RESEARCH ARTICLE

Isolation and Antimicrobial Resistance Mapping of *Escherichia coli* Isolates from Various Animal Species and Birds

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Abstract

In the present study, 257 faecal samples were collected from diarrheic and healthy cattle, buffaloes, dogs, and poultry in and around Anand, Gujarat. Based on cultural and biochemical characterization, 200 isolates (77.82%) were identified as *Escherichia coli*. Antibiotic susceptibility testing using 22 antimicrobials revealed high resistance to cephalothin (up to 100%), followed by pefloxacin (84%), erythromycin (72%), and cefoperazone (64%), while complete sensitivity was observed toward amoxyclav, colistin, and amikacin. Extended-spectrum β-lactamase (ESBL) production was phenotypically confirmed in 52.00% of isolates from diseased poultry, followed by 44.00% from diarrheic cattle, 40.0% from healthy cattle and diarrheic buffaloes, each and 20.00% from healthy dogs. PCR based screening of 200 isolates for 14 different antibiotic resistance genes (ARGs) detected multiple ARGs, with *tetA* (47.50%), followed by *tetB* (41.50%), *sulI* (39.00%), *aadA1* (32.50%) and *qnrS* (29.50), while β-lactamase genes (*blaTEM*, *blaOXA*, *blaSHV*) and integron gene *intI1* were detected in 10.50-27.00% of the isolates. The findings highlight widespread dissemination of multidrug-resistant and ESBL-producing *E. coli* among livestock and poultry, emphasizing the need for judicious antibiotic use and regular AMR surveillance in animal populations.

Key words: Animals, Antibiotic resistance genes, Antimicrobial resistance, Birds, *E. coli*, ESBL, Integron.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.1.12

Introduction

Escherichia coli is a Gram-negative, rod-shaped, flagellated, non-spore-forming, facultative anaerobic bacterium belonging to the family *Enterobacteriaceae*. It is typically motile with peritrichous flagella and often fimbriated. Based on virulence properties and clinical manifestations, *E. coli* strains are classified into several pathotypes: Enterohemorrhagic (*EHEC*), Enterotoxigenic (*ETEC*), Necrotoxigenic (*NTEC*), Enteroinvasive (*EIEC*), Enteropathogenic (*EPEC*), Attaching and Effacing (*AEEC*), Enteroaggregative (*EAEC*), Avian Pathogenic (*APEC*), Septicemic (*SEPEC*), and Uropathogenic (*UPEC*) *E. coli* (Shahrani *et al*., 2014). *E. coli* can survive for weeks in the environment under favourable conditions, particularly through faecal contamination, thereby facilitating the spread of resistant strains from one host to another. The potential transfer of antimicrobial resistance (AMR) from enteric bacteria of food animals to humans is an emerging public health concern (Helmuth and Hensel, 2004). In cases of diarrhoea in animals, the precise role of *E. coli* remains uncertain, as it is part of the normal intestinal flora in both healthy and diseased hosts (Begum *et al*., 2016). Calf diarrhoea remains one of the most economically significant and prevalent health issues in the livestock industry. *E. coli* is a major causative agent, particularly the *ETEC* pathotype, which commonly affects neonatal calves during the first four weeks of life (Nguyen *et al*., 2011). In poultry, *E. coli* is commonly found in the intestinal tract, and while most strains are non-pathogenic, pathogenic variants can cause severe diarrhoea and systemic infections (El-Mongy *et al*., 2017).

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How to cite this article: Khant, R. B., Pathan, F. A., Mathakiya, R. A., Rana, N. M., Nimavat, V. R., & Vahora, A. S. (2026). Isolation and Antimicrobial Resistance Mapping of *Escherichia coli* Isolates from Various Animal Species and Birds.. Ind J Vet Sci and Biotech, 22(1), 62-67.

Source of support: Nil

Conflict of interest: None

Submitted 08/11/2025 **Accepted** 17/11/2025 **Published** 10/01/2026

In recent years, an alarming increase in antibiotic resistance among *E. coli* isolates has been observed and AMR, often referred to as the "silent pandemic," poses a severe threat to global public health (Laxminarayan, 2022). The WHO categorizes antimicrobial resistance as one of the top ten public health threats. Deaths from antimicrobial resistance are projected to rise from 7 lakh to 10 million annually by 2050, costing about USD 100 billion in healthcare resources, surpassing diseases such as cancer (Adebisi and Ogunkola, 2023). The emergence of acquired antibiotic resistance poses a serious challenge, as both pathogenic and commensal bacteria. Commensal bacteria act as reservoirs of AMR genes and can disseminate these genes to pathogenic bacteria, even in the absence of antibiotic pressure (Nikolich *et al*.,

1994). Integrons, which are genetic elements capable of capturing and expressing gene cassettes, play a major role in the horizontal transfer of resistance determinants and act as a "genetic construction kit" for bacteria (Bennett, 1999). *E. coli* also produces extended-spectrum beta-lactamases (ESBLs), enzymes that confer resistance to β-lactam antibiotics such as penicillins, first-, second-, and third-generation cephalosporins, and monobactams (Bush and Jacoby, 2010). Detection of β-lactamase-mediated resistance is crucial to selecting effective therapeutic agents and preventing treatment failure. The β-lactamase (*bla*) genes, located either on plasmids or chromosomal DNA, encode these enzymes and facilitate their dissemination among bacterial populations (Bush *et al*., 1995). Therefore, the present study was conducted to isolate, identify, and characterize *E. coli* from multiple animal species and birds, both healthy and diseased, determine their antimicrobial susceptibility pattern, detect ESBL-producing isolates, and screen for antibiotic resistance genes including integron gene.

Materials and Methods

Sample Collection

A total of 257 fecal samples were collected aseptically from cattle, buffaloes, dogs, and poultry in and around Anand, Gujarat, India. Samples were obtained randomly from both diarrhoeic/diseased and healthy animals or birds (Table 1) and transported under a maintained cold chain to the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat (India), for further processing.

Table 1: Results of *E. coli* isolation from different animal species and bird

Species	Status	Samples	*E. coli* isolated	% Positive
Cattle	Diarrheic	30	25	83.33
	Healthy	31	25	80.65
Buffaloes	Diarrheic	28	25	89.28
	Healthy	40	25	62.50
Dogs	Diarrheic	32	25	78.12
	Healthy	37	25	67.57
Poultry	Diseased	25	25	100.00
	Healthy	34	25	73.52
Total		**257**	**200**	**77.82**

Isolation and Identification of *E. coli*

Samples were processed for bacterial isolation following the methods described by Barrow and Feltham (2004) and Markey *et al*. (2013). The faecal samples were cultured on MacConkey agar and incubated aerobically at 37°C for 24 h. Lactose-fermenting colonies were subcultured on Eosin Methylene Blue (EMB) agar, where colonies exhibiting a characteristic greenish metallic sheen were presumptively identified as *E. coli*. These isolates were further confirmed by standard biochemical tests including KOH, catalase, and oxidase tests. Confirmed isolates were preserved on Brain Heart Infusion (BHI) agar slants at 4°C for subsequent analysis.

Antibiotic Susceptibility Testing

Antibiotic susceptibility of *E. coli* isolates was determined by the disc diffusion method on MuellerHinton agar as per Bauer *et al*. (1966). The bacterial inoculum turbidity was standardized to 0.5 McFarland (~1.5×10^8 CFU/mL). The antibiotic discs used (HiMedia Laboratories Pvt. Ltd., Mumbai) are listed in Table 4. Following overnight incubation at 37°C, inhibition zone diameters were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Detection of Extended-Spectrum β-Lactamase (ESBL) Producing *E. coli*

Phenotypic detection of ESBL production was carried out following the two-step protocol recommended by CLSI (2015). Isolates were initially screened using indicator antibiotics (Table 2). Isolates showing reduced susceptibility to at least one antibiotic were subjected to the combination disc method for confirmation. An increase of ≥5 mm in inhibition zone diameter for cephalosporin/clavulanic acid combination compared to cephalosporin alone was considered indicative of ESBL production (Drieux *et al*., 2008).

Table 2: List of the antibiotics used for detection of ESBL

Name of Antibiotic	Code	Content (µg/disc)	Zones below may indicate ESBL production (mm) (CLSI*)
Ceftazidime	CAZ	30	≤ 22
Ceftriaxone	CTR	30	≤ 25
Ceftazidime + clavulanic acid	CAC	30/10	A ≥5-mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanate vs. the zone diameter of the agent when tested alone
Cefotaxime + clavulanic acid	CEC	30/10	A ≥5-mm increase in zone diameter for eitherantimicrobial agent tested in combination with clavulanate vs. the zone diameter of the agent when tested alone

Genomic DNA Extraction

Genomic DNA was extracted from *E. coli* isolates using a simple boiling method. A single colony was suspended in 100 µL of Milli-Q water, heated at 95°C for 15 min, and centrifuged at 10,000 rpm for 1 min. The supernatant was used as the DNA template. DNA concentration and purity were determined spectrophotometrically ($A_{260}/A_{280} \approx 1.8$ indicates purity).

Molecular Based Confirmation of *E. coli* and Detection of Antibiotic Resistance Genes

The *E. coli* isolates were confirmed by PCR targeting the *phoA* gene (Hu *et al*., 2011). Detection of antibiotic resistance genes (ARGs) associated with resistance to tetracyclines, sulfonamides, chloramphenicol, trimethoprim, quinolones, aminoglycosides, and β-lactams was performed by PCR. A total of 200 isolates were screened for 14 ARGs (*tetA*, *tetB*,

sulI, *cat1*, *cmlA*, *dhfrI*, *dhfrV*, *qnrS*, *aac(3)-IV*, *aadA1*, *blaOXA*, *blaSHV*, *blaTEM*, and *blaCMY*) along with the integron gene *intI1* using primers shown in Table 3. PCR reactions (25 µL) were prepared using 2X Takara PCR Master Mix 12.5 µL, 10 pmol primer 1.0 µL each, 3 µL of template DNA and nuclease free water 7.5 µL. Each primer set, along with its sequence, expected amplicon size, and reference, was selected based on previously published studies.

Amplified PCR products were analyzed by electrophoresis on 2% agarose gels containing 0.5 µg/mL ethidium bromide in 0.5X TBE buffer. Gels were run at 80 V and visualized under UV light using a gel documentation system (Genetix Biotech Pvt. Ltd., Delhi).

Results and Discussion

Isolation and Identification of *E. coli*

Out of a total of 257 faecal samples collected from diarrheic and healthy cattle, buffaloes, dogs, and poultry, 200 isolates (77.82%) were presumptively identified as *E. coli* based on their cultural and biochemical characteristics as shown in Table 1. On MacConkey agar, colonies appeared pink due to lactose fermentation, while on EMB agar, they showed a characteristic metallic green sheen. All isolates exhibited the typical IMViC pattern "++--" and produced a yellow slant and butt on TSI agar, confirming *E. coli*. Among the individual species, the highest prevalence was recorded in healthy buffaloes (85.00%), followed by diarrheic buffaloes (82.14%), healthy dogs (83.78%), diarrheic dogs (81.25%), healthy cattle (80.64%), and diarrheic cattle (83.33%). In poultry, *E. coli* was isolated from 80.00% of diseased and 82.35% of healthy birds. Overall, the findings indicated that *E. coli* was consistently present across all species and health statuses, suggesting its widespread distribution in animal and poultry gut flora.

Antibiotic Susceptibility Testing

All *E. coli* isolates were evaluated for their susceptibility against 22 commonly used antimicrobial agents. The isolates from diarrheic cattle exhibited a high level of resistance to several antibiotics as shown in Table 4. Specifically, 100.00% of isolates were resistant to cephalothin, followed by pefloxacin (84.00%), erythromycin (72.00%),

Table 3: Primers used for the detection of *E. coli* specific gene, ARG and integron gene

Target Genes	Name of Primers	Sequences (5'→3')	Expected Product Size (bp)	References
phoA	F	CGATTCTGGAAATGGCAAAAAG	720	Hu *et al*. (2011)
	R	CGT GAT CAG CGG TGA CTA TGA C		
sulI	F	TTC GGC ATT CTG AAT CTC AC	822	Shehata *et al*. (2016)
	R	ATG ATC TTA ACC CTC GGT CTC		
tetA	F	GTG AAA CCC AAC ATA CCC C	887	
	R	GAA GGC AAG CAG GAT GTA G		
tetB	F	CCT TAT CAT GCC AGT CTT GC	773	
	R	ACT GCC GTT TTT TCG CC		
blaOXA	F	GCA GGC CAG TGC ATA CAC AC	710	
	R	CCG CAT CAA ATG GCC ATA AGT G		
bla_{SHV}	F	TCG CCT GGT GTT ATT ATC TCCC	768	
	R	CGC AGA TAA TAC CCA CAC AAT G		
bla_{TEM}	F	GAG TAT TCA ACA TTT TCG T	698	
	R	ACC AAT GCT TAA CAT GGA		
dhfrV	F	CTG CAA AAG CGA AAA ACG G	432	
	R	AGC AAA TGT TAA GTT TGA CAA AG		
dhfrI	F	AAG AAT GGA GTT ATC GGA T	391	
	R	GGG TAA AAA CTG GGC TAA ATG		
cat1	F	AGT TGC TCA ATG TAC CTA ACC	551	
	R	TTG TAA TTC ATT AAG CAT TTC GCC		
cmlA	F	CCG CCA CGG TGT GTT GTA TC	699	
	R	CAC CTT GCC TGC CCA TCA TAG		
aadA1	F	TAT CCA GCT AAG CGG CAA CT	490	
	R	ATT TGC CGA CTA CCT TGG TC		
aac(3)-IV	F	CTT CAG GAT GGC AAG TGT GT	286	Van *et al*. (2008)
	R	TCA CTC GTT CTT CCC GTC AT		
bla_{CMY}	F	TGG CCA GAA CTG ACA GGC AAA	462	
	R	TTT CTC CTG AAC TGG CTG GC		
qnrS	F	ACG ACA TTG CTA ACT GCAA	417	Robicsek *et al*. (2006)
	R	TAA ATT GGC ACC CTG TAG GC		
intI1	F	ACG AGC GCA AGG TTT CGG T	565	Goudarzi and Eftekhar (2014)
	R	GAA AGG TCT GGT CAT ACA TG		

cefoperazone (64.00%), and tetracycline and moxifloxacin (60.00% each). A moderate level of intermediate response (8.00-16.00%) was observed for pefloxacin, erythromycin, cefoperazone, and tetracycline. In contrast, all isolates showed complete sensitivity (100.00%) to amoxyclav, colistin, and amikacin, while high sensitivity was also noted toward spectinomycin (88.00%), cefixime (80.00%), and gentamicin and chloramphenicol (76.00%).

Similarly, *E. coli* isolates obtained from healthy cattle also exhibited extensive resistance, showing 100% resistance to ampicillin, cefoperazone, cephalothin, co-trimoxazole, erythromycin, pefloxacin, and streptomycin as shown in Table 4, while maintaining complete susceptibility to amoxyclav, colistin, and amikacin. The overall antibiogram indicated a dominant resistance pattern against β-lactams, fluoroquinolones, and macrolides, whereas aminoglycosides and combination drugs remained the most effective therapeutic options. Overall, a higher resistance trend was observed among isolates from diarrheic animals compared to those from healthy counterparts across all species. The degree and spectrum of resistance varied among animal groups, indicating possible differences in antimicrobial exposure and bacterial adaptation.

Detection of ESBL Producing *E. coli*

All *E. coli* isolates that showed reduced inhibition zone diameters during the screening test were subjected to the combination disc method for phenotypic confirmation of extended-spectrum β-lactamase (ESBL) production. ESBL activity was confirmed when the inhibition zone increased by ≥5 mm in the presence of clavulanic acid compared to the corresponding cephalosporin alone. Out of the total isolates tested, the occurrence of ESBL producers varied among animal species. In bovines, 44.00% (11/25) isolates were confirmed as ESBL producers in diarrheic cattle, whereas 40.00% (10/25) of isolates were positive from healthy cattle. Among buffalo isolates, 36.00% (9/25) of diarrheic buffalo samples showed ESBL production, whereas 40.00% (10/25) of isolates were positive from healthy buffaloes. In dogs, the corresponding figures were 20.00% (5/25) and 16.00% (4/25), and in poultry 52.00% (13/25) and 16.00% (4/25), respectively. Extended-spectrum β-lactamases are characterized as highly potent bacterial enzymes that exhibit resistance to β-lactam group antibiotics and often other antibiotic classes (Lemlem *et al*., 2023). Overall, these results indicate a substantial prevalence of ESBL producing *E. coli* across all animal species tested, with higher frequencies in poultry, cattle and

Table 4: Prevalence of antibiotic resistance percentage in *E. coli* isolated from healthy animals and birds, and diarrheic animals and diseased birds

Name of antibiotic	Healthy animals and bird				Diarrheic animals and diseased birds			
	Cattle (25)	Buffalo (25)	Dogs (25)	Poultry (25)	Cattle (25)	Buffalo (25)	Dogs (25)	Poultry (25)
Amikacin (30 µg)	0	0	0	2 (8.0)	0	0	1 (4.0)	4 (16.0)
Amoxyclav (30 µg)	0	0	1 (4.0)	6 (24.0)	0	0	0	0
Ampicillin (10 µg)	25 (100.0)	16 (64.0)	3 (12.0)	20 (80.0)	6 (24.0)	19 (64.0)	10 (40.0)	21 (84.0)
Aztreonam (30 µg)	2 (8.0)	2 (8.0)	7 (28.0)	4 (16.0)	4 (16.0)	4 (16.0)	13 (52.0)	11 (44.0)
Cefixime (5 µg)	17 (68.0)	1 (4.0)	6 (24.0)	7 (28.0)	5 (20.0)	5 (20.0)	13 (52.0)	8 (32.0)
Cefoparazone (75 µg)	25 (100.0)	0	6 (24.0)	6 (24.0)	10 (40.0)	11 (44.0)	16 (64.0)	9 (36.0)
Cefpodoxime (10 µg)	7 (28.0)	4 (16.0)	13 (52.0)	15 (60.0)	15 (60.0)	11 (44.0)	25 (100.0)	17 (68.0)
Ceftriaxone (30 µg)	13 (52.0)	4 (16.0)	10 (40.0)	9 (36.0)	11 (44.0)	10 (40.0)	9 (36.0)	22 (88.0)
Cephalothin (30 µg)	25 (100.0)	6 (24.0)	19 (76.0)	19 (76.0)	15 (60.0)	9 (36.0)	9 (36.0)	15 (40.0)
Chloramphenicol (30 µg)	0	0	1 (4.0)	6 (24.0)	4 (16.0)	0)	0	3 (12.0)
Ciprofloxacin (5 µg)	17 (68.0)	3 (12.0)	9 (36.0)	19 (76.0)	4 (16.0)	1 (40.0)	11 (44.0)	25 (100.0)
Colistin (10 µg)	0	0	2 (8.0)	0	0	0	2 (8.0)	0
Co-trimpxazole (25µg)	25 (100.0)	25 (100.0)	2 (8.0)	11 (44.0)	16 (64.0)	12 (48.0)	5 (20.0)	16 (64.0)
Erythromycin (15 µg)	25 (100.0)	25 (100.0)	25 (100.0)	24 (96.0)	25 (100.0)	25 (100.0)	18 (72.0)	14 (56.0)
Gentamicin (10 µg)	10 (40.0)	0	12 (48.0)	5 (20.0)	19 (76.0)	0	11 (44.0)	8 (32.0)
Levofloxacin (5 µg)	19 (76.0)	0	9 (36.0)	18 (72.0)	13 (52.0)	0	9 (36.0)	23 (92.0)
Moxifloxacin (5 µg)	19 (76.0)	2 (8.0)	7 (28.0)	25 (100.0)	15 (60.0)	8 (42.0)	15 (60.0)	23 (92.0)
Pefloxacin (5 µg)	25 (100.0)	8 (32.0)	12 (48.0)	23 (92.0)	21 (84.0)	8 (32.0)	21 (84.0)	25 (100.0)
Spectinomycin (100µg)	2 (8.0)	0)	4 (16.0)	3 (12.0)	2 (8.0)	0	14 (56.0)	3 (12.0)
Streptomycin (10 µg)	25 (100.0)	14 (56.0)	12 (48.0)	5 (20.0)	19 (76.0)	13 (52.0)	11 (44.0)	14 (56.0)
Sulphadiazine (300 µg)	25 (100.0)	2 (8.0)	11 (44.0)	17 (68.0)	20 (80.0)	9 (36.0)	11 (44.0)	17 (68.0)
Tetracycline (30 µg)	11 (44.0)	0	10 (40.0)	23 (92.0)	11 (44.0)	12 (48.0)	15 (60.0)	23 (92.0)

buffaloes compared to dogs, suggesting their potential role as reservoirs for β-lactamase-producing bacteria.

Detection of Antibiotic Resistance Genes (ARGs)

The extracted DNA from all bacterial isolates was subjected to PCR for the detection of 14 antibiotic resistance genes (ARGs) along with the integron gene. The targeted ARGs included β-lactam resistance genes (bla_{OXA}, bla_{SHV}, bla_{TEM}, and bla_{CMY}) with expected product sizes of 710 bp, 768 bp, 698 bp, and 462 bp, respectively; tetracycline resistance genes (*tetA* and *tetB*); sulfonamide resistance gene (*sulI*); chloramphenicol resistance genes (*cat1* and *cmlA*); trimethoprim resistance genes (*dhfrI* and *dhfrV*); plasmid-mediated quinolone resistance gene (*qnrS*); and aminoglycoside resistance genes (*aadA1* and *aac(3)-IV),* in addition to the integron gene (*intI1*). Amplification of *phoA* confirmed species identity, while detection of *tetB* indicated the presence of tetracycline resistance determinants among the isolates. The PCR amplification results revealed specific bands corresponding to the expected amplicon sizes. The agarose gel electrophoresis confirmed the presence of amplified products for *phoA* (773bp), *tetB* (720bp) and *intI1* (565 bp) genes among the bacterial isolates (Fig. 1).

Out of 200 *E. coli* isolates screened by PCR for the presence of antibiotic resistance genes (ARGs), a wide range of resistance determinants were identified as shown in Table 5. The most frequently detected genes were *tetA* (47.50%) and *tetB* (41.50%), both associated with tetracycline resistance, followed by *sulI* (39.00%), conferring sulfonamide resistance. Among aminoglycoside resistance genes, *aadA1* was detected in 32.50% of isolates, while *qnrS*, responsible for quinolone resistance, was present in 29.50%. β-lactamase encoding genes were also detected at varying frequencies, with *blaTEM* (27.00%), *blaOXA* (24.00%), and *blaSHV* (20.50%) contributing to β-lactam resistance. The integron gene *intI1*, which facilitates horizontal gene transfer, was observed in 10.50% of isolates. The frequency and distribution of detected antibiotic resistance genes (ARGs) among *E. coli* isolates are detailed in Table 5. The detection of ARGs highlights the predominance of tetracycline resistance genes (*tetA* and *tetB*), followed by sulfonamide (*sulI*), aminoglycoside (*aadA1*), and β-lactamase

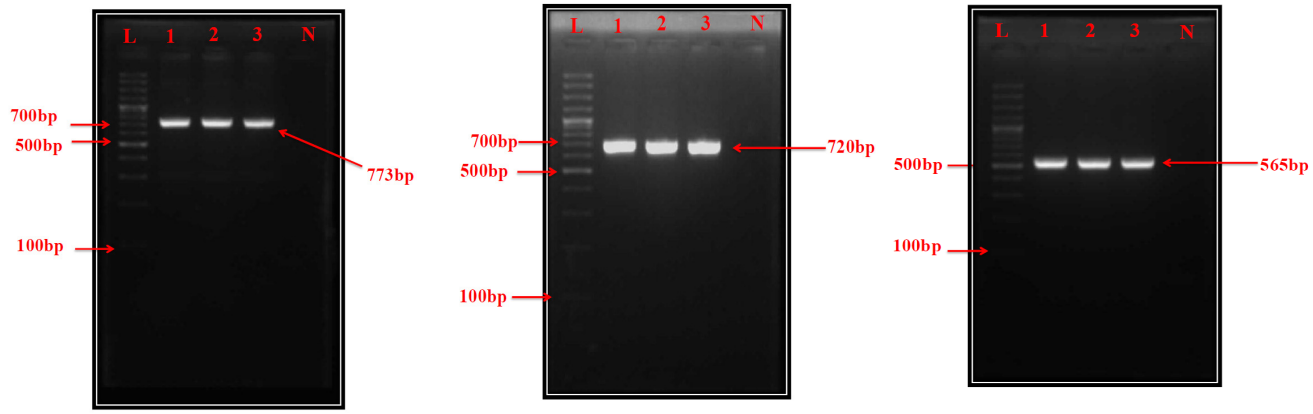

Fig. 1: Agarose gel showing amplified product for *phoA* (773 bp), *tetB* (720 bp) and *intI1* (565 bp) for bacterial isolates. L: DNA ladder-100bp; 1-3: Isolates (Sample); N: Negative control

Table 5: ARGs' percentage in *E. coli* isolated from healthy animals and birds, and diarrheic animals and diseased birds

Name of ARG	Healthy animals and bird				Diarrheic animals and Diseased birds			
	Cattle (25)	Buffalo (25)	Dogs (25)	Poultry (25)	Cattle (25)	Buffalo (25)	Dogs (25)	Poultry (25)
qnrS	1 (4.0)	0	1 (4.0)	4 (16.0)	0	9 (36.0)	2 (8.0)	6 (24.0)
aac(3)-IV	2 (8.0)	0	0	0	2 (8.0)	2 (8.0)	2 (8.0)	0 (0.0)
aadA1	0	0	2 (8.0)	8 (32.0)	0	1 (4.0)	2 (8.0)	12 (48.0)
dhfrI	0	0	2 (8.0)	0	0	2 (8.0)	2 (8.0)	7 (28.0)
tetB	2 (8.0)	3 (12.0)	6 (24.0)	7 (28.0)	1 (4.0)	1 (4.0)	2 (8.0)	4 (16.0)
sulI	0	1 (4.0)	1 (4.0)	6 (24.0)	0	7 (28.0)	1 (4.0)	6 (24.0)
tetA	1 (4.0)	1 (4.0)	4 (16.0)	7 (28.0)	2 (8.0)	6 (24.0)	3 (12.0)	16 (64.0)
dhfrV	1 (4.0)	0	1 (4.0)	3 (12.0)	0	2 (8.0)	1 (4.0)	3 (12.0)
cat1	0	0	5 (20.0)	0	0	0	1 (4.0)	3 (12.0)
cmlA	0	0	3 (12.0)	4 (16.0)	0	0	3 (12.0)	2 (8.0)
bla_{OXA}	2 (8.0)	0	0	0	2 (8.0)	2 (8.0)	0 (0.0)	4 (16.0)
bla_{SHV}	2 (8.0)	0	0	0	2 (8.0)	0	0	0
bla_{CMY}	1 (4.0)	0	4 (16.0)	0	1 (4.0)	0	4 (16.0)	5 (20.0)
bla_{TEM}	0	0	0	0	0	0	0	0
intI1	2 (8.0)	2 (8.0)	0	2 (8.0)	7 (28.0)	3 (12.0)	0	5 (20.0)

genes (bla_{TEM}, bla_{OXA}, bla_{SHV}). Overall, the predominance of tetracycline, sulfonamide, and aminoglycoside-resistance genes indicates extensive dissemination of multidrug resistance determinants among *E. coli* isolates from different animal species, posing a potential risk for transmission of these genes through the food chain and environment. Similar studies in the poultry industry have reported the detection of various CTX genes in ESBL isolates. For instance, Wibisono *et al*. (2021) reported that among 130 cloacal samples collected from four laying hen farms in Indonesia, 8.69% harbored ESBL-producing *E. coli*, and $bla_{CTX\text{-}M}$ genes were detected in 80% of the ESBL-positive isolates.

Conclusion

The present study revealed a high prevalence of *E. coli* across cattle, buffalo, dog, and poultry samples, with significant multidrug resistance, particularly to β-lactam and fluoroquinolone antibiotics. The detection of ESBL-producing isolates and multiple resistance genes (*tetA*, *sull*, *aadA1*, *blaTEM*) highlights the potential for horizontal gene transfer and environmental dissemination of AMR determinants. Amoxycillin clavulanic acid, colistin, and amikacin remained highly effective, indicating their continued therapeutic value. These findings emphasize the urgent need for judicious antibiotic use, biosecurity enforcement, and continuous resistance monitoring to mitigate zoonotic and public health risks.

Acknowledgement

The authors are grateful to the Principal, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand for the facilities provided.

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