Molecular Mechanism Research (2025) Volume 3 Issue 1
doi: 10.59429/mmr.v3i1.8574
ISSN: 3029-2212 (O)

RESEARCH ARTICLE

Prevalence, Antimicrobial Resistance and Molecular Characterization of *Escherichia coli* in Milk Samples from Jashore District, Bangladesh

Elma Siddique Esha[1], Shohanur Rahaman[2], Sadia Islam[3], Md. Ashiqur Rahman[4*]

[1] *Elma Siddique Esha, Department of Microbiology, Jashore University of Science and Technology, Jashore, Bangladesh, elmaesha23@gmail.com*

[2] *Shohanur Rahaman, Department of Microbiology, Popular Diagnostic Limited, Dhaka, Bangladesh, shohanbuhs@gmail.com*

[3] *Sadia Islam, Department of Laboratory Medicine, Bangladesh Specialized Hospital Limited, Dhaka, Bangladesh, sadia.buhs.6700@gmail.com*

[4] *Md. Ashiqur Rahman, Department of Laboratory Medicine, Novus Clinical Research Services Limited, Dhaka, Bangladesh, ararashiqur@gmail.com*

***Corresponding author:** Md. Ashiqur Rahman, ararashiqur@gmail.com

ABSTRACT

Background: *Escherichia coli* is a common microbial flora in milk and milk products and is known to be a leading cause of illness in humans. It can lead to severe infections such as cellulitis, septicemia, and airsacculitis. Due to its potential health risks, monitoring and control of *E. coli* in milk are crucial for public health.

Objective: The study aimed to determine the prevalence and antimicrobial resistance patterns of *E. coli* from milk samples collected from various areas in Jashore District, Bangladesh.

Methods: A total of sixty milk samples were collected from different areas in Jashore. The samples were enriched in saline water, followed by serial dilution, and plated on MacConkey agar. Isolates were further cultured on EMB agar plates, identified using biochemical methods, and tested for antimicrobial resistance against seven antibiotics using MHA agar plates. DNA extraction and PCR amplification were performed to detect specific virulence genes using multiplex primers.

Results: Out of sixty isolates, 100% of *E. coli* isolates were resistant to erythromycin. Resistance was also observed to chloramphenicol (15%), gentamicin (18%), trimethoprim (0%), streptomycin (12%), cefoxitin (56%), and aztreonam (0%). The highest susceptibility was observed with cefoxitin and aztreonam. PCR results showed no amplification of virulence genes in the isolates.

Conclusion: The study found high antimicrobial resistance in *E. coli* from milk in Jashore, posing a public health risk. While virulence genes were not detected, the resistance highlights the need for better milk handling and stricter antibiotic use regulations in the dairy industry.

Keywords: *Escherichia coli*; Antimicrobial Resistance; Milk; PCR

ARTICLE INFO
Received: 13 January 2025|Accepted: 16 March 2025|Available online: 28 March 2025

CITATION
Esha, E. S.; Rahaman, S.; Islam, S.; Rahman, M. A.Prevalence, Antimicrobial Resistance, and Molecular Characterization of Escherichia coli in Milk Samples from Jashore District, Bangladesh. *Molecular Mechanism Research* 2025; 3(1): 8574.doi: 10.59429/mmr.v3i1.8574

1. Introduction

Food safety and the quality of food are critical topics of global public health concern today. Well-publicized and widespread foodborne disease outbreaks have raised awareness about the potential risks to human health from contaminated food products. These illnesses impose a heavy toll on human life, especially among vulnerable groups such as infants, children, the elderly, and other susceptible populations. Beyond human suffering, foodborne diseases also create significant social, cultural, and economic burdens on communities and their healthcare systems. In particular, milk and milk products are significant in both rural and urban diets worldwide, providing essential nutrients for people of all ages.

Milk is a well-known medium for the growth of numerous microorganisms, which can lead to spoilage or cause infections and intoxication in consumers. This makes milk a potential source of foodborne pathogens that can contribute to illness in humans. Milk is composed of water, proteins, fats, lactose, and minerals, which makes it an excellent environment for bacterial growth. Raw milk, in particular, is often consumed for its perceived nutritional advantages and traditional health benefits, though it also poses significant risks due to potential contamination by pathogenic microorganisms. The consumption of raw milk and raw milk products like cheese has been linked to outbreaks of foodborne illnesses caused by pathogens such as *Salmonella enterica*, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Escherichia coli* O157:H7.

The potential for disease transmission is especially high when milk is consumed in its raw form or when dairy products made from raw milk are consumed without proper pasteurization. Raw milk may contain bacteria introduced from the cow's udder during milking, which can then contaminate the milk. *Escherichia coli (E. coli)* is one of the most common bacterial contaminants found in milk, and its presence in milk has raised serious public health concerns. Although *E. coli* is a normal inhabitant of the intestines of both animals and humans, certain strains of the bacterium can cause severe gastrointestinal and extra-intestinal diseases in humans. The pathogens associated with *E. coli* contamination in milk are often entero-pathogenic or enterotoxigenic strains, which can cause serious illnesses ranging from mild gastrointestinal distress to life-threatening diseases, particularly among infants and those with weakened immune systems.

E. coli is a significant milk borne pathogen, capable of producing toxins like Shiga toxin, which can lead to severe conditions such as kidney failure. The presence of pathogenic *E. coli* in raw milk indicates poor hygiene during milking and handling. Additionally, the overuse of antibiotics in animal husbandry has led to antibiotic-resistant strains, further complicating treatment and increasing public health risks. This highlights the need for better dairy farm practices, improved hygiene, controlled antibiotic use, and promotion of milk pasteurization.

To address these concerns, various molecular techniques, including Polymerase Chain Reaction (PCR), have been developed for the rapid and specific detection of pathogenic *E. coli* strains and their virulence genes. PCR allows for the identification of multiple virulence factors simultaneously, helping to detect strains of *E. coli* that produce toxins or exhibit pathogenic properties. This method is useful for identifying and differentiating various *E. coli* pathotypes such as enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), and others. PCR-based approaches provide a more accurate and timely method for detecting potentially harmful pathogens in milk, improving the monitoring of food safety and public health protection.

This study aims to evaluate the prevalence and antimicrobial resistance of *E. coli* in milk from Jashore, Bangladesh, and to explore its molecular characterization using PCR to detect virulence genes. The goal is to improve milk safety and control the spread of antibiotic-resistant bacteria in dairy products.

2. Materials and methods

The experimental study was carried out in the Microbiology laboratory of the Department of Microbiology. The main aim of the study was to isolate and identify *Escherichia coli* in milk samples collected from coastal communities in Jashore, Khulna, Bangladesh. The antimicrobial sensitivity test and multiplex PCR were used to determine the prevalence of *E. coli* in different milk samples from different areas of Jashore.

2.1. Handling of apparatus and glassware in the laboratory

Laboratory glassware was properly cleaned using an appropriate detergent, followed by rinsing with tap water and finally distilled water. Petri plates, glassware, and other tools such as glass rods and beads were sterilized at 180°C for one hour in a hot air oven. Cotton buds, micropipette tips, and Eppendorf tubes were sterilized using moist heat at 121°C for 15 minutes in an autoclave. Inoculating loops and spreaders were sterilized using a spirit lamp.

2.2. Sample collection and study area

Milk samples were collected from various areas of the Jashore district of Bangladesh between June and October 2021. The primary areas included Ambortola, Sajiali, Ghope, Churamonkathi, Rajarhat, Borobazar, and Khajura. The samples were collected from farms, tea stalls, and milk vendors. The sample types included regular raw milk, refrigerated milk, frozen milk, and pasteurized milk.

2.3. Sample processing

Milk samples were placed into sterile test tubes with saline broth using a sterile micropipette. These test tubes were capped to prevent contamination and incubated. For serial dilution, 10 ml of each sample was diluted in sterile saline broth, followed by a 10-fold dilution series. After dilution, 10 µl from each test tube was dropped onto MacConkey agar plates. The plates were incubated at 37°C for 24 hours. Colonies were counted using the drop plate method, and CFU (colony-forming units) were calculated using the formula:

CFU/ml = (No. of colonies × Dilution factor) / Volume of culture plate.

2.4. Subculture and growth on agar plates

Isolated colonies were subcultured on MacConkey agar plates for further analysis. MacConkey agar is a selective and differential medium that allows for the growth of *E. coli* and other Gram-negative bacteria, which form pink colonies on the agar. For additional confirmation, growth on Eosin Methylene Blue (EMB) agar was also observed, as EMB is selective for Gram-negative bacteria and differentiates between lactose fermenters and non-fermenters.

2.5. Antimicrobial susceptibility testing

The antimicrobial susceptibility of the isolates was determined using the agar disc diffusion method (Kirby-Bauer method). Ten antibiotic discs representing different groups of antimicrobial agents were used. The antibiotic agents tested included chloramphenicol, gentamicin, trimethoprim, streptomycin, cefoxitin, erythromycin, and aztreonam. The test was performed by applying antibiotic discs to inoculated plates of Mueller-Hinton agar and incubating at 37°C for 18-24 hours. After incubation, the zone of inhibition was measured to assess the resistance, intermediate, or sensitive status of the isolates.

2.6. Molecular detection of *E. coli* by PCR

DNA extraction was performed using a boil method. A loop of bacterial culture was added to 0.5 ml of nuclease-free water, vortexed, and centrifuged. The supernatant was discarded, and the pellet was resuspended

in deionized water. The sample was boiled at 100°C for 10 minutes and then cooled on ice. The DNA was then extracted, centrifuged again, and the supernatant was stored at -20°C for further use.

Multiplex PCR was used to detect the presence of *E. coli* and other enteropathogenic bacteria. Multiple primer sets were selected to amplify different DNA sequences, producing distinct amplicon sizes. A typical PCR reaction mixture contained AmpliTaq Gold, dNTPs, MgCl2, and primers specific for *E. coli*. The thermal cycling program consisted of an initial denaturation at 93°C for 15 minutes, followed by 35 cycles of denaturation at 92°C for 40 seconds, annealing at 57°C for 1 minute, and extension at 72°C for 1.5 minutes. A final extension step was performed at 72°C for 7 minutes. PCR products were analyzed by gel electrophoresis using a 1.5% agarose gel and visualized under UV light after staining with ethidium bromide.

2.7. Preparation of agarose gel and electrophoresis

A 1.5% agarose gel was prepared by dissolving agarose in deionized water and boiling it. After cooling, the gel was poured into a tray with combs to form wells. The gel was then placed in an electrophoresis chamber, and TAE buffer was added to cover the gel. The PCR samples, along with a DNA ladder, were loaded into the wells, and the gel was run at 80V for 1 hour. After the run, the gel was stained with ethidium bromide and observed under a UV transilluminator.

3. Results

Samples were collected from cow farms, tea shops, and milk vendors using regular water bottles while wearing gloves to avoid contamination. The samples were then cultured on MacConkey agar and EMB media to isolate *E. coli* and observe its cultural characteristics.

MacConkey agar is selective for Gram-negative bacteria like *E. coli*, producing pink colonies due to lactose fermentation. EMB agar also selects for Gram-negative bacteria and differentiates coliforms like *E. coli* with a metallic green sheen, while inhibiting Gram-positive bacteria. This table summarizes the colony appearances of *E. coli* on both media (**Table 1**).

Table 1. Describe the culture characteristics of *Staphylococcus aureus* on mannitol salt agar.

Selective Media	Name of the Organism	Shape & Rearrangement	Nature of the Colony	Appearance
MacConkey ager media	*E. coli*	Pink colony and deep pink center pink colony	Smooth shiny surface with pink colony	Pinkish to deep pink
Eosin methyl blue media	*E. coli*	Light green colony and media made red to green	A distinctive metallic green sheen	Smooth green sheen

By distributing the sample in drops, colony counting can be done faster and perhaps more accurately. Generally, 3-30 colonies are used to count in drop plate method. A colony-forming unit (CFU) is a unit used to estimate the number of fungal or bacteria cells in a sample which are viable, able to multiply via binary fission under the controlled conditions (**Table 2**).

Table 2. Total colony count by drop plate method.

Sample No	Dilution No	TVC Count (CFN)
S1	10^{-3}	TNTC
S1	10^{-4}	7×10^{-5}
S2	10^{-3}	9×10^{-5}
S3	10^{-3}	9×10^{-5}
S3	10^{-4}	7×10^{-5}
S4	10^{-3}	TNTC
S4	10^{-4}	1.8×10^{-5}
S5	10^{-3}	TNTC
S6	10^{-3}	5.3×10^{-5}
S7	10^{-4}	3.3×10^{-5}
S8	10^{-3}	8.9×10^{-5}
S9	10^{-3}	9.4×10^{-5}
S9	10^{-4}	TFTC
S10	10^{-3}	TNTC
S11	10^{-3}	TNTC
S11	10^{-4}	9×10^{-5}
S13	10^{-3}	9.8×10^{-5}
S13	10^{-4}	TFTC
S14	10^{-3}	TNTC
S14	10^{-4}	3.65×10^{-5}
S16	10^{-3}	2.6×10^{-5}
S17	10^{-3}	7.3×10^{-5}
S20	10^{-4}	TNTC
S22	10^{-3}	39.5×10^{-5}
S24	10^{-3}	19.6×10^{-5}
S24	10^{-4}	TFTC
S28	10^{-3}	TNTC
S29	10^{-3}	TNTC
S30	10^{-4}	TNTC
S30	10^{-5}	4.6×10^{-5}
S31	10^{-5}	4.8×10^{-5}
S34	10^{-3}	TNTC
S35	10^{-5}	23.5×10^{-5}
S36	10^{-3}	TNTC
S37	10^{-5}	7.3×10^{-5}
S40	10^{-4}	TFTC
S39	10^{-3}	9.5×10^{-5}
S41	10^{-3}	9.6×10^{-5}

Sample No	Dilution No	TVC Count (CFN)
S44	10^{-4}	TFTC
S48	10^{-3}	4.8×10^{-5}
S49	10^{-3}	8.3×10^{-5}
S50	10^{-4}	TNTC
S45	10^{-5}	8×10^{-5}
S41	10^{-5}	16×10^{-5}
S54	10^{-5}	TNTC
S51	10^{-3}	5×10^{-5}
S46	10^{-5}	TFTC
S57	10^{-3}	3×10^{-5}
S52	10^{-5}	TFTC
S59	10^{-4}	35×10^{-5}
S51	10^{-3}	26×10^{-5}
S55	10^{-3}	5.8×10^{-5}

Table 2. (*Continued*)

In the biochemical tests, *E. coli* showed a negative result for the oxidase test, as there was no color change when exposed to Kovac's reagent. The catalase test was positive, indicated by the production of bubbles when hydrogen peroxide was added. In the motility test, *E. coli* demonstrated positive motility, showing growth throughout the soft agar. The Kligler Iron Agar test showed yellow coloration in both the butt and slant, indicating fermentation of glucose, sucrose, and lactose, with gas production and no hydrogen sulfide formation. Lastly, the citrate test was negative, as there was no color change in the medium, indicating that *E. coli* could not utilize citrate (**Table 3**).

Table 3. Biochemical result.

Sample no.	Oxidase test	Catalase test	Motility test	Citrate test	KIA test				Presumptive bac.
					Butt	Co_2	Slant	H_2S	
S2 I1	-	+	+	-	Acid	+	Acid	-	*E. coli*
S2 I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S3 I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S2 I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S3 I1	-	+	+	-	Acid	+	Acid	-	*E. coli*
S5 I1	-	+	+	-	Acid	+	Acid	-	*E. coli*
S4 I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S2 I5	-	+	+	-	Acid	+	Acid	-	*E. coli*
S6 I1	-	+	+	-	Acid	+	Acid	-	*E. coli*
S4 I1	-	+	+	-	Acid	+	Acid	-	*E. coli*
S16I1 r	-	+	+	-	Acid	+	Acid	-	*E. coli*
S17 I1	-	+	+	-	Acid	+	Acid	-	*E. coli*
S14 I1	-	+	+	-	Acid	+	Acid	-	*E. coli*

Sample no.	Oxidase test	Catalase test	Motility test	Citrate test	KIA test				Presumptive bac.
					Butt	Co_2	Slant	H_2S	
S16 I1y	-	+	+	-	Acid	+	Acid	-	*E. coli*
S21 I1	-	+	+	-	Acid	+	Acid	-	*E. coli*
S14 I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S46I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S54I1	-	+	+	-	Acid	+	Acid	-	*E. coli*
S51I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S13 I4	-	+	+	-	Acid	+	Acid	-	*E. coli*
S13I3	-	+	+	-	Acid	+	Acid	-	*E. coli*
S12I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S9I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S10I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S27I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S40I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S33I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S34I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S31I1	-	+	+	-	Acid	+	Acid	-	*E. coli*
S49I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S51I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S39I1	-	+	+	-	Acid	+	Acid	-	*E. coli*
S15I1	-	+	+	-	Acid	+	Acid	-	*E. coli*
S44I1	-	+	+	-	Acid	+	Acid	-	*E. coli*
S57I3	-	+	+	-	Acid	+	Acid	-	*E. coli*
S29I2	-	+	+	-	Acid	+	Acid	-	*E. coli*
S40I2	-	+	+	-	Acid	+	Acid	-	*E. coli*

Table 3. (*Continued*)

In this experiment, were Chloramphenicol (30μg), Gentamicin (10μg), Trimethoprime (05μg), Streptomycin (10μg), Cefoxitin (30μg), Erathromycin (15μg), Aztroeonam (30μg), used to measure the susceptibility & resistance level of *Escherichia coli* (**Table 4**).

Table 4. Antibiotic sensitivity test results.

Sample No.	CL	GEN	TMP	S	FOX	E	ATM
S2 I1	S(24nm)	S(20nm)	S(22nm)	I(14nm)	S(21.4nm)	R	S(32nm)
S2 I2	S(24nm)	S(22nm)	S(22.5nm)	I(14nm)	S(24nm)	R	S(31nm)
S3 I2	S(23.5nm)	S(20.5nm)	S(22nm)	R(13nm)	S(21nm)	R	S(35nm)
S2 I2	S(20.5nm)	S(21nm)	S(24nm)	I(17nm)	S(21nm)	R	S(29nm)
S3 I1	S(24nm)	R(14nm)	S(25nm)	I(17nm)	S(22.5nm)	R	S(31nm)
S5 I1	I(15.5nm)	R(13.2nm)	S(21nm)	I(14nm)	S(24.6nm)	R	S(31nm)

Sample No.	CL	GEN	TMP	S	FOX	E	ATM
S4 I2	S(20nm)	S(19nm)	R	I(14.5nm)	S(24nm)	R	S(34nm)
S2 I5	S(24nm)	I(16.5nm)	S(24.5nm)	S(19.5nm)	S(23nm)	R	S(29nm)
S6 I1	S(21nm)	R(14.2nm)	S(22nm)	S(17.5nm)	S(25.5nm)	R	S(32nm)
S4 I1	S(22nm)	S(24nm)	S(23nm)	R(12nm)	S(21nm)	R	S(30nm)
S16I1 r	S(24nm)	S(24nm)	S(24.2nm)	I(15.5nm)	S(21nm)	R	S(35nm)
S17 I1	S(24nm)	S(22nm)	S(24.5nm)	R(12.5nm)	S(24nm)	R	S(28nm)
S14 I1	I(16.5nm)	R	S(24nm)	R(11nm)	S(27nm)	R	S(30nm)
S16 I1y	S(24.6nm)	S(20nm)	S(21nm)	I(16.5nm)	S(22.5nm)	R	S(31nm)
S21 I1	S(20nm)	I(17nm)	S(22.4nm)	I(14nm)	S(21nm)	R	S(32nm)
S14 I2	S(23nm)	S(21nm)	S(22nm)	S(20nm)	S(24nm)	R	S(32nm)
S46I2	I(15nm)	S(21nm)	S(21.7nm)	I(16nm)	S(25nm)	R	S(33nm)
S54I1	I(16.8nm)	S(24nm)	S(21nm)	R(13.2nm)	S(21nm)	R	S(32nm)
S51I2	S(24.5nm)	I(17.2nm)	S(25nm)	R(09nm)	S(24.5nm)	R	S(29nm)
S13 I4	S(25nm)	S(23nm)	S(22nm)	R(11.4nm)	S(22.4nm)	R	S(31nm)
S13I3	S(25nm)	S(21nm)	R	R(13nm)	S(21.8nm)	R	S(31nm)
S12I2	S(21nm)	R(14nm)	S(24nm)	S(18.5nm)	S(23nm)	R	S(30nm)
S9I2	S(21.5nm)	S(22nm)	S(21nm)	I(16.5nm)	S(24nm)	R	S(32nm)
S10I2	S(26nm)	S(24nm)	S(28nm)	R(11nm)	S(24nm)	R	S(35nm)
S27I2	S(23.6nm)	S(22nm)	S(24nm)	I(14nm)	S(22.4nm)	R	S(32nm)
S40I2	S(20.5nm)	S(22nm)	S(21nm)	I(16nm)	S(21nm)	R	S(29nm)
S33I2	I(16nm)	R	S(26nm)	R(11.5nm)	S(24nm)	R	S(28nm)
S34I2	S(20nm)	S(24nm)	S(23nm)	R(11.5nm)	S(24nm)	R	S(35nm)
S31I1	S(26nm)	S(21nm)	S(23nm)	R(09nm)	S(21nm)	R	S(30nm)
S49I2	S(24nm)	S(19nm)	S(24nm)	I(16nm)	S(24.8nm)	R	S(34nm)
S51I2	S(24nm)	S(20nm)	S(24nm)	S(20nm)	S(27nm)	R	S(32nm)
S39I1	S(24.5nm)	I(17.5nm)	S(21nm)	R(10.5nm)	S(212nm)	R	S(32nm)
S15I1	S(20nm)	S(24.2nm)	S(28nm)	I(17nm)	S(24nm)	R	S(31nm)
S44I1	S(22nm)	S(21.7nm)	S(26nm)	I(15.4nm)	S(23.5nm)	R	S(34nm)
S57I3	S(22nm)	S(22.6nm)	S(23nm)	I(16nm)	S(22nm)	R	S(31nm)
S29I2	S(23nm)	S(24nm)	S(22nm)	R(11nm)	S(21nm)	R	S(32nm)
S40I2	S(24nm)	S(21nm)	S(22nm)	I(14nm)	S(22nm)	R	S(32nm)

Table 4. (*Continued*)

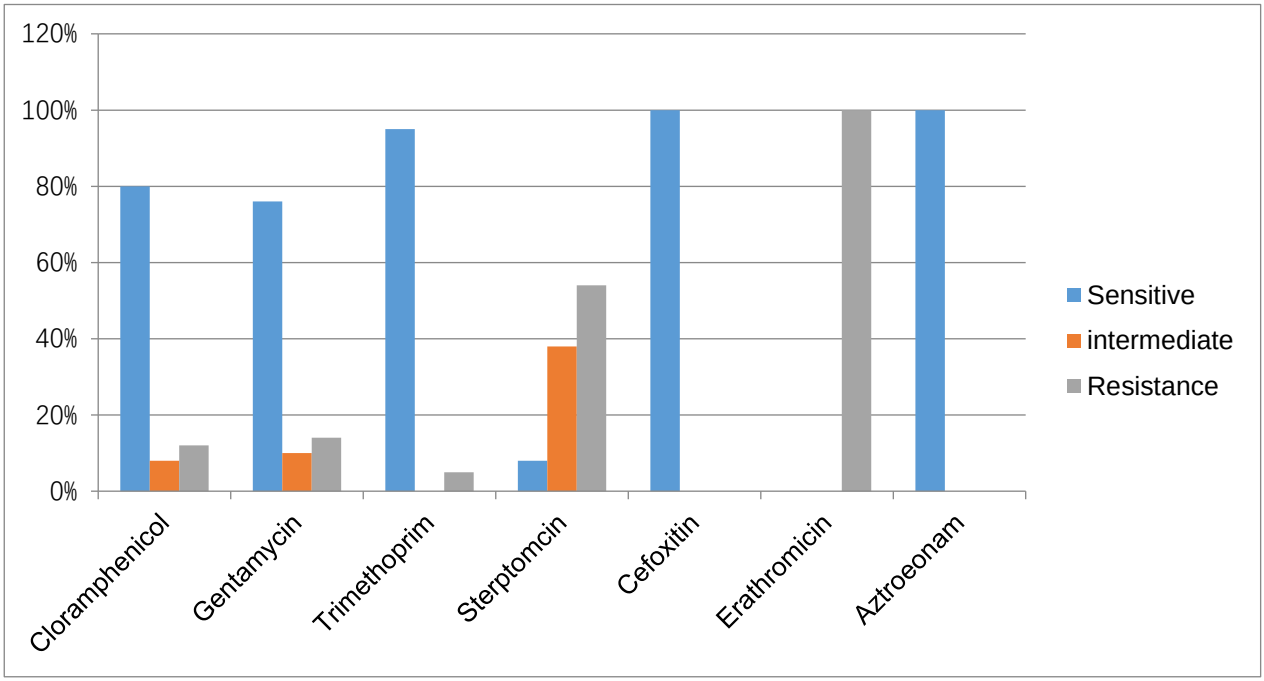

Figure 1. Result of antimicrobial sensitivity test in diagram.

The antimicrobial sensitivity test showed varying levels of susceptibility and resistance of *E. coli* to different antibiotics, with some antibiotics inhibiting growth while others showed resistance.

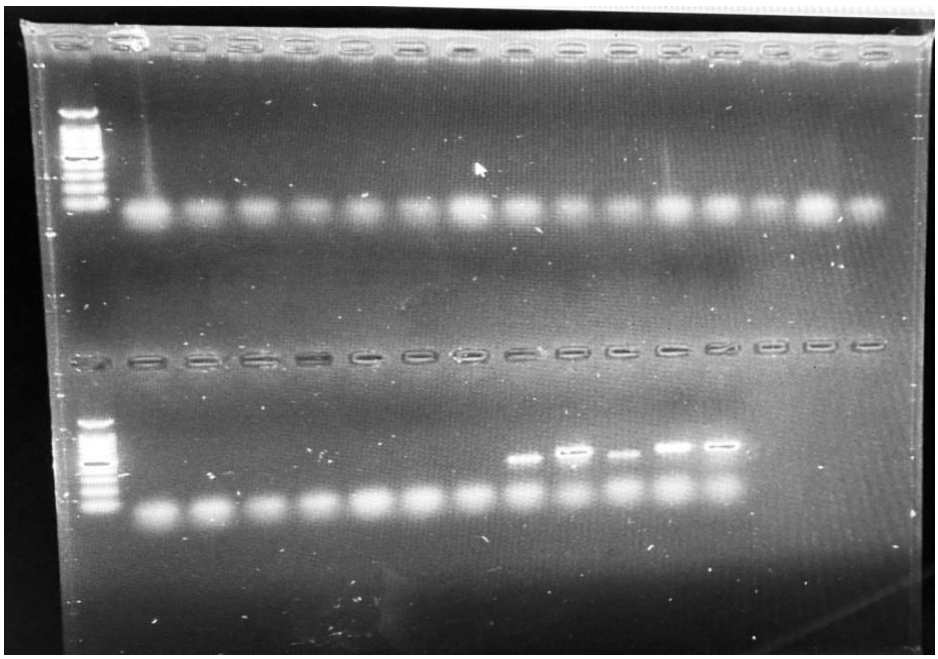

Figure 2. PCR results by gel electrophoresis.

The gel electrophoresis did not show any bands, indicating that none of the targeted genes (EstA, Ial, BfpA, Pcvd, eaeA, IpaH, eltB) were amplified in the PCR process (**Figure 2**).

4. Discussion

In this study, milk emerged as a significant source of *Escherichia coli*, particularly from cows, which are among the most common cattle worldwide. The relatively high prevalence of *E. coli* isolated in this study highlights an important concern for health authorities, particularly in densely populated areas. An outbreak could have severe health implications for the local population. Additionally, the use of antibiotics in veterinary medicine, although common, raises concerns due to the potential for antibiotic residues to enter the human food chain or lead to reduced drug efficacy, especially when administered improperly by unqualified personnel.

A total of 60 milk samples were collected from different markets in Jashore, Bangladesh, with *E. coli* being isolated from 48 of these samples, resulting in a 65% prevalence rate. This prevalence is relatively high compared to similar studies conducted in other regions, which reported lower levels of contamination in milk samples. This suggests that milk, particularly in regions with unregulated animal husbandry practices, could act as a reservoir for *E. coli* contamination.

Following isolation, the *E. coli* strains were subjected to a series of biochemical tests, which confirmed their identity. The subsequent antibiotic susceptibility tests revealed alarming resistance patterns: 28% of the isolates were resistant to Chloramphenicol, 34% to Gentamicin, 22% to Trimethoprim, 60% to Streptomycin, and 100% to Erythromycin. This high level of antibiotic resistance is concerning, as it could pose a significant threat to both animal and human health. The development of resistance in *E. coli* strains has been linked to the excessive and often unregulated use of antibiotics in livestock treatment and feed.

The study also aimed to assess the presence of multiple genes in the *E. coli* isolates using Polymerase Chain Reaction (PCR) to better understand the molecular characteristics of these strains. However, despite the use of five different primers targeting specific genes, no bands were detected in the PCR, indicating that none of the isolates harbored the targeted genes. This finding contrasts with other studies where specific virulence genes were identified in *E. coli* strains isolated from milk or other sources, suggesting that the strains in this study may not possess the same virulence factors.

Overall, the results of this study demonstrate a significant presence of multi-drug resistant *E. coli* strains in milk samples from the Jashore District. This highlights the importance of monitoring antibiotic use in livestock, as the presence of multi-drug resistant strains could serve as a potential source of resistance transmission to humans. Given the potential public health risks, further research and strict regulations on antibiotic use in animal husbandry are essential to mitigate the spread of resistant pathogens.

5. Conclusion

This study reveals a high prevalence of *Escherichia coli* in milk samples from Jashore, Bangladesh, with significant antibiotic resistance, particularly to Erythromycin, Chloramphenicol, and Streptomycin. The presence of multi-drug-resistant strains poses a public health risk, as these bacteria can transfer resistance to human pathogens. Although no virulence genes were detected, the findings highlight the need for better regulation of antibiotic use in livestock and improved surveillance to protect public health.

Conflict of interest

The authors declare no conflict of interest.

References

1. Akond MA, Alam S, Hassan SM, Shirin M. Antibiotic resistance of Escherichia coli isolated from poultry and poultry environment of Bangladesh. Internet Journal of food safety. 2009 Jan;11:19-23.
2. Rahman MA, Rahman AK, Islam MA, Alam MM. Antimicrobial resistance of Escherichia coli isolated from milk, beef and chicken meat in Bangladesh. Bangladesh Journal of Veterinary Medicine. 2017 Jul 1;15(2).
3. Akbar A, Sitara U, Ali I, Khan MI, Phadungchob T, Anal AK. Presence of Escherichia coli in poultry meat: A potential food safety threat. International Food Research Journal. 2014 May 1;21(3):941.
4. Altalhi AD, Hassan SA. Bacterial quality of raw milk investigated by Escherichia coli and isolates analysis for specific virulence-gene markers. Food control. 2009 Oct 1;20(10):913-7.
5. Islam MA, Roy S, Nabi A, Solaiman S, Rahman M, Huq M, Siddiquee NA, Ahmed N. Microbiological quality assessment of milk at different stages of the dairy value chain in a developing country setting. International Journal of Food Microbiology. 2018 Aug 2;278:11-9.
6. Ntuli V, Njage PM, Buys EM. Characterization of Escherichia coli and other Enterobacteriaceae in producer-distributor bulk milk. Journal of dairy science. 2016 Dec 1;99(12):9534-49.
7. Kumar R, Prasad A. Detection of E. coli and Staphylococcus in milk and milk products in and around Pantnagar. Veterinary World. 2010 Nov 1;3(11):495.
8. Vidal M, Kruger E, Durán C, Lagos R, Levine M, Prado V, Toro C, Vidal R. Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic Escherichia coli associated with enteric infections. Journal of clinical microbiology. 2005 Oct;43(10):5362-5.
9. El-Leboudy AA, Amer AA, El-Mohsen SA. Detection of some pathogenic organisms from dairy farm milk. Alexandria Journal of Veterinary Sciences. 2014 Dec 1;44(3):111-8.
10. Megersa R, Mathewos M, Fesseha H. Isolation and identification of Escherichia coli from dairy cow raw milk in Bishoftu town, Central Ethiopia. Arch Vet Anim Sci. 2019;1(1).
11. Yohannes G. Isolation, identification, and antimicrobial susceptibility testing of Escherichia coli isolated from selected dairy farms in and around Mekelle, Tigray, Ethiopia. J Vet Sci Technol. 2018;9(518):10-4172.
12. Dadi S, Lakew M, Seid M, Koran T, Olani A, Yimesgen L. Isolation of Salmonella and E. coli (E. coli O157: H7) and its Antimicrobial Resistance Pattern from Bulk Tank Raw Milk in Sebeta Town, Ethiopia. J Anim Res Vet Sci. 4: 021. of. 2020;7:2.
13. Amenu K, Grace D, Nemo S, Wieland B. Bacteriological quality and safety of ready-to-consume milk and naturally fermented milk in Borana pastoral area, southern Ethiopia. Tropical animal health and production. 2019 Sep 1;51:2079-84.
14. Gray-Hammerton C. Beta-Lactamase Resistance Harboured by Escherichia coli isolated from a Dairy Farm (Doctoral dissertation, University of Nottingham).
15. Ibrahim DR, Dodd CE, Stekel DJ, Ramsden SJ, Hobman JL. Multidrug resistant, extended spectrum β-lactamase (ESBL)-producing Escherichia coli isolated from a dairy farm. FEMS microbiology ecology. 2016 Apr 1;92(4):fiw013.