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Prevalence and Antimicrobial Susceptibility Profiles of Escherichia Coli Isolated from Raw Cow Milk on Selected Intensive Dairy Farms in Bishoftu, Ethiopia

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Abstract

Background

Raw milk can serve as a vehicle for foodborne pathogens, including *Escherichia coli*, and may pose a public health risk, particularly where unpasteurized milk is commonly consumed.

Objective

This study aimed to determine the prevalence of *E. coli* in raw cow milk and characterize the antimicrobial susceptibility profiles of the recovered isolates from selected intensive dairy farms in Bishoftu, Ethiopia.

Methods

A cross-sectional study was conducted from October to May 2025 in three selected dairy farms in Bishoftu. A total of 210 milk samples were collected and examined using bacteriological and biochemical methods. Antimicrobial susceptibility was assessed using the Kirby–Bauer disk diffusion method. The susceptibility results were summarized for six antimicrobial agents.

Results

From 210 milk samples examined, 113 (53.8%) were positive for *E. coli*. The prevalence was 55.8% (53/95) at EMDID, 48.9% (22/45) at EDF, and 54.3% (38/70) at CVMA. The difference among farms was not statistically significant ($p = 0.743$). Contamination was more frequent in bulk-tanker milk (80.0%, 32/40) than in individual cow milk (47.6%, 81/170; $p = 0.0004$). The highest susceptibility was observed to amikacin (92.0%), gentamicin (85.8%), and ceftriaxone (77.9%). Resistance was highest to ampicillin (69.9%) and trimethoprim–sulfamethoxazole (61.9%). Based on the study's reported classification, 51 isolates (45.1%) were categorized as multidrug-resistant.

Conclusion

E. coli was detected in more than half of the milk samples examined, and the isolates exhibited varying levels of resistance to the antimicrobial agents tested. These findings underscore the importance of hygienic milk production and handling, appropriate antimicrobial use, and continued surveillance of antimicrobial resistance in dairy production systems.

INTRODUCTION

Ethiopia has the largest livestock population in Africa. According to the Central Statistical Agency of Ethiopia [1], the national cattle population was estimated at approximately 55.03 million in 2013. Female cattle accounted for 55.38% of the population, while males represented 44.62%. The country also had substantial numbers of dairy and milking cows. Most cattle were indigenous breeds (98.71%), whereas hybrid and exotic breeds accounted for 1.15% and 0.14%, respectively. Annual cow milk production was estimated at approximately 2.9 billion litres [1]. Cattle are the principal source of milk in Ethiopia, contributing approximately 83% of national production, while camels and goats account for the remainder [2].

Milk production and consumption patterns in Ethiopia are largely shaped by household use and traditional handling practices. An estimated 82.9% of milk produced is consumed by households, while 10% is processed into products with longer shelf lives, 6.61% is sold, and 0.43% is used as payment in kind [3]. Per capital milk consumption was estimated at approximately 17 litres per year [2]. The widespread consumption of raw milk and traditionally prepared dairy products makes milk hygiene an important food safety concern.

Raw milk can support the survival and growth of microorganisms that cause food spoilage and human illness. Potential sources of contamination include manure, soil, water, contaminated equipment, and inadequate milking and handling practices [4, 5]. Foodborne pathogens associated with milk and dairy products include *Campylobacter jejuni*, pathogenic *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, and *Yersinia enterocolitica* [5].

Among these microorganisms, *E. coli* is particularly relevant to milk hygiene and public health [6]. It is a Gram-negative, facultative anaerobic, rod-shaped bacterium that normally inhabits the intestinal tract of humans and animals [7, 9]. Although most *E. coli* strains are harmless, some possess virulence factors that enable them to cause intestinal or extra intestinal disease. Consequently, detecting *E. coli* in milk may indicate fecal contamination and inadequate hygiene, although species-level identification alone does not establish that an isolate is pathogenic [9].

Pathogenic *E. coli* strains are classified into pathotypes according to their virulence characteristics and mechanisms of disease. These include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), and entero aggregative *E. coli* (EAEC). Related terminology includes Shiga toxin-producing *E. coli* (STEC) and verotoxin-producing *E. coli* (VTEC) [9, 11]. Identifying these pathotypes requires additional characterization beyond routine bacteriological and biochemical identification.

Milk from a healthy mammary gland is generally expected to contain few microorganisms. However, contamination can occur during milking, collection, storage, transportation, and subsequent handling, particularly when udder hygiene, equipment sanitation, or storage conditions are inadequate [12]. These risks highlight the importance of maintaining hygienic practices throughout the milk supply chain.

Antimicrobial resistance is another concern associated with bacterial contamination of foods of animal origin. Resistant *E. coli* may circulate among animals, humans, and the environment, while the inappropriate or excessive use of antimicrobials can contribute to the selection and spread of resistant bacteria. Resistance may compromise treatment options when infections occur and complicate efforts to control bacterial diseases in both veterinary and human medicine [13].

Although studies have investigated bacterial contamination and antimicrobial susceptibility in Bishoftu and other parts of Ethiopia, evidence specifically addressing *E. coli* isolated from raw cow milk produced on intensive dairy farms remains limited. Local data are needed to characterize the extent of contamination and the antimicrobial susceptibility profiles of recovered isolates.

Therefore, this study investigated the prevalence of *E. coli* in raw cow milk collected from selected intensive dairy farms in Bishoftu, Ethiopia, assessed the distribution of positive samples across farms and sample types, examined associations with selected animal level factors, and characterized the antimicrobial susceptibility profiles of the isolates.

OBJECTIVES OF THE STUDY

- To isolate and identify *E. coli* from raw cow milk samples collected from selected intensive dairy farms.
- To determine the antimicrobial susceptibility profiles of the recovered isolates.
- To compare the prevalence of *E. coli* among the selected dairy farms and milk sample types.
- To assess associations between *E. coli* positivity and selected factors, including cow age and body condition score.

MATERIALS AND METHODOLOGY

Study Area

The study was conducted in Bishoftu, Ethiopia, approximately 47 km southeast of Addis Ababa. The town is located at 8°45' N latitude and 38°59' E longitude, at an elevation of approximately 1,920 m above sea level. Bishoftu comprises nine kebeles, peri-urban areas, and surrounding rural settlements. Dairy production is an important agricultural activity in the area, with farms ranging from smallholder operations to larger commercial enterprises. Three dairy farms-EMDID, CVMA, and EDF were purposively selected for the study. Figure 1 presents the location of the study area.

Study Animals

The study involved apparently healthy lactating dairy cows kept under intensive management on the selected farms.

Study Design and Period

A cross-sectional study was conducted from October, 2025 to May, 2025 to determine the prevalence of *E. coli* in raw cow milk and assess the antimicrobial susceptibility profiles of the recovered isolates

Sample Size Determination

A total of 210 milk samples were collected from three purposively selected dairy farms. The sample size was determined primarily by logistical considerations, including farm accessibility, available resources, and the time allocated for fieldwork. Although the sample size was not established through a formal prevalence-based calculation, samples were collected from the selected farms to characterize *E. coli* occurrence under the study conditions.

Sampling Methods and Sample Collection

A simple random sampling technique was used to collect raw milk samples from individual cows and farm bulk tanks. Milk samples were collected aseptically in the morning. The reason for collecting morning samples was that most dairy farms deliver milk to collection centers shortly after milking.

During sampling, the first 3–4 streams of milk were discarded to minimize contamination. Then, an adequate volume of milk was collected from the teats and placed into sterile universal bottles and tanker milk were collected. A proportional number of samples was collected from each farm.

The samples were labeled and immediately transported to the Microbiology Laboratory of the College of Veterinary Medicine and Agriculture under refrigeration using ice boxes. Bacteriological analysis was initiated within one hour of sample collection. Isolation of *E. coli* and antimicrobial susceptibility testing were conducted in the laboratory.

Isolation and Identification of Escherichia Coli

The isolation procedure was described as following ISO 16649-2:2001. Samples were inoculated into 9 mL of modified tryptone soya broth and incubated at 41°C for 24 hours. Following enrichment, the samples were streaked onto MacConkey agar and incubated at 37°C for 24 hours.

Pink, lactose-fermenting colonies were considered presumptive *E. coli* and subcultured onto eosin methylene blue (EMB) agar. Colonies exhibiting a characteristic metallic-green sheen were considered presumptive *E. coli*. Further identification involved Gram staining and biochemical tests. The expected profile comprised Gram-negative rods, a negative oxidase reaction, positive indole and methyl red reactions, a negative Voges-Proskauer reaction, and a triple sugar iron (TSI) reaction with an alkaline slant and acid butt, without hydrogen sulfide production.

Antimicrobial susceptibility testing and multidrug resistance classification

Antimicrobial susceptibility testing was performed using the disk diffusion method. The susceptibility results were interpreted according to [insert the standard and edition actually used in the study]. Isolates were categorized as resistant, intermediate, or susceptible according to the applicable interpretive criteria.

Multidrug resistance (MDR) was defined as non-susceptibility to at least one antimicrobial agent in three or more antimicrobial classes. For each *Escherichia coli* isolate, the susceptibility profile was assessed across the antimicrobial classes represented in the study panel. Gentamicin and amikacin were treated as members of the same antimicrobial class and were not counted as two separate classes. Isolates meeting the class-based definition were classified as MDR, while those that did not meet the criterion were classified as non-MDR. The number and proportion of isolates in each category were calculated using the 113 confirmed *E. coli* isolates as the denominator.

A 0.5 McFarland standard bacterial suspension was prepared and uniformly spread on the agar surface. The plates were incubated at 37 ^{0}C for 16 to 18 hours. Zone of inhibition were measured and interpreted as susceptible, intermediate, or resistant according to CLSI standards.

Data Analysis

Data were entered into Microsoft Excel and analysed using IBM SPSS Statistics, version 16. Descriptive statistics, including frequencies and percentages, were used to summarize the results. Associations between *E. coli* positivity and selected categorical variables were assessed using the chi-square test. Statistical significance was set at $p < 0.05$, with a 95% confidence level.

RESULTS

Prevalence and Distribution of E. coli

Of the 210 raw cow milk samples examined, 113 were positive for *E. coli*, giving an overall prevalence of 53.8%. The prevalence varied across the three dairy farms, although the difference was not statistically significant ($p = 0.743$).

The highest prevalence was recorded at EMDID (55.8%, 53/95), followed by CVMA (54.3%, 38/70) and EDF (48.9%, 22/45). These findings indicate that *E. coli* was detected on all three farms, with relatively small differences in the observed prevalence.

Table 1
Prevalence of *E. coli* by dairy farm and milk sample type

Factor	Category	Total	Number of positive (n)	Prevalence (%)	95% CI (%)	p-value
Farm	EMDID	95	53	55.8	45.8–65.4	
	EDF	45	22	48.9	35.0–63.0	0.743
	CVMA	70	38	54.3	42.7–65.4	
Milk sample type	Individual cow milk	170	81	47.6	40.3–55.1	0.0004
	Bulk tanker milk	40	32	80.0	65.2–89.5	

The prevalence of *E. coli* was significantly higher in bulk-tanker milk (80.0%) than in individual-cow milk (47.6%; $p = 0.0004$). This difference suggests that contamination may be more frequent in pooled milk, although the cross-sectional design does not establish the source or timing of contamination.

Association between E. Coli Positive and Selected Factors

The prevalence of *E. coli* was similar between the two age groups. Among young cows, 36 of 68 samples were positive (52.9%), compared with 77 of 142 samples from adult cows (54.2%). The association between age group and *E. coli* positivity was not statistically significant ($p = 0.979$).

Prevalence differed across body condition categories. All 15 samples from cows classified as having poor body condition were positive (100.0%). The corresponding prevalence was 27.5% (22/80) among cows with fair body condition, 76.7% (46/60) among those with good body condition, and 54.5% (30/55) among those with excellent body condition. The association was statistically significant ($p = 0.0001$).

Table 2
Associations between *E. coli* positivity and body condition score, age, sample type, and farm

Factor	Category	Positive (n)	Total (n)	Prevalence (%)	p-value
Age	Young	36	68	52.9	0.979
	Adult	77	142	54.2	
Body condition	Poor	15	15	100.0	
	Fair	22	80	27.5	0.0001
	Good	46	60	76.7	
	Excellent	30	55	54.2	
Sample type	Cow milk	81	170	47.6	0.0004
	Tanker milk	32	40	80.0	
Farm	EMDID	53	95	55.8	0.743
	EDF	22	45	48.9	
	CVMA	38	70	54.3	

Antimicrobial Susceptibility profiles of E. coli isolates.

The antimicrobial susceptibility profiles of the 113 *E. coli* isolates are presented in Table 3. Resistance was highest to ampicillin (69.9%, 79/113), followed by trimethoprim–sulfamethoxazole (61.9%, 70/113) and ciprofloxacin (43.4%, 49/113). An additional 18.6% (21/113) of isolates showed an intermediate response to ciprofloxacin. When resistant and intermediate results were combined, non-susceptibility was 80.5% for ampicillin, 62.0% for ciprofloxacin, and 61.9% for trimethoprim–sulfamethoxazole.

In contrast, susceptibility was highest to amikacin (92.0%, 104/113), followed by gentamicin (85.8%, 97/113) and ceftriaxone (77.9%, 88/113). Ceftriaxone resistance was detected in 13 isolates (11.5%), while 12 (10.6%) were classified as intermediate. These findings demonstrate substantial variation in susceptibility among the six antimicrobial agents tested.

Using the study's class-based definition of multidrug resistance (MDR), 51 isolates (45.1%) were non-susceptible to at least one agent in three or more antimicrobial classes. The remaining 62 isolates (54.9%) did not meet this criterion. All 13 ceftriaxone-resistant isolates belonged to the MDR group. In the isolate-level analysis, ceftriaxone resistance was also associated with ciprofloxacin resistance (Fisher's exact test, $p = 0.002$; odds ratio = 9.0), suggesting a clustering of resistance phenotypes. However, the underlying resistance mechanisms require further laboratory confirmation.

Table 3
Antimicrobial susceptibility profile of *E. coli* isolate

Drug	Resistant n (%)	Intermediate n (%)	Susceptible n (%)	Non-susceptible R + I (%)
Ampicillin	79(69.9)	12 (10.6)	22 (19.5)	91 (80.5)
Trimethoprim-sulfamethoxazole	70 (61.9)	0 (0.0)	43 (38.1)	70 (61.9)
Ciprofloxacin	49 (43.4)	21 (18.6)	43 (38.1)	70 (62.0)
Ceftriaxone	13 (11.5)	12 (10.6)	88 (77.9)	25 (22.1)
Gentamicin	2 (1.8)	14 (12.4)	97 (85.8)	25 (14.2)
Amikacin	1 (0.9)	8 (7.1)	104 (92.0)	9 (8.0)

Multidrug resistance

Of the 113 *E. coli* isolates tested, 51 (45.1%) were classified as multidrug-resistant (MDR) using the class-based definition, while 62 (54.9%) did not meet the MDR criterion (Table 4). MDR was defined as non-susceptibility to at least one antimicrobial agent in three or more antimicrobial classes. These findings indicate that nearly half of the isolates met the study's class-based MDR criterion.

Table 4
Class-based multidrug resistance among *E. coli* isolates recovered from raw cow milk

MDR classification	Number of isolates (n)	Percentage (%)
MDR	51	45.1
Non-MDR	62	54.9
Total	113	100.0

DISCUSSION

This study detected *E. coli* in 113 of 210 raw cow milk samples, corresponding to a prevalence of 53.8%. The finding indicates that contamination was common among the sampled dairy farms in Bishoftu. The observed prevalence was higher than the 18.6% reported by Biruke and Shimeles, but comparisons with other studies should account for differences in sampling methods, study populations, laboratory procedures, and farm management. [15]

The detection of *E. coli* in raw milk is relevant to food hygiene because the organism may indicate fecal contamination and inadequate sanitation during milk production or handling [16]. Potential contamination sources include unclean udders, contaminated water, equipment, and the farm environment [17, 18]. Nevertheless, routine identification of *E. coli* does not establish the presence of a

pathogenic strain. Because molecular characterization was not performed, the pathogenic potential of the isolates cannot be determined from the present results.

The prevalence varied across the three farms, ranging from 48.9% to 55.8%. However, this variation was not statistically significant ($p = 0.743$). The findings therefore do not provide sufficient evidence of a difference in prevalence among the sampled farms. Differences in sanitation, milking procedures, equipment cleaning, and milk storage may contribute to variation in contamination, but these factors were not directly measured and should be investigated in future studies.

A significant difference was observed between individual-cow milk and bulk-tanker milk. The prevalence was 47.6% in individual-cow milk and 80.0% in bulk-tanker milk ($p = 0.0004$). Pooling milk from multiple cows may increase the opportunity for contamination to enter a larger volume of milk, particularly when one or more contributing samples are contaminated. Body condition score was significantly associated with *E. coli* positivity ($p = 0.0001$). Prevalence was highest among cows classified as having poor body condition and lowest among those with fair body condition. Although nutritional status and general health may be associated with susceptibility to infection, the cross-sectional design does not establish a causal relationship. Moreover, the small number of animals in the poor-condition category and the variation across the four categories warrant cautious interpretation. Further studies should account for potential confounding factors, including management practices, lactation stage, and health status.

No statistically significant association was observed between age group and *E. coli* positivity ($p = 0.979$). The prevalence was similar in young and adult cows, suggesting that age was not associated with contamination in this sample. Environmental exposure and milking hygiene may be relevant, but the available data do not establish which factors account for the observed pattern.

The antimicrobial susceptibility results revealed substantial resistance to ampicillin and trimethoprim–sulfamethoxazole among the *E. coli* isolates. Resistance to these agents was 69.9% and 61.9%, respectively, while ciprofloxacin resistance was 43.4%. The additional 18.6% of isolates classified as intermediate to ciprofloxacin further emphasizes the importance of reporting all three susceptibility categories rather than resistance percentages alone. These findings indicate that susceptibility varied considerably among the antimicrobial agents tested.

Amikacin and gentamicin showed the highest susceptibility rates, at 92.0% and 85.8%, respectively. Ceftriaxone susceptibility was 77.9%, although 11.5% of isolates were resistant and a further 10.6% were intermediate. The marked difference between ampicillin and ceftriaxone resistance suggests that resistance was not uniform across the beta-lactam agents examined. However, the susceptibility results alone cannot establish the enzymes or genetic mechanisms responsible for this pattern.

An important finding was that 45.1% of the isolates met the study's class-based definition of MDR. Furthermore, all 13 ceftriaxone-resistant isolates were classified as MDR. Ceftriaxone resistance was significantly associated with ciprofloxacin resistance in the isolate-level analysis, suggesting that resistance to these agents may co-occur within a subset of isolates. This pattern raises the possibility of

linked resistance determinants, including mechanisms associated with extended-spectrum beta-lactamases (ESBLs). Nevertheless, an ESBL phenotype cannot be confirmed from these susceptibility patterns alone. Confirmatory phenotypic testing and, where feasible, molecular characterization are needed to establish the underlying mechanism.

The MDR findings should also be interpreted in light of the classification method. Under the class-based definition, 51 isolates (45.1%) were MDR because they were non-susceptible to at least one agent in three or more antimicrobial classes. When a stricter criterion based on resistance to at least three individual drugs was applied, 29 isolates (25.7%) met the threshold. These estimates represent different classification approaches and should be clearly distinguished in the manuscript.

Overall, the findings support continued antimicrobial resistance surveillance and improved hygiene throughout milk production and handling. Because the isolates were recovered from raw milk, the results describe the susceptibility profiles of bacteria in the sampled milk and should not be used alone to recommend antimicrobial treatment for animal or human infections.

CONCLUSION

Escherichia coli was detected in 53.8% of the 210 raw cow milk samples examined from three selected intensive dairy farms in Bishoftu, Ethiopia. Prevalence differed significantly between individual-cow milk and bulk-tanker milk, whereas differences among farms and between age groups were not statistically significant. Body condition score was significantly associated with *E. coli* positivity, although the cross-sectional design does not establish a causal relationship.

The antimicrobial susceptibility results showed high resistance to ampicillin and trimethoprim–sulfamethoxazole, alongside relatively high susceptibility to amikacin, gentamicin, and ceftriaxone. These findings highlight the importance of maintaining hygienic practices during milk production and handling and monitoring antimicrobial resistance in dairy production systems. Further research involving additional farms, validated susceptibility testing, and molecular characterization would help clarify the distribution, pathogenic potential, and resistance mechanisms of the isolates.

RECOMMENDATION

Based on the findings, the following measures are recommended:

- Strengthen udder preparation, hand hygiene, equipment sanitation, and cleaning of milk collection and storage containers.
- Maintain hygienic conditions during milk pooling, transportation, and storage to reduce opportunities for contamination.
- Encourage veterinary oversight and evidence-based antimicrobial use in dairy production, supported by appropriate diagnostic testing.

- Conduct larger studies involving additional dairy farms and sampling points along the milk supply chain to better characterize contamination and antimicrobial resistance.
- Where resources permit, use molecular methods to investigate virulence determinants and antimicrobial resistance genes.
- Apply validated interpretive criteria and clearly defined MDR classifications to facilitate comparison with other studies.

Abbreviations

CLSI	Clinical and Laboratory Standards Institute
CVMA	College of Veterinary Medicine and Agriculture
EDF	Ethiopian Defense Force
EMB	Eosin Methylene Blue
EMDID	Ethiopian Meat and Dairy Industry Development
MDR	Multidrug Resistant
MM	Millimeter
MR	Methylene red
PW	Peptone Water
VP	Vogues-Proskeaur

Declarations

Ethics Approval and Consent to Participate

Verbal informed consent was obtained from all dairy farm owners before specimen collection. Permission to access the farms and collect raw cow milk samples was obtained from farm management and responsible authority and ethical approval for this study was obtained from the institutional review board (IBR) of Addis Ababa University, college of veterinary medicine and agriculture. The study was conducted in accordance with applicable institutional and local requirements for field specimen collection.

Consent for Publication

Not applicable

FUNDING

No specific funding was received for this study.

Author Contribution

Author ContributionsT.D.G. and A.K.M. conceptualized the study and contributed to the study design. T.D.G. and A.E.J.. contributed to data collection and laboratory investigation. A.K.M. conducted the data analysis and interpretation and drafted the main manuscript text. D.A.B contributed to the interpretation of the findings and critically revised the manuscript.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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Figures

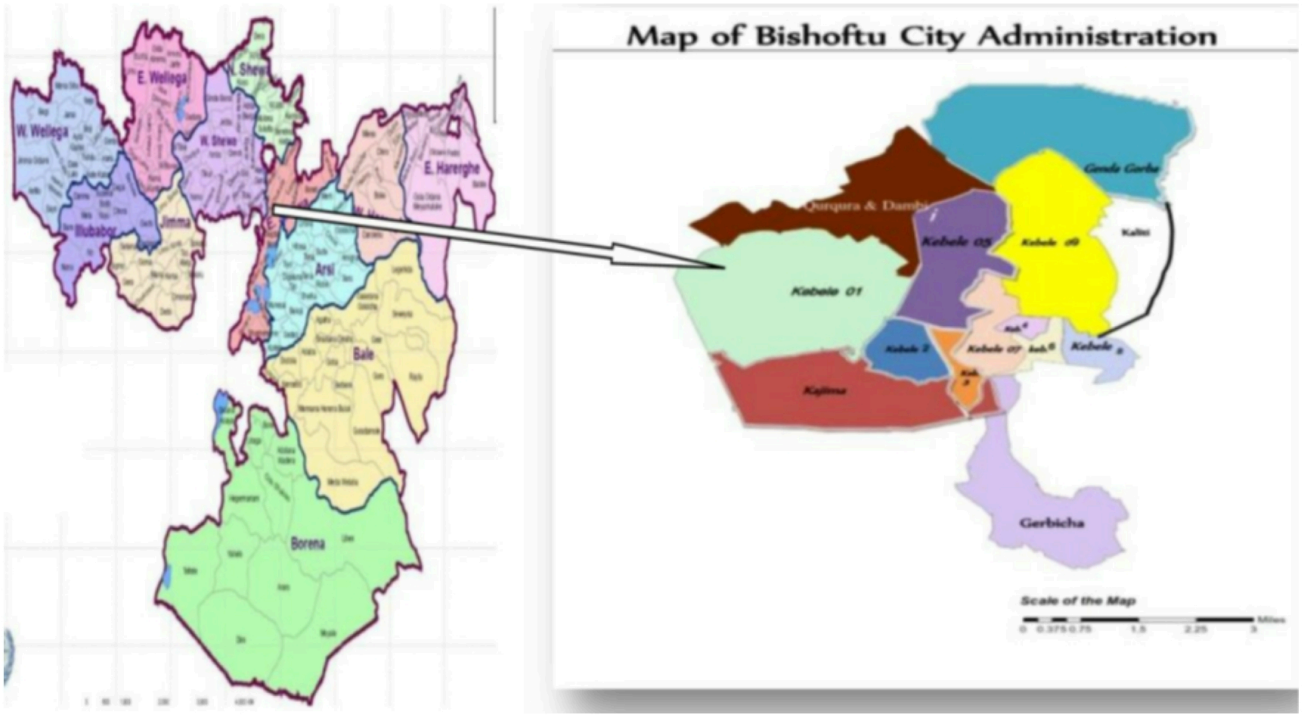


Figure 1

Map of the study area, Bishoftu Town **(Source:** Bishoftu town Municipal office, (2017)

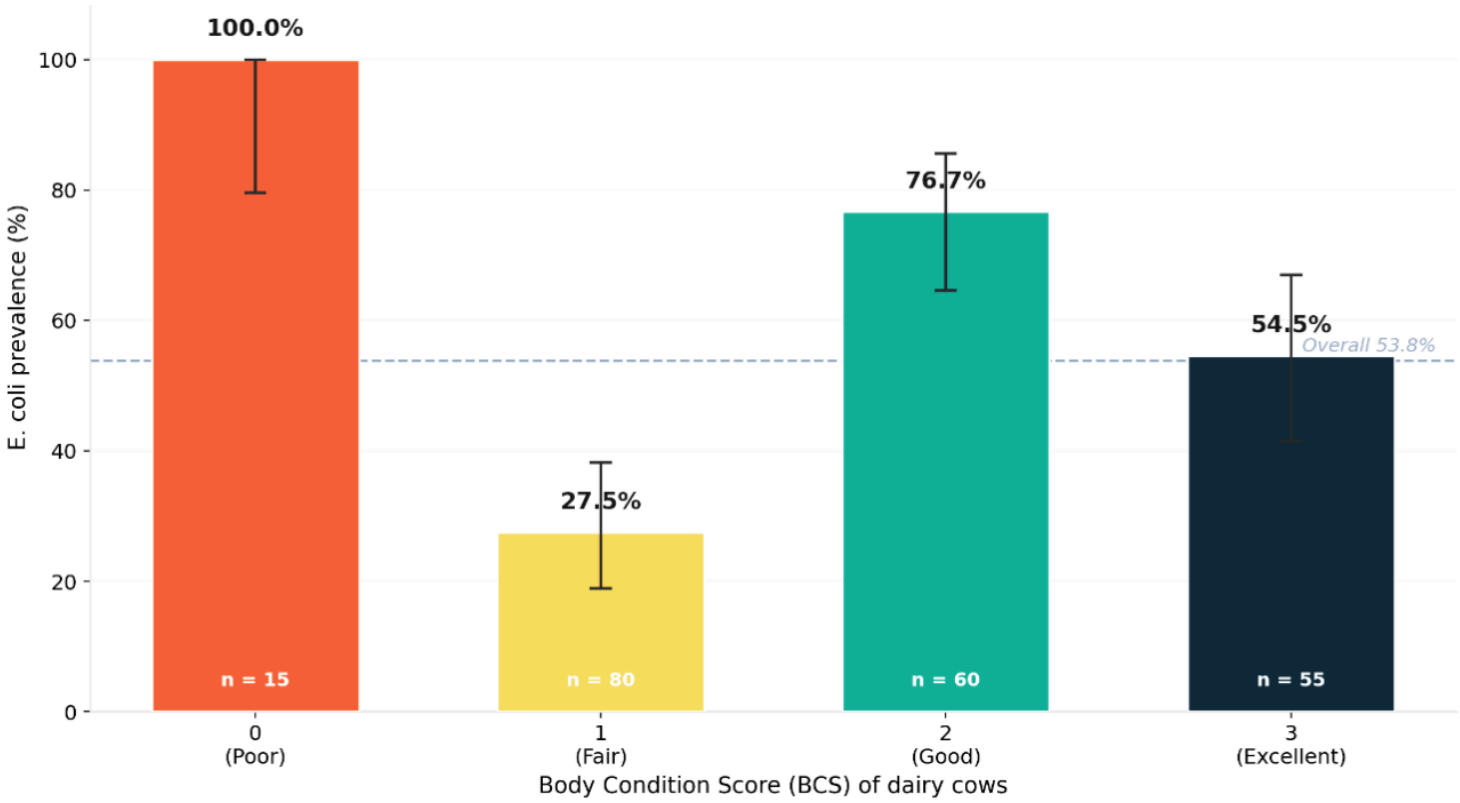


Figure 2

Prevalence of *E. coli* by cow body condition score, with 95% Wilson confidence intervals.

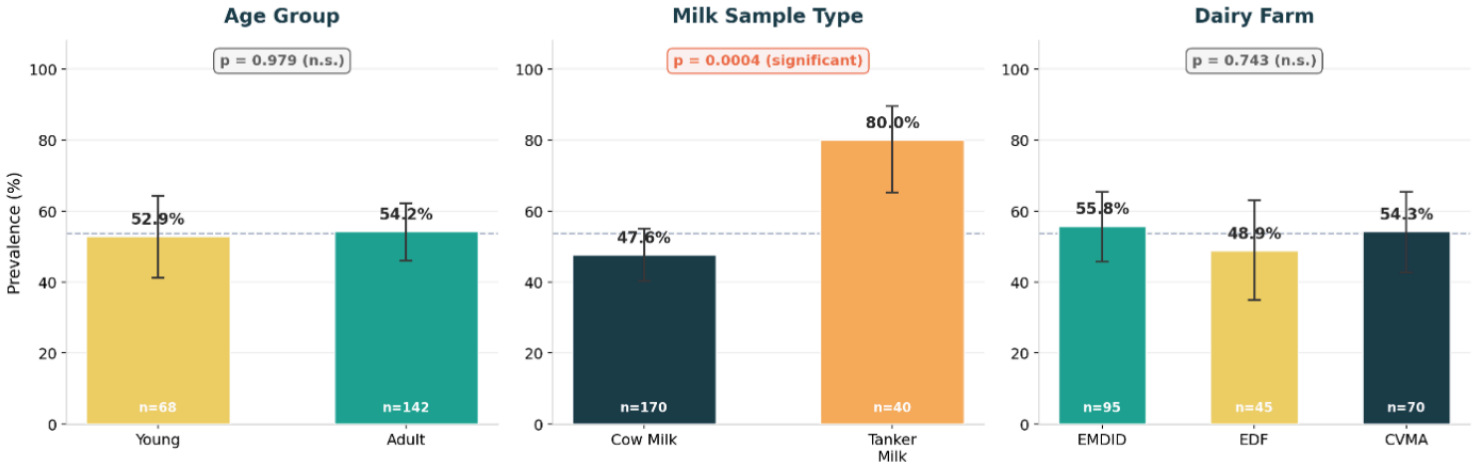


Figure 3

Prevalence of *E. coli* by age group, milk sample type, and dairy farm, with 95% Wilson confidence intervals.

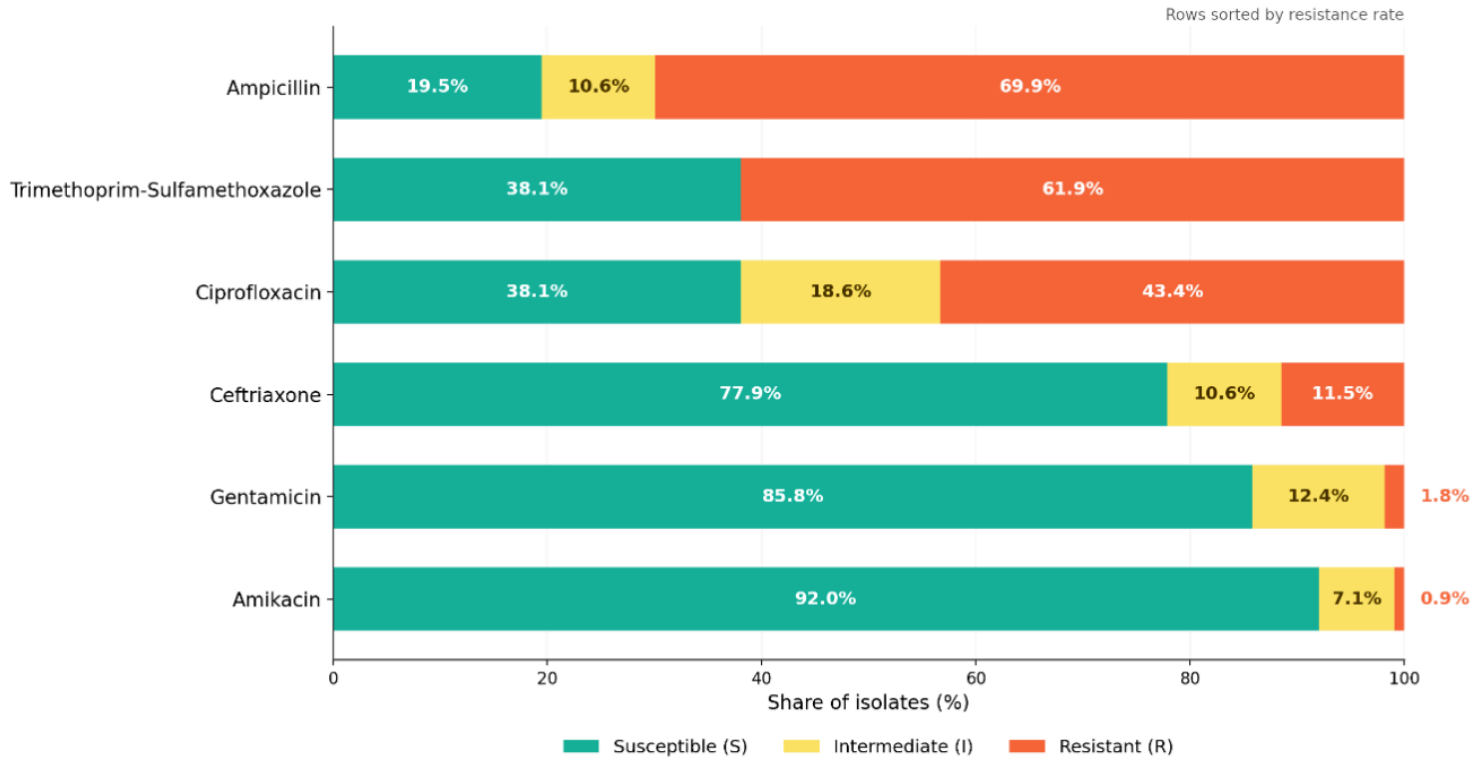


Figure 4

Antimicrobial Susceptibility profiles of *E. coli* isolates.