

# Antibiotic Resistance Genes Detection in *Escherichia coli* Isolated from Raw Meat in Rajshahi Division of Bangladesh

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**Abstract** In Bangladesh, *Escherichia coli* strains show a wide range of antibiotic resistance, due to uncontrolled antibiotic use in animals and insufficient surveillance, which poses serious public health risks and potentially fatal outcomes. This study aimed to identify antimicrobial resistance patterns and the prevalence of antibiotic resistance genes in *Escherichia coli* isolated from raw meat sold in retail outlets across the Rajshahi division of Bangladesh.

**Methods:** 92 raw meat samples (broiler chicken: 20, layer chicken: 20, cattle: 20, goat: 20, buffalo: 12) were collected from four districts in Rajshahi division of Bangladesh. Phenotypic identification was done by using different cultural characteristics and biochemical tests and, the Kirby-Bauer disc diffusion method was used to detect antibiotic susceptibility of *Escherichia coli*. Molecular conformation and detection of antibiotic resistance genes were performed by PCR analysis. **Results:** In phenotypic detection, the prevalence of *Escherichia coli* in raw meat was 25%, with varying rates across types: cattle meat (25%), goat meat (10%), buffalo meat (8.33%), broiler chicken meat (45%), and layer chicken meat (30%). Ceftriaxone, sulphonamide, enrofloxacin, gentamycin, ciprofloxacin, levofloxacin, neomycin, and colistin sulfate showed sensitivity ranging from 52.17% to 100%. Whereas, penicillin, tetracycline, and oxytetracycline showed no sensitivity. Conversely, erythromycin, doxycycline, tetracycline, amoxicillin, ampicillin, and penicillin all exhibited resistance in 47.83% to 100%. In addition, levofloxacin, neomycin, and colistin sulfate all showed no resistance. The study found the prevalence of antibiotic resistance genes: streptomycin (*aadA1*) 4.35%, erythromycin (*ereA*) 4.35%, gentamicin (*aac(3)-IV*) 8.70%, tetracycline (*tetB*) 17.39%, sulfonamide (*sul1*) 21.80%, and tetracycline (*tetA*) 43.48%. No *tetC*, *bla<sub>TEM</sub>*, *bla<sub>SHV</sub>*, or *bla<sub>CMY</sub>* was detected.

**Conclusion:** Detection of antibiotic resistant genes highlights contamination complexity, urging continuous research and monitoring measures to ensure public health safety across the food supply chain.

**Keywords:** *Escherichia coli*, prevalence, antibiotic resistant pattern, resistance genes, raw meat

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## 1. Introduction

Meats obtained from cattle, buffalo, goat, and chicken, provide high-quality protein, rich in essential amino acids, fatty acids, and micronutrients. Although meat contains few or no microorganisms, contamination occurs during unhygienic slaughtering, processing, handling, transportation, and sale points [1]. Contaminated meat, providing ideal conditions and nutrients for pathogenic microorganisms, is a major source of food-borne diseases and a significant food safety concern, leading to various health problems [2,3,4]. However, foodborne diseases

result from various pathogens, including resistant bacteria, viruses, parasites, toxins, and along with their metabolites. The common food-borne bacteria that are found and transmitted through meat include *Salmonella* spp., *Shigella* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Vibrio* spp., *Proteus* spp., *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Campylobacter* spp., *Listeria monocytogenes* and *Clostridium perfringens* [5,6,7].

*Escherichia coli* is a natural inhabitant of the gastrointestinal tracts of humans and animals, primarily transmitted to humans through the consumption of contaminated raw and uncooked meat, especially affecting immune-compromised individuals, such as infants, children, pregnant women, and those with chronic

illnesses like diabetes [8,9,10]. *Escherichia coli* has been listed by the World Health Organization (WHO) as one of the leading antibiotic-resistant pathogens across twelve families, presenting significant risks to the health of both humans and animals [11]. In Bangladesh, like many developing countries, uncontrolled antibiotics used in food animals, outdated food processing technology, and inadequate surveillance for antimicrobial resistance raise the threat of resistant *E. coli* strains [12]. *Escherichia coli* strains displayed a wide array of antimicrobial resistance in Bangladesh, featuring 24 distinct resistance genes [13]. These genes confer resistance to various classes of antibiotics, including beta-lactams (such as *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M-1</sub>, *bla*<sub>CTX-M-2</sub>, and *bla*<sub>CTX-M-9</sub>), ampicillin (*CITM*), tetracyclines (*tetA*, *tetB*, and *tetC*), fluoroquinolones (*qnrB* and *qnrS*), colistin (*mcr1* and *mcr3*), sulfonamides (*sul1* and *sul2*), gentamicin (*aac-3-IV*), aminoglycosides (*rmtB*), streptomycin (*aadA1*), and erythromycin (*ereA*) [13,14,15,16]. High levels of antibiotic resistance *Escherichia coli* strains tend to cause more severe and prolonged illnesses that can lead to fatal outcomes, weakening the body's immunity and increasing susceptibility to other diseases [12,17]. The current study was conducted to investigate the prevalence of *Escherichia coli*, its antibiogram profiles, and antibiotic resistance genes from raw meat purchased from retail stores in the Rajshahi division of Bangladesh.

## 2. Materials and Method

### 2.1. Collection and Transportation of Sample

A total of 92 raw meat samples, including broiler chicken meat 20, layer chicken meat 20, cattle meat 20, goat meat 20, and buffalo meat 12, were collected from randomly selected retail meat shops under the area of four districts (Rajshahi, Naogaon, Sirajgonj, and Bogura district) in Rajshahi division of Bangladesh. Samples were collected with care to prevent cross-contamination, stored in labeled zip-lock bags and transported in ice-filled thermos flasks to the Microbiology Lab, Department of Veterinary and Animal Sciences, University of Rajshahi for immediate examination and characterization.

### 2.2. Samples Preparation

A 10 g portion of raw meat samples was transferred under sterile conditions to a mortar containing 90 ml of phosphate-buffered saline (PBS) and homogenized using a sterile pestle. Then, 1 ml of this homogenate was mixed with 9 ml of Nutrient broth and incubated for 18-24 hours at 37°C for enrichment as described by Sangeetha *et al.*, [18] and Shrestha *et al.*, [19].

### 2.3. Isolation and Identification of *E. coli*

After enrichment, a loopful of the enriched cultures was streaked onto MacConkey Agar (HiMedia, India) and incubated for 18-24 hours at 37°C to obtain pink color colonies. The pink colonies were observed then streaked onto Eosin Methylene Blue (EMB) agar (HiMedia, India) and further incubated at 37°C for 18-24 hours. Dark

colonies with a greenish metallic sheen after inoculation were identified as typical *Escherichia coli* as described by Da Silva *et al.*, [20] and Senapati *et al.*, [21]. Pure colonies were selected and subjected to Gram staining and a series of biochemical tests, including fermentation of five basic sugars, Methyl Red, Voges-Proskauer, Triple sugar iron, urease production, indole production, and citrate utilization using standard methods as described by Marchant and Packer, [22] and Buxton and Fraser, [23]. Preservation of isolates was done in 50% buffered glycerol and stored at -20°C.

### 2.4. Antibiotic Sensitivity and Resistance Patterns of Isolated *Escherichia coli*

The Kirby-Bauer disc diffusion method [24] was employed to conduct the antibiotic susceptibility and resistance pattern of isolated *Escherichia coli*. A total of 23 *E. coli* isolates were subjected to susceptibility testing against fifteen commercially available antibiotic discs (Liofilchem, Italy): ampicillin (AMP, 10 µg), ceftriaxone (CRO, 30 µg), ciprofloxacin (CIP, 5 µg), erythromycin (E, 15 µg), gentamicin (CN, 10 µg), tetracycline (TE, 30 µg), amoxicillin (AML, 30 µg), colistin sulfate (CS, 30 IU), doxycycline (DXT, 30 µg), enrofloxacin (ENR, 5 µg), levofloxacin (LEV, 5 µg), neomycin (N, 30 µg), oxytetracycline (OT, 30 µg), penicillin (P, 10IU), and sulfonamide (S3, 300 µg). In brief, pure *Escherichia coli* colonies were incubated at 37°C for 24 hours in nutrient broth (HiMedia, India). The turbidity of the broth was adjusted to a 0.5 McFarland standard using sterile nutrient broth and 200 µl of broth was taken by micropipette and spread onto Muller Hinton agar (HiMedia, India) plates using a sterile glass rod spreader. The antibiotic discs were then dispensed 1cm apart to avoid overlapping of inhibition zones. The plates were subsequently placed in an incubator at 37°C for 18-24 hours, after that, the diameter of inhibition zones was measured and compared by the protocols of the Clinical and Laboratory Standards Institute, CLSI [25].

### 2.5. Molecular Confirmation of *E. coli*

Molecular confirmation of *Escherichia coli* isolates was done by polymerase chain reaction (PCR) using specific primers were Forward: GAC CTC GGT TTA GTT CAC AGA and Reverse: CAC ACG CTG ACG CTG ACC A amplicon size 585 bp as described by Schippa *et al.*, [26]. Bacterial DNA was extracted using a commercial DNA extraction kit (QIAamp® DNA Mini Kit, Qiagen, Germany, Catalog no. 51304) according to the manufacturer's instructions. PCR was performed in a thermal cycler (T100™ Bio-Rad, USA) as described by Rahman *et al.*, [12] and Momtaz *et al.*, [27]. After amplification PCR products were analyzed by gel electrophoresis on a 1.5% agarose gel stained with ethidium bromide (Sigma-Aldrich, USA) and visualized using UVP GelSolo (Analytik Jena, US).

### 2.6. Detection of Antibiotic Resistance Genes in Isolated *Escherichia coli* by PCR Analysis

PCR amplification was used to determine the presence of antibiotic resistance genes as previously described by Titilawo *et al.*, [28] and Iwu *et al.*, [29] associated with various antibiotics, including streptomycin (*aadA1*), tetracycline (*tet(A)*, *tet(B)*, *tet(C)*), gentamicin (*aac(3)-IV*), sulfonamides (*sul1*), beta-lactams (*bla<sub>TEM</sub>*, *bla<sub>SHV</sub>*, *bla<sub>CMY</sub>*), and erythromycin (*ere(A)*). Specific primers used for each gene from the published article are listed in Table 1. A 100-bp DNA ladder (Promega, USA) was used to estimate the DNA band sizes of the amplicons. Following amplification, the PCR products (15µl) were electrophoresed in a 1.5% agarose gel stained with ethidium bromide fluorescence (Sigma-Aldrich, USA) in 1X TBE buffer at 80V for 30 minutes and visualized using UVP GelSolo (Analytik Jena, US).

**Table 1. Primers Used for the Detection of Antimicrobial Resistance Genes in isolated *Escherichia coli***

| Target Genes             |   | Sequence (5'–3')       | Size (bp) | References                   |
|--------------------------|---|------------------------|-----------|------------------------------|
| <i>tet(A)</i>            | F | GGTTCACTCGAACGACGTCA   | 577       | Randall <i>et al.</i> , [31] |
|                          | R | CTGTCCGACAAGTTGCATGA   |           |                              |
| <i>tet(B)</i>            | F | CCTCAGCTTCTCAACGCGTG   | 634       |                              |
|                          | R | GCACCTTGCTGATGACTCTT   |           |                              |
| <i>tet(C)</i>            | F | AAC AAT GCG CTC ATC GT | 1138      |                              |
|                          | R | GGA GGC AGA CAA GGT AT |           |                              |
| <i>bla<sub>TEM</sub></i> | F | ATA AAA TTC TTG AAG AC | 1076      | Van <i>et al.</i> , [30]     |
|                          | R | TTA CCA ATG CTT AAT CA |           |                              |
| <i>bla<sub>SHV</sub></i> | F | TCGCCTGTGTATTATCTCCC   | 768       |                              |
|                          | R | CGCAGATAAATCACCACAATG  |           |                              |
| <i>bla<sub>CMY</sub></i> | F | TGGCCAGAACTGACAGGCAAA  | 462       |                              |
|                          | R | TTTCTCTGAACGTGGCTGGC   |           |                              |
| <i>ere(A)</i>            | F | GCCGGTGCTCATGAACCTTGAG | 419       |                              |
|                          | R | CGACTCTATTCGATCAGAGGC  |           |                              |
| <i>sul1</i>              | F | TTCGGCATTCTGAATCTCAC   | 822       |                              |
|                          | R | ATGATCTAACCCTCGGTCTC   |           |                              |
| <i>aadA1</i>             | F | TATCCAGCTAAGCGCGAACT   | 447       |                              |
|                          | R | ATTTGCCGACTACCTTGGTC   |           |                              |
| <i>aac(3)-IV</i>         | F | CTTCAGGATGGCAAGTTGGT   | 286       |                              |
|                          | R | TCATCTCGTTCTCCGCTCAT   |           |                              |

### 3. Results

#### 3.1. Prevalence of *Escherichia coli* in Raw Meat in the Rajshahi Division

**Table 2. Prevalence of *Escherichia coli* in raw meat samples in the Rajshahi division**

| Types of raw meat  | Samples tested | Rajshahi district | Naogaon district | Sirajganj district | Bogura district |
|--------------------|----------------|-------------------|------------------|--------------------|-----------------|
| Cattle             | 20             | 1                 | 1                | 2                  | 1               |
| Goat               | 20             | 1                 | 0                | 0                  | 1               |
| Buffalo            | 12             | 1                 | 0                | 0                  | 0               |
| Broiler            | 20             | 2                 | 2                | 3                  | 2               |
| Layer              | 20             | 2                 | 1                | 1                  | 2               |
| Overall prevalence | 92             | 7 (7.61%)         | 4 (4.35%)        | 6 (6.52%)          | 6 (6.52%)       |

The overall prevalence of *Escherichia coli* was 25% in raw meat samples in the Rajshahi division of Bangladesh. In different districts of the Rajshahi division 4.35%, 6.52%, 6.52%, and 7.61% prevalence of *E. coli* was found

in Naogaon, Sirajganj, Bogura, and Rajshahi districts respectively (Table 2). The *Escherichia coli* prevalence was 8.33%, 10%, 25%, 30%, and 45% in buffalo meat, goat meat, cattle meat, layer chicken meat, and broiler chicken meat, respectively (Table 3).

**Table 3. Prevalence of *Escherichia coli* in raw meat samples based on meat types**

| Types of raw meat    | No. of samples tested | Prevalence of <i>E. coli</i> |
|----------------------|-----------------------|------------------------------|
| Cattle meat          | 20                    | 5 (25%)                      |
| Goat meat            | 20                    | 2 (10%)                      |
| Buffalo meat         | 12                    | 1 (8.33%)                    |
| Broiler chicken meat | 20                    | 9 (45%)                      |
| Layer chicken meat   | 20                    | 6 (30%)                      |
| Overall prevalence   | 92                    | 23 (25%)                     |

#### 3.2. Antibiotic Sensitivity and Resistant Patterns of Isolated *Escherichia coli*

The results of antibiotic sensitivity patterns of the isolated *Escherichia coli* showed 8.70%, 13.04%, 21.74%, 39.13%, 52.17%, 60.87%, 73.91%, 78.26%, 86.96%, 86.96%, 91.30% and 100% sensitive to ampicillin, doxycycline, amoxycillin, erythromycin, ceftriaxone, sulphonamide, enrofloxacin, gentamycin, ciprofloxacin, levofloxacin, neomycin, and colistin sulfate, respectively. There was no sensitivity found in the case of penicillin, tetracycline, and oxytetracycline. *Escherichia coli* isolates showed 4.35%, 8.70%, 8.70%, 8.70%, 13.04%, 13.04%, 17.39%, 17.39%, 26.09%, 30.43% and 60.87% intermediate sensitive to sulphonamide, ampicillin, gentamycin, neomycin, levofloxacin, erythromycin, doxycycline, enrofloxacin, ceftriaxone, tetracycline, and oxytetracycline, respectively. There was no intermediate sensitivity found in the case of penicillin, amoxicillin, ciprofloxacin, and colistin sulfate. Resistant patterns were found 8.70%, 13.04%, 13.04%, 21.74%, 34.78%, 39.13%, 47.83%, 69.57%, 69.57%, 78.26%, 82.60% and 100%, resistant to enrofloxacin, gentamycin, ciprofloxacin, ceftriaxone, sulphonamide, oxytetracycline, erythromycin, doxycycline, tetracycline, amoxicillin, ampicillin, and penicillin, respectively. There was no resistance found in the case of levofloxacin, neomycin, and colistin sulfate. (Table 4, Figure 1).

**Table 4. Antibiotic sensitivity and resistant patterns of *E. coli* isolates**

| Name of antibiotics used | Sensitivity pattern |                            |               |
|--------------------------|---------------------|----------------------------|---------------|
|                          | Sensitive (%)       | Intermediate sensitive (%) | Resistant (%) |
| Ampicillin               | 2 (8.70%)           | 2 (8.70%)                  | 19 (82.60%)   |
| Amoxycillin              | 5 (21.74%)          | 0                          | 18 (78.26%)   |
| Ciprofloxacin            | 20 (86.96%)         | 0                          | 3 (13.04%)    |
| Colistin sulfate         | 23 (100%)           | 0                          | 0             |
| Ceftriaxone              | 12 (52.17%)         | 6 (26.09%)                 | 5 (21.74%)    |
| Doxycycline              | 3 (13.04%)          | 4 (17.39%)                 | 16 (69.57%)   |
| Enrofloxacin             | 17 (73.91%)         | 4 (17.39%)                 | 2 (8.70%)     |
| Erythromycin             | 9 (39.13%)          | 3 (13.04%)                 | 11 (47.83%)   |
| Gentamycin               | 18 (78.26%)         | 2 (8.70%)                  | 3 (13.04%)    |
| Levofloxacin             | 20 (86.96%)         | 3 (13.04%)                 | 0             |
| Neomycin                 | 21 (91.30%)         | 2 (8.70%)                  | 0             |
| Oxytetracycline          | 0                   | 14 (60.87%)                | 9 (39.13%)    |
| Penicillin               | 0                   | 0                          | 23 (100%)     |
| Sulphonamide             | 14 (60.87%)         | 1 (4.35%)                  | 8 (34.78%)    |
| Tetracycline             | 0                   | 7 (30.43%)                 | 16 (69.57%)   |

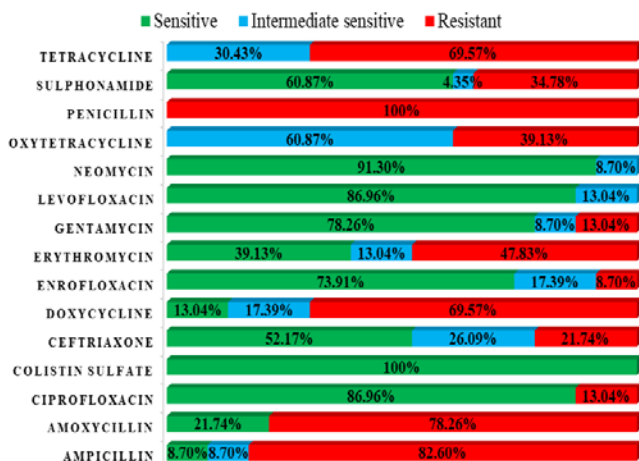


Figure 1. Antibiogram profile of *Escherichia coli* isolates

### 3.3. Genotypic Detection of *E. coli* isolates

All 23 bacterial isolates initially identified as *Escherichia coli* based on phenotypic characteristics were subjected to PCR analysis targeting a 585 bp amplicon using genus-specific primers. Each of the 23 isolates was 100% confirmed as *E. coli* by PCR analysis (Figure 2).

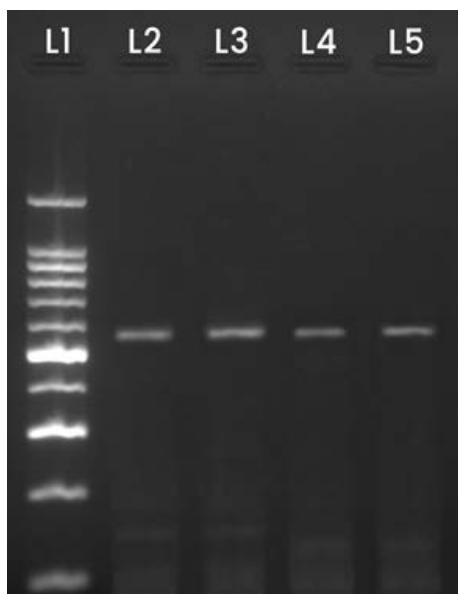


Figure 2. Agarose gel profile of *Escherichia coli* (Lane 1 indicated marker (100 bp) and Lane 2-5 indicated PCR products (585bp)

### 3.4. Detection of Antibiotic Resistance Genes in Isolated *Escherichia coli*

Genotypically identified isolates of *Escherichia coli* were further characterized by PCR to detect antibiotic-resistance genes. PCR analysis of 23 *E. coli* isolates revealed the presence of antibiotic resistance genes as follows: streptomycin (*aadA1*) and erythromycin (*ereA*) in 1 (4.35%) isolate each, gentamicin (*aac(3)-IV*) in 2 (8.70%) isolates, tetracycline (*tetB*) in 4 (17.39%) isolates, and sulfonamide (*sul1*) in 5 (21.80%) isolates. The most prevalent tetracycline resistance gene (*tetA*) was found in 10 (43.48%) isolates. No antibiotic resistance genes were detected in the case of tetracycline (*tetC*), and  $\beta$ -lactamases (*bla<sub>TEM</sub>*, *bla<sub>SHV</sub>*, and *bla<sub>CMY</sub>*) (Figure 3).

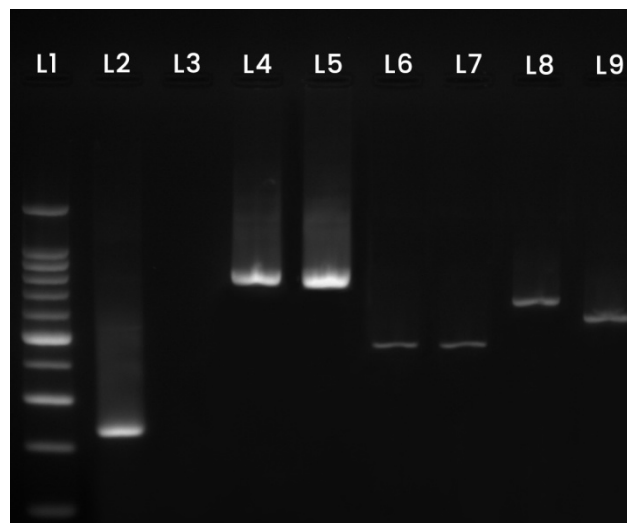


Figure 3. Agarose gel profile of antibiotic resistance genes of *E. coli*, Lane 1 indicated marker (100 bp), Lane 2 *aac(3)-IV* (286), Lane 3 negative control, Lane 4-5 *sul1* (822bp), Lane 6 *aadA1* (447), Lane 7 *ereA* (419), Lane 8 *tetB* (634), and Lane 9 *tetA* (577bp)

## 4. Discussion

In the present study, the prevalence of *Escherichia coli* in raw meat samples was 25%, corresponding with Matubber *et al.*, [32] at 29.7% in southern districts of Bangladesh and Messele *et al.*, [33] at 21.6% in Ethiopia. However, Chepkemei *et al.*, [7] found a higher rate of *Escherichia coli* contamination of 60% in Nairobi, Kenya, and Bantawa *et al.*, [6] reported a prevalence of 53% in eastern Nepal contrasting with our findings. Adzitey *et al.*, [34] identified *Escherichia coli* in various meat samples of 84% in Tamale, Ghana, marking the highest prevalence.

Various studies have demonstrated that the prevalence of *Escherichia coli* varies depending on both the types of meat and the geographical region. In the present investigation, *Escherichia coli* was detected in cattle meat (25%), goat meat (10%), buffalo meat (8.33%), broiler chicken meat (45%), and layer chicken meat (30%). On the contrary, Chepkemei *et al.*, [7] observed higher rates of 89.6%, 88.6%, and 76.2% in beef, chicken, and goat meat, respectively, in Nairobi, Kenya. Rahman *et al.*, [12] reported rates of 49.02% in chicken meat and 70% in beef in the Mymensingh district, Bangladesh. Messele *et al.*, [33] found *Escherichia coli* in chicken meat (37%), chevon (20.6%), and beef (5.5%) in Ethiopia. Bantawa *et al.*, [35] revealed contamination rates of 66.6%, 40%, and 46.7% in chicken, buffalo, and goat meat, respectively, in eastern Nepal. Saud *et al.*, [5] found rates of 31.6% and 33% in buffalo and chicken meat, respectively in Nepal. Adzitey *et al.*, [34] found that *E. coli* was prevalent in beef (86.67%), local chicken (80%), and chevon (75.56%) samples in Ghana. However, our results are higher than Zarei *et al.*, [36], Hessain *et al.*, [37], and Zhang *et al.*, [38]. Zarei *et al.*, [36] recognized a prevalence of 2.8% in beef and 1.4% in buffalo meat samples in Ahvaz, Iran. Hessain *et al.*, [37] found 2% in raw beef, 2.5% in chicken, and 2.5% in mutton meat samples in Riyadh, Saudi Arabia. Similarly, Zhang *et al.*, [38] stated that beef at 13.32%, chicken at 3.28%, and mutton at 0% in fresh raw meat from South China. *Escherichia coli* contamination in raw

meat in developing countries like Bangladesh, Nepal, Ghana, Kenya, and Ethiopia can indeed result from a combination of various factors. Such as close contact between meat carcasses with potentially contaminated sources like infected individuals, animals (dogs, rats, cats, and birds), and unsanitary environments in slaughterhouses can introduce the bacteria. Additionally, during the slaughtering process, transmission from the digestive tract, blood, and external surfaces of the animal's body can occur. The use of contaminated water for washing meat carcasses and unclean equipment such as knives can further contribute to the spread of *Escherichia coli*.

The antibiotic sensitivity test results for the isolated *E. coli* bacteria indicated varying degrees of susceptibility. Ceftriaxone, sulphonamide, enrofloxacin, gentamycin, ciprofloxacin, levofloxacin, neomycin, and colistin sulfate showed sensitivity ranging from 52.17% to 100%. Conversely, penicillin, tetracycline, and oxytetracycline exhibited no sensitivity. Resistance was observed in 47.83% to 100% of cases for erythromycin, doxycycline, tetracycline, amoxicillin, ampicillin, and penicillin. Notably, levofloxacin, neomycin, and colistin sulfate demonstrated no resistance. The antibiotic resistance profiles of *Escherichia coli*, as documented by Zhang *et al.*, [38], Rahman *et al.*, [12], Messele *et al.*, [33], and Adzitey *et al.*, [34], show consistent trends. Zhang *et al.*, [38] reported high resistance to penicillin (100%), and ampicillin (57.14%), but sensitivity to gentamicin (100%), and ciprofloxacin (96.43%) in China. Messele *et al.*, [33] observed notable resistance to ampicillin (71.4%) and tetracycline (47.6%). Whereas, Rahman *et al.*, [12] found *Escherichia coli* from chicken meat resistant to oxytetracycline (92%), sulphonamide-trimethoprim (84%), amoxycillin (76%), and erythromycin (60%), while those from beef were resistant to erythromycin (85.71%) and oxytetracycline (71.43%), yet 100% sensitive to ciprofloxacin, gentamicin, and neomycin. Additionally, Adzitey *et al.*, [34] noted significant resistance to erythromycin (85%), tetracycline (73.33%), and ampicillin (71.67%).

In the current study, antibiotic resistance genes were notably prevalent, with 4.35% for streptomycin (*aadA1*), 4.35% for erythromycin (*ereA*), 8.70% for gentamicin (*aac(3)-IV*), 17.39% for tetracycline (*tetB*), 21.80% for sulfonamide (*sul1*), and 43.48% for tetracycline (*tetA*). Interestingly, no resistance genes were detected for tetracycline (*tetC*) or beta-lactamases (*bla<sub>TEM</sub>*, *bla<sub>SHV</sub>*, *bla<sub>CMY</sub>*). Noticeably, our findings are lower than previous studies: Messele *et al.*, [33] found a higher prevalence for *tet(A)* (65.1%), *bla<sub>CMY</sub>* (65.1%), and *sul1* (54.0%) in *Escherichia coli* from raw meat in Ethiopia, while Momtaz *et al.*, [27] reported *tet(A)* and *tet(B)* at 52.63%, and *sul1* and *ereA* at 47.36%. Moreover, Momtaz *et al.*, [17] found resistance genes in beef meat, including *bla<sub>SHV</sub>* (70.14%), *aac(3)-IV* (64.17%), *tetA* (58.20%), and *aadA1* (49.25%). Additionally, Mashak, [39] highlighted the high prevalence of resistance genes for gentamicin (*aac(3)-IV*) (94.44%), and tetracycline (*tetA*) (61.11%).

In summary, our study revealed the presence of antibiotic resistance genes in *Escherichia coli* isolated from various raw meat sources such as bovine, caprine, and chicken displayed resistance to tetracycline, sulfonamide, and gentamicin. The high incidence of

resistant *E. coli* indicates problems in meat inspection and the possibility of cross-contamination in Bangladeshi slaughterhouses. Maintaining strict hygiene standards throughout the meat production process is crucial to mitigate the risk of contamination. While resistance to certain antibiotics like ceftriaxone, gentamycin, colistin sulfate, and quinolone antibiotics remains low, it's important to note that infections caused by *Escherichia coli* in meat can still be treated with these antibiotics. However, thorough proper cooking of meat is crucial to prevent *E. coli* infections in humans.

## 5. Conclusion

*Escherichia coli* observed high levels of antibiotic resistance among meat samples in the Rajshahi division of Bangladesh underscoring the urgent need for robust monitoring and mitigation strategies to ensure food safety and safeguard public health. Our findings highlight the necessity for continuous research and vigilant monitoring to adapt strategies in response to evolving microbial patterns and emerging challenges. Effective measures must be implemented across the food supply chain to mitigate the spread of antibiotic resistance and reduce the incidence of foodborne illnesses.

## Conflicts of Interest

The authors have declared that they have no conflicts of interest.

## Authors' Contributions

MSH designed the study. MSH, MAHJ, NS, and BI performed sample collection, sample processing, data collection, laboratory work, and interpretation. KAS, PB, MAN, and MMH performed the data analysis, statistical analysis, visualization, and literature searches. SM made critical comments on the manuscript. KMMH supervised the present study. All authors contributed to the reviewed the manuscript and approved the final manuscript.

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