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Article

Escherichia coli O157:H7 and non-O157:H7 in broiler meat of Mymensingh, Bangladesh: a study of isolation, identification and antibiogram

Md. Zarif Hossain[1#], M. Rafiqul Islam[1#], Sayed Abdullah-Al-Mamun[1], Fatema Islam[1], Sk Shaheenur Islam[2], Yosef Deneke[3], Mohammad Ferdousur Rahman Khan[1] and S. M. Lutful Kabir[1*]

[1]Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Department of Livestock Services, Ministry of Fisheries and Livestock, Dhaka-1215, Bangladesh
[3]Jimma University College of Agriculture and Veterinary Medicine, P.O. Box 307, Jimma, Ethiopia

[#]These authors contributed equally to this work

*Corresponding author: S. M. Lutful Kabir, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. E-mail: lkabir79@bau.edu.bd

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Abstract: *Escherichia coli* is a major foodborne pathogen that plays a critical role in the onset of gastrointestinal illnesses, especially hemorrhagic diarrhea. This bacterium is frequently transmitted through contaminated food and water sources. Its impact is particularly concerning in low- and mid-income countries, where inadequate sanitation, restricted access to clean water, and poor food safety standards contribute to its widespread occurrence. This research focused on identifying *E. coli* strains and analyzing their antibiotic resistance profiles from broiler meat samples collected across different locations in the Mymensingh district between January and July 2020. A total of 100 broiler meat samples were gathered and subjected to standard microbiological techniques for *E. coli* detection, including selective culturing, Gram staining, latex agglutination, and PCR. The antimicrobial susceptibility of the isolated strains was assessed using the disk diffusion technique. Results showed that *E. coli* was detected in 49 out of 100 analyzed samples, corresponding to a prevalence rate of 49%. The 95% confidence interval for this estimate ranged from 39.42% to 58.65%, with a *P*-value of 0.4908. Among these, 7% were identified as the O157:H7 serotype (7/100; 95% CI: 2.86–13.89; P=0.1495), while 42% were non-O157:H7 strains (42/100; 95% CI: 32.80–51.79; P=0.4543). Analysis of the O157:H7 isolates showed complete resistance to erythromycin (100%), with a majority also resistant to amoxicillin (83.33%). However, high sensitivity was observed for ceftriaxone (85.71%), followed by ciprofloxacin (50.00%) and streptomycin (45.24%). Multidrug resistance (MDR) was observed in 48.98% of the *E. coli* isolates (24/49; 95% CI: 35.58–62.53), which were found to exhibit eight different resistance profiles, including four MDR patterns. The predominant resistance pattern identified among the isolates was AMX-S-TE, observed in 20.41% of cases (10 out of 49 isolates). Additionally, four isolates exhibited resistance to four different antibiotics representing four distinct antimicrobial classes—specifically amoxicillin, tetracycline, erythromycin, and gentamicin (AMX-TET-ER-GEN). The multiple antibiotic resistance (MAR) index among the isolates varied between 0.13 and 0.50. These findings underscore the presence of antibiotic-resistant *E. coli* in broiler meat, indicating a possible pathway for the transmission of resistance genes to humans via the food chain.

Keywords: *E. coli*; isolation and identification; antimicrobial resistance; MDR; MARI; broiler meat

1. Introduction

The poultry sector in Bangladesh began to develop as a formal agribusiness in the 1980s and has experienced remarkable expansion, particularly during the period from 1996 to 2006 (Begum *et al*., 2010; Uddin *et al*., 2019). Over the years, this industry has become a cornerstone of the country's economy due to its significant role in employment generation, income enhancement, and nutritional improvement. It is estimated that around 20% of rural residents are engaged in poultry farming on a full-time basis, while nearly half are involved part-time (Orsini *et al*., 2013). Poultry products, particularly meat and eggs, are highly nutritious and serve as excellent sources of quality protein. These foods are characterized by their relatively low fat content and favorable fatty acid profile. Compared to most red meats, chicken generally contains two to three times more polyunsaturated fatty acids per gram. Skinless, boneless chicken breast, for example, offers a lean protein option—100 grams of roasted chicken breast typically contains about 31 grams of protein and only 4 grams of fat. In contrast, a similar portion of broiled lean skirt steak contains approximately 27 grams of protein and 10 grams of fat (De Oliveira *et al*., 2016).

Foodborne illnesses, encompassing food poisoning and infections acquired through contaminated food, are significant public health concerns. While numerous cases remain mild, certain infections can progress to severe and potentially life-threatening conditions, including hemorrhagic colitis, septicemia, meningitis, joint inflammation, renal failure, neurological complications such as paralysis, pregnancy-related issues like miscarriage, and various other critical health problems (Elbehiry *et al.*, 2023). These illnesses result from the ingestion of unsafe or contaminated food items, leading to both substantial morbidity and, in some instances, mortality (Abebe *et al.*, 2020). Poultry products, particularly meat and eggs, can become contaminated at various stages, including production, processing, distribution, and storage, potentially causing foodborne outbreaks. Common bacterial pathogens linked to poultry include *Salmonella* spp., *Escherichia coli*, *Staphylococcus* spp., and *Campylobacter* spp., all of which are known to trigger gastrointestinal diseases in humans (Wessels *et al*., 2021). The risk of infection increases with more handling and time elapsed between slaughter and consumption. A major issue arises from the frequent and inappropriate use of antibiotics in poultry production, which plays a significant role in the development of antibiotic-resistant bacteria capable of posing health risks to humans (Singh *et al.*, 2025).

To ensure food safety, robust monitoring and control strategies are essential, and numerous interventions have been proposed to mitigate these risks (Vergis *et al.*, 2025). Pathogenic bacteria such as *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. are not only associated with foodborne illness in humans but also with growth suppression in poultry, including ducks (Golden *et al*., 2020). These effects may arise from toxin production, competition for nutrients, and interference with beneficial microbial populations in the host (Gonzalez and Mavridou, 2019). Among *E. coli* strains, the O157:H7 serotype is particularly dangerous, as it can cause life-threatening complications like hemolytic uremic syndrome (HUS), resulting in severe anemia and kidney failure (Bavaro, 2012). Another strain, enteropathogenic *E. coli* (EPEC), is a frequent cause of diarrheal illness, known to disrupt intestinal cell structure and function, increase permeability, and trigger inflammation (Razmi *et al*., 2020; Savkovic *et al*., 2005). Shiga toxin-producing *E. coli* (STEC) strains produce potent toxins (Stx) that can enter the bloodstream and induce HUS (Tong *et al*., 2015). Improper handling or undercooking of meat, particularly failing to reach a core temperature of 71 °C (160 °F), allows *E. coli* to survive and infect consumers. Cross-contamination from raw meat to other foods and surfaces, as well as poor hand hygiene, can also facilitate transmission from person to person (Islam *et al.*, 2024).

Antibiotics are frequently employed in poultry production, not only to treat diseases but also to promote growth (Beceiro *et al*., 2013). This widespread use has raised alarm due to the potential for antibiotic-resistant bacteria to colonize humans and share resistance genes with native gut microbes via plasmids and other mobile genetic elements (Muhammad *et al*., 2020). The growing prevalence of such resistant pathogens poses a serious threat to public health. Consequently, several countries have restricted the prophylactic use of antibiotics in livestock to combat this issue (Miller and Selgelid, 2007). Studies show that withdrawing antibiotics from animal feed alters gut microbial communities, favoring fast-growing, non-resistant strains over antibiotic-tolerant ones (Allen *et al*., 2011). Nevertheless, the pervasive use of antibiotics in veterinary and human medicine—as well as in livestock feed—has contributed to the evolution of bacteria resistant to multiple drugs, complicating treatment options (Allen *et al*., 2011). Therefore, continuous surveillance of bacterial resistance profiles in poultry is crucial for guiding effective treatment and minimizing the spread of resistant strains.

Today, broiler chickens are one of the fastest-growing sources of meat worldwide, as modern farming methods allow them to reach market weight in less than six weeks. In Bangladesh, poultry farming plays a vital role in the national economy. However, the economic impact of bacterial infections, particularly in terms of poultry mortality and reduced productivity, remains a serious concern for farmers and policymakers. The study was

conducted to identify *E. coli* strains, including O157:H7, in broiler meat samples and assess their antibiotic resistance patterns to evaluate the prevalence of multidrug resistance. The findings are intended to contribute to understanding the antimicrobial resistance burden and inform appropriate strategies for antibiotic usage in public health and livestock.

2. Materials and Methods

2.1. Ethical approval

The research received ethical clearance from the Animal Welfare and Ethical Committee at Bangladesh Agricultural University, Mymensingh, Bangladesh. This approval affirms that the study met the required ethical guidelines and holds potential to contribute to advancements in sustainable agricultural practices. The approval was issued under reference number AWEEC/BAU/2020(12).

2.2. Collection and preparation of samples

A total of 100 samples of broiler meat—comprising breast, thigh muscles, and skin—were obtained from various markets across five upazilas in the Mymensingh district namely, Mymensingh Sadar, Muktagachha, Trishal, Bhaluka, and Fulbaria (Figure 1). The collected samples were immediately placed in cool thermos containers and transported to the Bacteriology Laboratory at Bangladesh Agricultural University, Mymensingh. There, both *E. coli* O157:H7 and non-O157:H7 strains were isolated and identified. All specimens were analyzed within 24 hours of collection, with strict hygienic measures maintained throughout sampling, transport, and laboratory procedures.

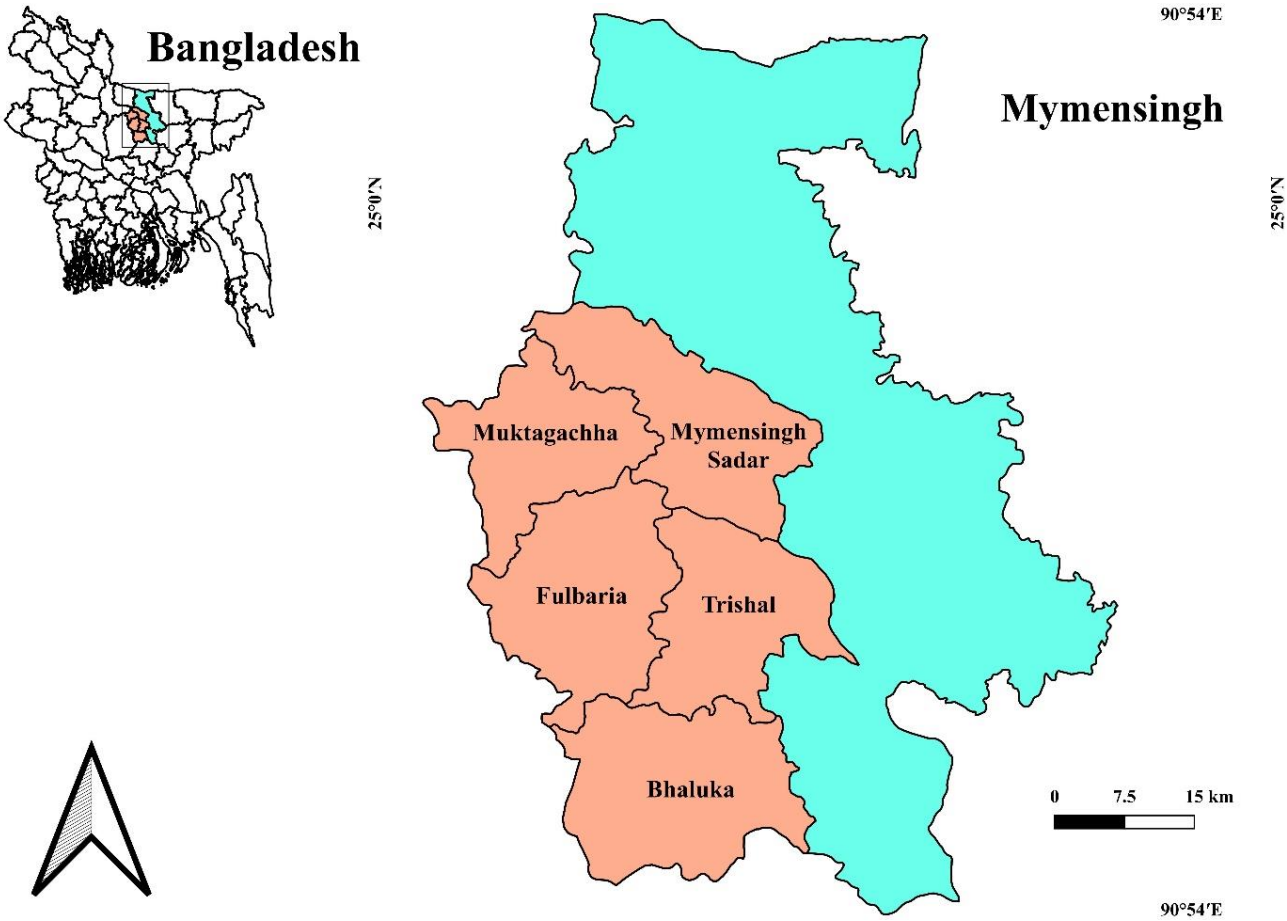

Figure 1. Map showing the areas of the study in Mymensingh district of Bangladesh.

2.3. Isolation and identification of *E. coli* O157:H7 and non-O157:H7

The procedure for isolating and identifying *E. coli* O157:H7 from broiler meat samples followed the protocol outlined by Islam *et al*. (2007). Briefly, 200 µl of a homogenized meat sample—prepared by blending 10 grams of tissue with 90 ml of 0.1% peptone water—was transferred into 3 ml of tryptone soya broth and incubated at 37 °C overnight. Following enrichment, cultures were streaked onto sorbitol MacConkey agar (CT-SMAC)

supplemented with cefixime and tellurite (Oxoid), where suspected *E. coli* O157:H7 colonies appeared as pale or colorless. These presumptive colonies were subjected to microscopic analysis using Gram staining techniques. Colonies transferred to tryptic soy agar (TSA) plates underwent further confirmation through the Wellcolex *E. coli* O157:H7 Rapid Latex Agglutination Test (Oxoid, UK). Additionally, PCR assays were conducted to detect the *rfbE* gene, which is specific for *E. coli* O157, and the *malB* promoter region to distinguish between O157:H7 and non-O157:H7 strains. Information regarding the primers and specific PCR conditions applied in this study is provided in Tables 1 and 2.

Table 1. The primers employed for identifying *E. coli* isolates.

Primer	Target gene	Sequence (5'-3')	Amplicon size	Reference
*rfb*O157F	*rfb*E_{O157}	CGGACATCCATGTGATATGG	259 bp	Paton and Paton (1998)
*rfb*O157R		TTGCCTATGTACAGCTAATCC		
ECO-1	*mal*B	GACCTCGGTTTAGTTCACAGA	585 bp	Wang *et al.* (1996)
ECO-2		CACACGCTGACGCTGACCA		

Table 2. The thermal profile for amplifying various target genes using PCR.

Target genes	Thermal profile					
	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Number of cycles
rfbE_{O157}	94°C/10min	94°C/1min	56°C/1min	72°C/1min	72°C/7min	35
*mal*B	95°C/5min	94°C/45sec	52°C/45sec	72°C/1min	72°C/5min	30

2.4. Extraction of DNA

Genomic DNA from the bacterial isolates was extracted using a streamlined heat-based lysis method, modified from the protocol outlined by Kabir *et al*. (2011). In this approach, three to five well-isolated colonies grown on Tryptone Soya Agar plates were carefully selected and suspended in 250 µl of TE buffer inside sterile Eppendorf tubes. To lyse the bacterial cells, the suspension was incubated in a boiling water bath for 10 minutes, which helps break down the cell walls and release the DNA. Immediately following the heat treatment, the tubes were rapidly cooled on ice for 10 minutes to prevent DNA degradation and stabilize the lysate. Subsequently, the samples were centrifuged at 12,000 rpm for 10 minutes to pellet cellular debris, allowing the DNA-containing supernatant to be separated. Approximately 100 µl of this clear supernatant was then carefully transferred and directly utilized as the DNA template in subsequent PCR reactions for molecular analysis. This simple yet effective technique provides a rapid means of obtaining bacterial DNA suitable for amplification and further genetic characterization.

2.5. Amplification of genes using PCR

PCR amplification was performed using a 20 µl reaction mixture designed to detect specific genetic markers within the *E. coli* isolates. Each reaction included 10 µl of a master mix (Promega, USA) that contained the necessary components for DNA synthesis, such as DNA polymerase, deoxynucleotide triphosphates (dNTPs), and an optimized reaction buffer. To achieve targeted amplification, 1.0 µl of each primer (both forward and reverse), each at a concentration of 10 pmol, was incorporated to facilitate specific binding to the intended DNA regions. A volume of 3.0 µl of the extracted bacterial DNA served as the template, while the remaining 5.0 µl of the reaction volume was made up with nuclease-free deionized water to reach the final volume. The entire PCR setup was run on a thermal cycler (2720 Thermal Cycler, Applied Biosystems, USA), which ensured precise temperature control during the cycling phases, including initial denaturation, primer annealing, and extension. The exact sequences of the primers used for *E. coli* identification are detailed in Table 1, while the step-by-step thermal cycling conditions—including times and temperatures for each stage—are outlined in Table 2.

2.6. Electrophoresis of the PCR products

The PCR products were analyzed by electrophoresis using a 2% agarose gel prepared with agarose powder (Invitrogen, USA) to effectively separate DNA fragments based on size. After performing agarose gel electrophoresis, the gel was stained with ethidium bromide at a concentration of 0.5 µg/ml (Sigma-Aldrich, USA). This fluorescent dye binds to DNA, enabling the visualization of amplified DNA fragments under UV

light. Following electrophoresis, the gel was placed on a UV transilluminator (BDA Digital, Biometra GmbH, Germany), where the DNA bands fluoresced under ultraviolet light.

2.7. Assessment of antimicrobial susceptibility

The antibiotic resistance patterns of the *E. coli* isolates were evaluated using the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar plates (Oxoid, UK), following the standardized protocol originally described by Bauer *et al*. (1966). In this assay, antibiotic-impregnated discs containing specific concentrations of drugs were applied to the inoculated agar surface to determine bacterial susceptibility. The antibiotics tested included Amoxicillin (30 µg), Azithromycin (30 µg), Ciprofloxacin (5 µg), Erythromycin (30 µg), Gentamicin (10 µg), Ceftriaxone (10 µg), Streptomycin (10 µg), and Tetracycline (30 µg). After incubation, the zones of inhibition surrounding each disc were measured, and bacterial responses were categorized as susceptible, intermediate, or resistant according to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI, 2018). Furthermore, isolates exhibiting resistance to antibiotics from three or more distinct antimicrobial classes were identified as multidrug-resistant (MDR), following the classification criteria outlined by Sweeney *et al*. (2018). This method provided a comprehensive understanding of the resistance profiles among the tested *E. coli* strains.

2.8. Multiple antibiotic resistance index (MARI)

The Multiple Antibiotic Resistance (MAR) index of the *E. coli* isolates was calculated using the formula presented by Msolo *et al*. (2020),

$$\text{MARI} = \frac{a}{b}$$

In this formula, “b” denotes the total count of antibiotics tested against each isolate, and “a” refers to the number of antibiotics to which the particular isolate shows resistance.

2.9. Data management and statistical analysis

All collected data were systematically entered and organized using Microsoft Excel 2016 (Microsoft Office 2016, Microsoft, Los Angeles, CA, USA), ensuring efficient data management and ease of access for subsequent analyses. For statistical evaluation, SPSS software version 20—a robust and widely utilized statistical package—was employed to perform various analyses. The data were primarily summarized using descriptive statistics, including calculations of frequencies and percentages, to provide a clear overview of the dataset. To determine the significance of observed differences or associations, a P-value threshold of less than 0.05 ($P < 0.05$) was established as the criterion for statistical significance, in line with conventional scientific standards.

3. Results

Microscopic examination of the cultured bacterial colonies, conducted at 100X magnification, revealed the presence of small, Gram-negative, rod-shaped organisms consistent with the morphology of *E. coli*. These colonies were carefully isolated as individual strains for preliminary identification. To further confirm the identity of the presumptive *E. coli*, the isolates were subjected to a rapid latex agglutination assay specifically designed to detect the O157:H7 serotype. Subsequent molecular confirmation was carried out using polymerase chain reaction (PCR), which validated the presence of both *E. coli* O157:H7 and non-O157:H7 strains (Figures 2 and 3). The analytical results demonstrated that *E. coli* was present in 49% of the broiler meat samples (49 out of 100), with a 95% confidence interval (CI) ranging from 39.42% to 58.65%, and a P-value of 0.49, indicating no statistical significance. Among the positive samples, 7% (7/100; 95% CI: 2.86–13.89; P=0.15) were confirmed as *E. coli* O157:H7, a pathogenic strain associated with serious foodborne illnesses. In contrast, non-O157:H7 strains were detected in 42% of the samples (42/100; 95% CI: 32.80–51.79; P=0.45) (Table 3).

Antibiotic susceptibility testing was performed on all *E. coli* isolates, including both O157:H7 (n = 7) and non-O157:H7 strains (n = 42), to evaluate their resistance profiles. The analysis revealed that all isolates exhibited complete resistance to erythromycin (100%), indicating a uniform resistance trend across both serotypes. Additionally, a high proportion of the isolates (83.33%) were resistant to amoxicillin, although a few showed intermediate levels of susceptibility, suggesting varying degrees of tolerance to this antibiotic. On the other hand, several antibiotics demonstrated greater efficacy. Ceftriaxone emerged as the most effective agent, with 85.71% of the isolates showing sensitivity. Ciprofloxacin and streptomycin also showed moderate effectiveness, with sensitivity rates of 50.00% and 45.24%, respectively (Table 4).

Table 3. Prevalence of *E. coli* O157:H7 and *E. coli* non-O157:H7 in broiler meat.

Name of sample	No. of sample	Prevalence [No. (%)]		95% CI		*P* value	
		O157:H7	non-O157:H7	O157:H7	non-O157:H7	O157:H7	non-O157:H7
Broiler meat	100	7 (7)	42 (42)	2.86-13.89	32.80-51.79	0.1495	0.4543

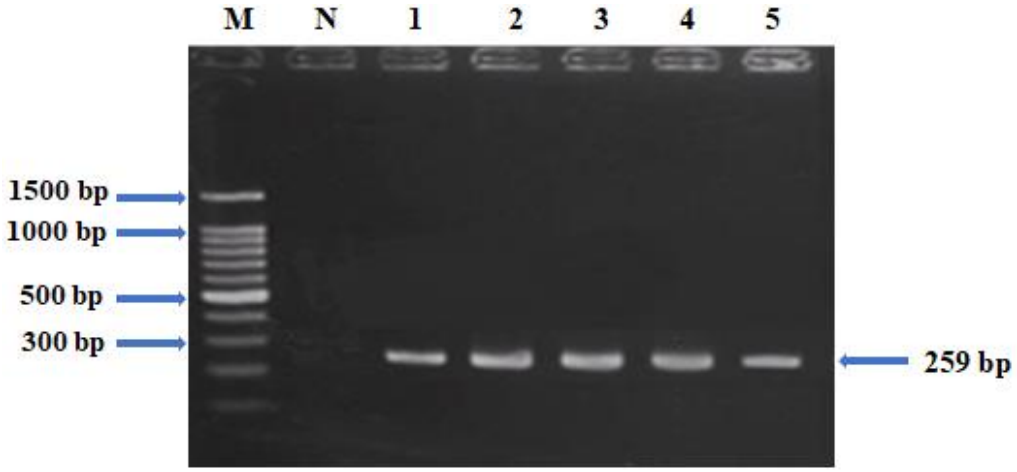

Figure 2. *rfb*E$_{O157}$ gene-based PCR for *E. coli* O157:H7. Here, M= 100 bp DNA ladder (Takara, Japan); N= Negative control; Lanes 1-5= *E. coli* O157:H7 isolates of broiler meat.

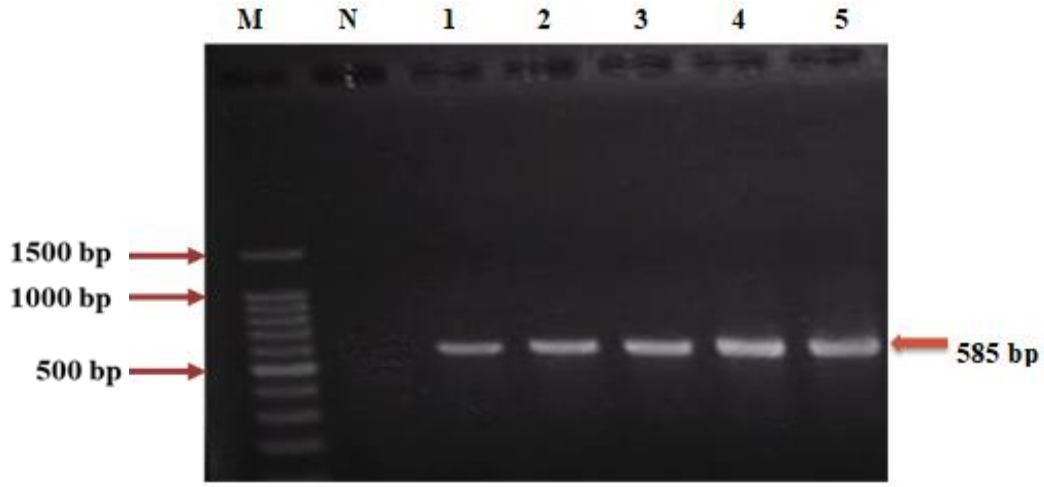

Figure 3. *mal*B gene-based PCR. Here, M= 100 bp DNA ladder (Takara, Japan); N= Negative control; Lanes 1-5= *E. coli* non-O157:H7 isolates of broiler meat.

Table 4. Antimicrobial susceptibility of *E. coli* isolates identified by disk diffusion method.

Antimicrobial Agents	No. (%) of antimicrobial susceptibility of *E. coli* isolates					
	E. coli O157:H7 (n=7)			*E. coli* non O157:H7 (n=42)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Amoxicillin(AMX)	1 (14.29)	2 (28.57)	4 (57.14)	0 (0)	7 (16.67)	35 (83.33)
Tetracycline (TE)	0 (0)	1 (14.29)	6 (85.71)	5 (11.90)	9 (21.43)	28 (66.67)
Gentamycin (GEN)	3 (42.86)	3 (42.86)	1 (14.29)	15 (35.71)	18 (42.86)	9 (21.43)
Streptomycin (S)	2 (28.57)	3 (42.86)	2 (28.57)	19 (45.24)	10 (23.81)	13 (30.95)
Erythromycin (E)	0 (0)	0 (0)	7 (100)	0 (0)	0 (0)	42 (100)
Azithromycin (AZM)	4 (57.14)	2 (28.57)	1 (14.29)	14 (33.33)	17 (40.48)	11 (26.19)
Ciprofloxacin (CIP)	4 (57.14)	1 (14.29)	2 (28.57)	21 (50.00)	11 (26.19)	10 (23.81)
Ceftriaxone (CTR)	6 (85.71)	0 (0)	1 (14.29)	23 (54.76)	7 (16.67)	12 (28.57)

Among the seven *E. coli* O157:H7 isolates, none were sensitive to all tested antibiotics. Specifically, one isolate (14.29%) showed resistance to two antibiotics (amoxicillin and tetracycline), while two isolates (28.57%) were resistant to three antibiotics (erythromycin, streptomycin, and ciprofloxacin). Additionally, one isolate (14.29%) resisted amoxicillin, streptomycin, and tetracycline; another (14.29%) resisted amoxicillin, tetracycline, and erythromycin; and one isolate (14.29%) exhibited resistance to four antibiotics (amoxicillin, tetracycline, erythromycin, and gentamicin) (Table 5). For the 42 *E. coli* non-O157:H7 isolates, none were susceptible to all antibiotics. Resistance to a single drug was observed in eight isolates (19.05%) against amoxicillin, and seven isolates (16.67%) against erythromycin. Resistance to two antibiotics was found in four isolates (9.52%) against amoxicillin and tetracycline, and five isolates (11.90%) against amoxicillin and streptomycin. Furthermore, nine isolates (21.43%) resisted three antibiotics (amoxicillin, streptomycin, and tetracycline), four isolates (9.52%) resisted erythromycin, streptomycin, and ciprofloxacin, and three isolates (7.14%) resisted amoxicillin, tetracycline, and erythromycin. Lastly, two isolates (4.76%) showed resistance to four antimicrobials (amoxicillin, tetracycline, erythromycin, and gentamicin).

Table 5. Results of antimicrobial resistance patterns of *E. coli* isolates obtained from broiler meat samples.

Isolates (n)	Antibiotic resistance patterns	No. (%) of isolates	95% CI	No. (class) of antibiotics
***E. coli* O157:H7 (7)**	AMX-TET	1 (14.29)	2.57 - 51.31	2 (2)
	AMX-S-TET	1 (14.29)	2.57 - 51.31	3 (3)
	E-S-CIP	2 (28.57)	8.22 - 64.11	3 (3)
	AMX-TET-ER	1 (14.29)	2.57 - 51.31	3 (3)
	AMX-TET-ER-GEN	2 (28.57)	8.22 - 64.11	4 (4)
***E. coli* non O157:H7 (42)**	AMX	8 (19.05)	9.98 - 33.3	1 (1)
	E	7 (16.67)	8.32 - 30.6	1 (1)
	AMX-TET	4 (9.52)	3.77 - 22.07	2 (2)
	AMX-S	5 (11.90)	5.19 - 25	2 (2)
	AMX-S-TET	9 (21.43)	11.71 - 35.94	3 (3)
	E-S-CIP	4 (9.52)	3.77 - 22.07	3 (3)
	AMX-TET-ER	3 (7.14)	2.46 - 19.01	3 (3)
	AMX-TET-ER-GEN	2 (4.76)	1.32 - 15.79	4 (4)

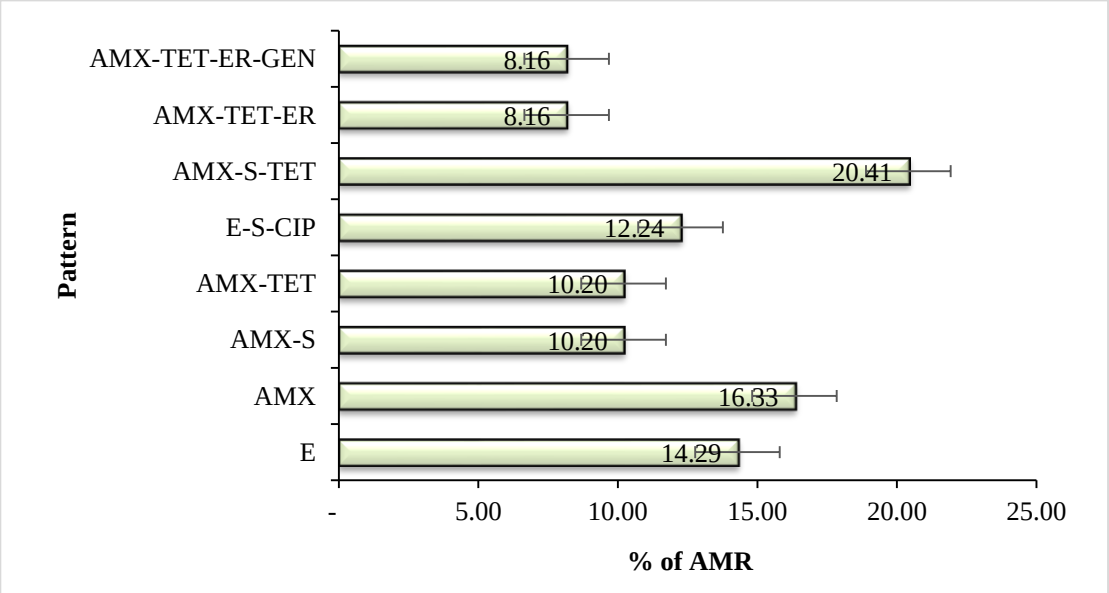

Figure 4. The overall AMR profiles of *E. coli* isolates from broiler meat in Mymensingh.

This study revealed that 48.98% (24 out of 49; 95% CI: 35.58–62.53) of the *E. coli* isolates were classified as MDR. Across the isolates, eight distinct antibiotic resistance patterns were identified, with four of these

categorized as MDR (Figure 4). The most frequently observed resistance pattern involved amoxicillin, streptomycin, and tetracycline (AMX-S-TE), found in 20.41% (10 out of 49) of the isolates. Moreover, four isolates exhibited resistance to four antibiotics from four different classes (AMX-TET-ER-GEN). The MAR index values for these isolates ranged between 0.13 and 0.50 (Figure 5).

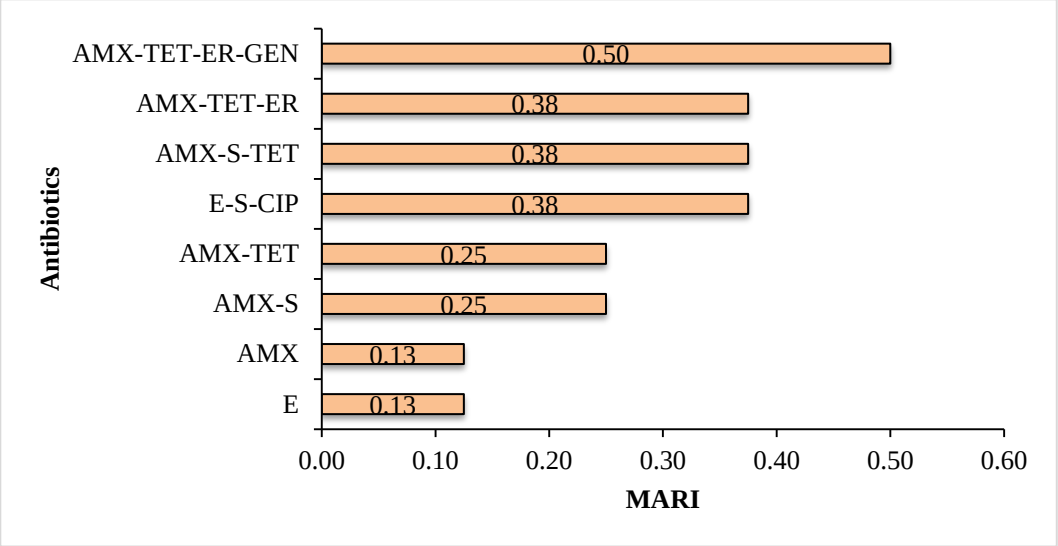

Figure 5. The MAR Index of *E. coli* isolates from broiler meat in Mymensingh.

4. Discussion

This study aimed to isolate and identify pathogenic bacteria from broiler meat samples collected from various markets in the Mymensingh district. Additionally, it assessed the antimicrobial resistance profiles of these bacteria and evaluated their potential public health implications. The characterization of the isolates involved cultural methods, morphological observation, staining techniques, and biochemical testing. Finally, PCR was used to amplify specific pathogenic genes in the bacterial isolates. An antibiogram was conducted on the pathogens to determine their susceptibility and resistance patterns against commonly used antibiotics available in the market.

This study detected the presence of *E. coli* in poultry meat, consistent with findings reported by Begum *et al*. (2010), Uddin *et al*. (2019), and Singh *et al.* (2025). Multiple selective culture media were employed concurrently to isolate the bacteria. The choice of media was informed by previous research in related fields, including studies by Kalin *et al*. (2012), Akter *et al*. (2016), Elbayoumi *et al*. (2018), and Rahman *et al*. (2020).

Meat provides a favorable environment for the growth of various bacteria, including those that can cause illness. Broiler meat, in particular, can harbor both pathogenic and non-pathogenic strains of *E. coli*, which may be passed to humans during handling or consumption. Studies, such as Kalin *et al*. (2012), have identified the presence of *E. coli* O157:H7 and non-O157:H7 strains in broiler meat. Consumption of undercooked meat contaminated with these bacteria poses health risks, and poor hygiene practices during meat processing can further increase the chance of disease transmission.

This study represents an important advancement in the investigation of *E. coli* O157:H7 and non-O157:H7 strains in Bangladesh (Fazle *et al*., 2014). The identification process involved detailed characterization of the isolates, including cultural and staining properties, latex agglutination testing PCR confirmation of *E. coli* O157:H7 (Rakotovao-Ravahatra *et al.*, 2022). Multiple selective culture media were employed simultaneously for organism cultivation, following approaches similar to those used by previous researchers (Islam *et al*., 2007; Fazle *et al*., 2014; Hassan *et al*., 2014).

The colony morphology of *E. coli* observed on sorbitol MacConkey agar in this study aligned with the observations reported by Kalin *et al*. (2012), Fazle *et al*. (2014), and Islam *et al.* (2024). Gram staining revealed the isolates as Gram-negative, short rods, typically arranged singly or in pairs, and exhibiting motility, consistent with descriptions provided by Hakkani *et al*. (2016) and Uddin *et al*. (2019).

Of the 49 *E. coli* isolates tested in this study, 7 produced positive results with a latex agglutination test designed to detect the O157:H7 serotype. This result confirmed their identification as *E. coli* O157:H7 and supports findings by Uddin *et al*. (2019), who used a similar approach for serotype verification. To validate these

findings at the molecular level, PCR assays were performed. All 7 latex-positive isolates were found to carry the *rfbE*O157 gene, a genetic marker specific to *E. coli* O157:H7. This molecular confirmation is consistent with the work of Fazle *et al.* (2014), who identified the *rfbE*O157 gene as a reliable target for distinguishing this pathogenic strain. In addition to the O157:H7 isolates, the remaining 42 isolates were PCR-positive for the *mal*B gene, a conserved gene commonly used to detect non-O157:H7 strains of *E. coli*. This observation corresponds with the findings of Islam *et al.* (2024), who highlighted the utility of the *mal*B gene in characterizing non-O157:H7 *E. coli* isolates. Overall, the combination of biochemical, serological, and molecular analyses provided robust confirmation of the identity and diversity of *E. coli* strains present in the studied samples.

In this study, *E. coli* isolates from broiler meat showed susceptibility to ciprofloxacin and gentamicin. Among the 7 *E. coli* O157:H7 isolates, 2 (28.57%) exhibited resistance to four antibiotics: amoxicillin, tetracycline, erythromycin, and gentamicin (AMX-TET-ER-GEN). These findings support previous reports by Mamun *et al*. (2016), Nesa *et al*. (2012), Veeraselvam *et al*. (2019), and Kundu *et al*. (2021). Similarly, among the 42 *E. coli* non-O157:H7 isolates, 2 (4.76%) showed resistance to the same four antibiotics. Resistance against erythromycin and amoxicillin was also observed, aligning with results from Islam *et al*. (2007), Fazle *et al*. (2014), Hassan *et al*. (2014), and Kundu *et al*. (2021). The presence of antibiotic resistance in these isolates suggests that indiscriminate antibiotic use may have contributed to this resistance development, as noted by Begum *et al*. (2010) and Uddin *et al*. (2019). The detection of resistant *E. coli* strains highlights a potential health hazard, emphasizing the need for measures to mitigate risk factors. Importantly, ciprofloxacin demonstrated high efficacy against *E. coli* infections in this study, corroborating findings from Fazle *et al*. (2014) and Kundu *et al*. (2021), who reported similar antibiotic susceptibility profiles.

These results are consistent with the findings of Haque *et al*. (2017), who reported similar patterns of multidrug resistance (MDR) in bacterial isolates. The observed high prevalence of MDR among *E. coli* strains presents a considerable challenge to public health, as it hampers the effectiveness of commonly used antibiotics in clinical treatment. Notably, the most frequent MDR profile identified in the current study was the combination of amoxicillin, streptomycin, and tetracycline resistance (AMX-S-TE), found in 20.41% (10 out of 49) of the isolates. This resistance pattern aligns with the observations made by Hassan *et al*. (2014), further highlighting its clinical relevance and widespread occurrence in *E. coli* populations. The emergence of the AMX-S-TE resistance pattern likely reflects the excessive or inappropriate use of these antibiotics in both human healthcare and livestock production systems. This underscores the urgent need to strengthen antibiotic stewardship and enforce guidelines to minimize the indiscriminate use of antimicrobials across various sectors. Additionally, the study found that 69.39% of the isolates had a MAR index above 0.2. This observation is in line with the findings of Osundiya *et al*. (2013), who noted that MAR index values above this threshold are generally indicative of exposure to environments where antibiotics are routinely used, such as in hospital settings or intensive farming operations. The presence of high MAR values among the isolates suggests significant antibiotic pressure, which can select for and propagate resistant bacterial strains. Further supporting this trend, Beshiru *et al*. (2022) and Fallah *et al*. (2021) documented notably high MAR indices of 0.60 and 0.75, respectively—well above the accepted benchmark for environmental contamination with antibiotic-resistant bacteria. Collectively, these findings from various studies point to a growing and alarming trend in antimicrobial resistance, likely driven by sustained and widespread antibiotic usage. This trend emphasizes the need for integrated surveillance and coordinated action to mitigate the further spread of MDR organisms.

5. Conclusions

The emergence of antibiotic-resistant bacteria on a global scale presents a significant challenge. The extensive application of widely used antimicrobials in food and livestock leads to the development of resistance. Evaluating health risks must encompass isolated incidents, and it is imperative that stringent rules and regulations be implemented without delay to mitigate risk factors. Implement the One Health Approach along with necessary sanitary standards for farm-to-plate meat to reduce the risk of antibiotic resistance. Contamination of broiler meat by *E. coli* is associated with the presence of antibiotics resistance, potentially contributing to an increase in antibiotic resistance among humans.

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Data availability
All relevant data are presented within the manuscript.

Conflict of interest
None to declare.

Authors' contribution
Md. Zarif Hossain: investigation, methodology, writing-original draft; M. Rafiqul Islam: investigation, methodology, writing-original draft; Sayed Abdullah-Al-Mamun: investigation, methodology; Fatema Islam: investigation; Sk Shaheenur Islam: investigation, writing-original draft; Yosef Deneke: writing-review & editing; Mohammad Ferdousur Rahman Khan: supervision, writing-review & editing; S. M. Lutful Kabir: conceptualization, funding acquisition, supervision. All authors have read and approved the final manuscript.

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