study may reflect antimicrobial selection pressure associated with their use in poultry production.

The erythromycin resistance observed in the present study (45.00%) was slightly lower than the 49.35% reported by Jaiswal *et al.* (2024) [18] among *E. coli* isolates recovered from broiler chicken meat in Prayagraj, Uttar Pradesh. In contrast, Amer *et al.* (2018) [19] reported a lower resistance rate of 35.00% among pathogenic *E. coli* isolated from diseased broiler chickens in Egypt. Resistance to amikacin (20.00%) and amoxicillin/clavulanic acid (15.00%) in the present study was higher than the absence of resistance reported by Khant *et al.* (2026) [20] among *E. coli* isolates obtained from faecal samples of poultry and other animal species in and around Anand, Gujarat. Conversely, cefoperazone resistance in the present study (15.00%) was substantially lower than the 64.00% reported by Khant *et al.* (2026) [20]. Such differences may be associated with variations in geographical location, host species, sample source, antimicrobial-use practices, and selection pressure.

The MAR index ranged from 0.17 to 0.75, with a mean value of 0.38, demonstrating considerable variation in the extent of multidrug resistance among the *E. coli* isolates. The highest MAR index of 0.75 was

recorded for isolate no. 3, which exhibited resistance to nine of the 12 antimicrobials tested. Isolates 2 and 17 also demonstrated relatively high MAR indices of 0.58. The high MAR values observed in several isolates indicate exposure to multiple antimicrobials and suggest substantial antimicrobial selection pressure within the study population. The occurrence of isolates resistant to multiple antimicrobial classes is of epidemiological importance because poultry-associated *E. coli* may serve as a reservoir of antimicrobial resistance determinants. Only one isolate (5.00%) was phenotypically identified as ESBL-positive in the present study, while 95.00% of the isolates were ESBL-negative. The low proportion of phenotypically ESBL-positive isolates indicates a relatively limited occurrence of the ESBL phenotype among the *E. coli* isolates examined. However, the detection of β -lactamase-associated resistance genes in some isolates highlights the importance of combining phenotypic susceptibility testing with molecular characterization for a better understanding of antimicrobial resistance.

The *sull* gene was detected in 1 (5.00%) *E. coli* isolate in the present study. This prevalence was comparable to the 6.3% reported by Kim *et al.* (2020) [21] among avian pathogenic *E. coli* isolates from broiler chickens, but considerably lower than the 59.15% reported by Rajkhowa *et al.* (2021) [22] among APEC isolates from Mizoram, India. Similarly, Racewicz *et al.* (2022) [23] reported a *sull* prevalence of 16.00% among *E. coli* isolates recovered from poultry cloacal swabs. The lower prevalence observed in the present study may be related to differences in geographical location, isolate characteristics, antimicrobial exposure, and poultry production systems.

The *tetA* gene was detected in 60.00% (12/20) of the *E. coli* isolates, which was considerably higher than the 16.00% reported by Racewicz *et al.* (2022) [23] among multidrug-resistant *E. coli* isolates recovered from cloacal swabs of poultry. In contrast, *tetB* was detected in only 10.00% (2/20) of the isolates, substantially lower than the 52.00% reported by Racewicz *et al.* (2022) [23]. The higher prevalence of *tetA* compared with *tetB* in the present study suggests that *tetA* was the predominant tetracycline resistance determinant among the isolates examined. Differences in the distribution of tetracycline resistance genes between studies may be influenced by antimicrobial usage patterns, geographical location, poultry production systems, and the characteristics of the bacterial populations investigated.

The *bla_{CMY}* gene was detected in 35.00% (7/20) of the *E. coli* isolates in the present study. This prevalence was comparable to the 34.55% (19/55) reported by Islam *et al.* (2022) [24] among *E. coli* isolates recovered from migratory birds travelling to Bangladesh. In that study, *bla_{CMY}* was detected in 19 of 21 phenotypically confirmed ESBL-positive isolates, corresponding to 90.00048% of the ESBL-positive subgroup. However, when calculated against the total number of *E. coli* isolates examined, the prevalence was 34.55%, which was similar to the 35.00% observed in the present study. The comparable prevalence suggests that CMY-type β -lactamase determinants may occur

among *E. coli* of avian origin. Nevertheless, *bla*_{CMY} detection should not be considered synonymous with the ESBL phenotype because CMY enzymes belong to the AmpC β -lactamase group. Differences in host type, geographical location, antimicrobial exposure, and isolate selection should also be considered when interpreting these findings.

The *int11* gene was detected in 30.00% (6/20) of the *E. coli* isolates in the present study, which was lower than the 60.00% reported by Racewicz *et al.* (2022) [23] among multidrug-resistant *E. coli* isolates recovered from broiler chicken cloacal swabs. The difference in prevalence may be related to variations in geographical location, poultry production systems, antimicrobial exposure, sample type, isolate characteristics, and selection pressure. The detection of *int11* among poultry-associated *E. coli* is epidemiologically relevant because class 1 integrons can facilitate the capture and dissemination of multiple antimicrobial resistance determinants.

Overall, the present findings demonstrate the occurrence of multidrug-resistant *E. coli* among layer birds, with particularly high resistance to tetracycline and fluoroquinolones. The detection of *tetA*, *tetB*, *sul1*, *bla*_{CMY}, and *int11* further indicates the presence of diverse antimicrobial resistance-associated genetic determinants. The coexistence of phenotypic antimicrobial resistance and resistance-associated genes emphasizes the potential role of poultry-associated *E. coli* as a reservoir of antimicrobial resistance and highlights the importance of prudent antimicrobial use and continued surveillance of antimicrobial resistance in poultry production systems.

Conclusion

The present study demonstrated the occurrence of antimicrobial-resistant *Escherichia coli* among layer birds, with 20 (40.00%) of the 50 cloacal samples examined confirmed as *E. coli*. The antimicrobial susceptibility profile revealed substantial resistance, particularly to tetracycline (90.00%), ciprofloxacin (70.00%), enrofloxacin (60.00%), and levofloxacin (60.00%). The mean Multiple Antibiotic Resistance (MAR) index of 0.38, ranging from 0.17 to 0.75, further indicated considerable variation in multidrug resistance among the isolates.

Phenotypic ESBL production was detected in one isolate (5.00%), whereas molecular characterization revealed the presence of several antimicrobial resistance-associated genes, including *tetA* (60.00%), *bla*_{CMY} (35.00%), *int11* (30.00%), *tetB* (10.00%), and *sul1* (5.00%). The predominance of *tetA* and the detection of AmpC β -lactamase-associated *bla*_{CMY} highlight the presence of diverse resistance determinants among poultry-associated *E. coli*. The findings also demonstrate that phenotypic ESBL detection alone may not fully characterize the antimicrobial resistance determinants present in these isolates.

Overall, the study highlights the potential role of poultry-associated *E. coli* as a reservoir of antimicrobial resistance and emphasizes the importance of continued AMR surveillance, prudent antimicrobial use, and improved antimicrobial stewardship in poultry production systems. Further studies involving larger sample sizes, multiple poultry production systems, and broader molecular characterization are warranted to better understand the distribution and dissemination of antimicrobial resistance in poultry-associated *E. coli*.

Acknowledgement

The authors express their sincere gratitude to the principal, College of Veterinary Science and Animal Husbandry, Anand, Gujarat for providing the necessary laboratory facilities and technical support to carry out this research work.

References

- O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. 2016.
- Department of Animal Husbandry and Dairying. Basic Animal Husbandry Statistics 2025. Ministry of Fisheries, Animal Husbandry and Dairying, Government of India; 2025.

- Gelband H, Miller, Petrie M, Pant S, Gandra S, Levinson J, Barter D, Laxminarayan R. The state of the world's antibiotics 2015. Wound Healing Southern Africa. 2015;8(2):30-34.
- Rawat N, Anjali, Jamwal R, Devi PP, Yadav K, Kumar N, Rajagopal R. Detection of unprecedented level of antibiotic resistance and identification of antibiotic resistance factors, including QRDR mutations in *Escherichia coli* isolated from commercial chickens from North India. Journal of Applied Microbiology. 2022;132(1):268-278.
- Tang B, Wang J, Zheng X, Chang J, Ma J, Wang J, Ding B. Antimicrobial resistance surveillance of *Escherichia coli* from chickens in the Qinghai Plateau of China. Frontiers in Microbiology. 2022;13:885132.
- Levine MM. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. Journal of Infectious Diseases. 1987;155(3):377-389.
- Shahrani M, Dehkordi FS, Momtaz H. Characterization of *Escherichia coli* virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. Biological Research. 2014;47(1):28.
- Dominick MA, Jensen AE. Colonization and persistence of *Escherichia coli* in axenic and monoxenic turkeys. American Journal of Veterinary Research. 1984;45(11):2331-2335.
- Hussain A, Shaik S, Ranjan A, Nandanwar N, Tiwari SK, Majid M, Ahmed N. Risk of transmission of antimicrobial resistant *Escherichia coli* from commercial broiler and free-range retail chicken in India. Frontiers in Microbiology. 2017;8:2120.
- Stromberg ZR, Johnson JR, Fairbrother JM, Kilbourne J, Van Goor A, Curtiss 3rd R, Mellata M. Evaluation of *Escherichia coli* isolates from healthy chickens to determine their potential risk to poultry and human health. PLoS One. 2017;12(7):e0180599.
- Masachessi G, Pisano MB, Prez VE, Martínez LC, Michelena JF, Martínez-Wassaf M, Ré V. Enteric viruses in surface waters from Argentina: molecular and viable-virus detection. Applied and Environmental Microbiology. 2018;84(5):e02327-17.
- Barrow GI, Feltham RKA. Cowan and Steel's Manual for the Identification of Medical Bacteria. Cambridge University Press, 3rd Ed; 2004.
- Markey B, Leonard F, Archambault M, Cullinane A, Maguire D. Clinical Veterinary Microbiology e-book: clinical veterinary microbiology e-book. Elsevier Health Sciences; 2013.
- Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1966;45(4_ts):493-496.
- Salehi TZ, Madani SA, Karimi V, Khazaeli FA. Molecular genetic differentiation of avian *Escherichia coli* by RAPD-PCR. Brazilian Journal of Microbiology. 2008;39:494-497.
- Patel SS, Patel AC, Mohapatra SK, Chauhan HC, Sharma KK, Shrimali MD, Prajapati BI. Antibiotic resistance and virulence gene patterns associated with multi drug resistant avian pathogenic *Escherichia coli* (APEC) isolated from broiler chickens in India. Indian Journal of Microbiology. 2024;64(3):917-926.
- Kaushik P, Anjay A, Kumari S, Dayal S, Kumar S. Antimicrobial resistance and molecular characterisation of *E. coli* from poultry in Eastern India. Veterinaria Italiana. 2018;54(3):197-204.
- Jaiswal A, Khan A, Yogi A, Singh S, Pal AK, Soni R, Tripathi V. Isolation and molecular characterization of multidrug-resistant *Escherichia coli* from chicken meat. 3 Biotech. 2024;14(4):107.
- Amer MM, Mekky HM, Amer AM, Fedawy HS. Antimicrobial resistance genes in pathogenic *Escherichia coli* isolated from diseased broiler chickens in Egypt and their relationship with the phenotypic resistance characteristics. Veterinary World. 2018;11(8):1082.

20. Khant RB, Pathan FA, Mathakiya RA, Rana NM, Nimavat VR, Vahora AS. Isolation and antimicrobial resistance mapping of *Escherichia coli* isolates from various animal species and birds. *Indian Journal of Veterinary Sciences and Biotechnology*. 2026;22(1):62–67.
21. Kim YB, Yoon MY, Ha JS, Seo KW, Noh EB, Son SH, Lee YJ. Molecular characterization of avian pathogenic *Escherichia coli* from broiler chickens with colibacillosis. *Poultry Science*. 2020;99(2):1088-1095.
22. Rajkhowa TK, Vanlalruati C, Hauhnar L, Jamoh K. Distribution of serotypes and molecular characterization of avian pathogenic *Escherichia coli* isolated from chicken died of colibacillosis. *Indian J. Anim. Sci.* 2021;90(11):1453-1460.
23. Racewicz P, Majewski M, Biesiada H, Nowaczewski S, Wilczyński J, Wystalska D, Madeja ZE. Prevalence and characterisation of antimicrobial resistance genes and class 1 and 2 integrons in multi-resistant *Escherichia coli* isolated from poultry production. *Scientific Reports*. 2022;12(1):6062.
24. Islam MS, Sobur MA, Rahman S, Ballah FM, Ievy S, Siddique MP, Rahman MT. Detection of blaTEM, blaCTX-M, blaCMY, and blaSHV genes among extended-spectrum beta-lactamase-producing *Escherichia coli* isolated from migratory birds travelling to Bangladesh. *Microbial Ecology*. 2022;83(4):942-950.
25. Goudarzi SM, Eftekhar F. Multidrug resistance and integron carriage in clinical isolates of *Pseudomonas aeruginosa* in Tehran, Iran. *Turkish Journal of Medical Sciences*. 2015;45(4):789–793.
26. Meena PR, Yadav P, Hemlata H, Tejavath KK, Singh AP. Poultry-origin extraintestinal *Escherichia coli* strains carrying the traits associated with urinary tract infection, sepsis, meningitis and avian colibacillosis in India. *Journal of Applied Microbiology*. 2021;130(6):2087-2101.
27. Mellata M, Johnson JR, Curtiss III R. *Escherichia coli* isolates from commercial chicken meat and eggs cause sepsis, meningitis and urinary tract infection in rodent models of human infections. *Zoonoses and Public Health*. 2018;65(1):103-113.
28. Riffon R, Sayasith K, Khalil H, Dubreuil P, Drolet M, Lagacé J. Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. *Journal of Clinical Microbiology*. 2001;39(7):2584-2589.
29. Shehata ME, Gamal M, Amira HM, Hesham MS. Molecular and phenotypic characterization of some antimicrobial resistance genes in *Escherichia coli* isolated from human and broiler chickens. *International Journal of Current Microbiology and Applied Sciences*. 2016;5(12):953-965.
30. Van TTH, Chin J, Chapman T, Tran LT, Coloe PJ. Safety of raw meat and shellfish in Vietnam: an analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. *International Journal of Food Microbiology*. 2008;124(3):217-223.

How to cite this article: Patel V, Mathakiya R, Javia B, Patel A. Phenotypic and molecular characterization of antimicrobial resistance in *Escherichia coli* isolated from poultry cloacal samples. *International Journal of Advanced Biochemistry Research*. 2026;10(9):703-709. doi: <https://www.doi.org/10.33545/26174693.2026.v10.i9i.10155>