Prevalence and Diversification of Enterotoxigenic Escherichia coli in Different Cow Farms of Chittorgarh: Implications for Community Health and Environmental Monitoring

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Abstract: **This study investigates the prevalence and antibiotic resistance of Enterotoxigenic *Escherichia coli* (ETEC) in two cow farms in Chittorgarh, Rajasthan, India. Environmental samples, including soil, water, feed, and feces, were collected and analyzed for bacterial contamination and resistance patterns. Gram staining revealed a predominance of Gram-negative bacteria across most samples, with notable differences in the feed and diarrheal fecal samples between the farms. E. coli was detected in 66.7% of soil samples from Farm A, with a significant difference in water contamination levels between the two farms. ETEC was identified in 10% of the total samples, exclusively in diarrheal feces, with a higher prevalence in Farm B. The antibiotic resistance analysis revealed varying efficacy of Amoxicillin and Gentamicin against the ETEC strains. Seasonal variations were observed, with higher ETEC concentrations during the rainy season. The findings underscore the need for improved farm management practices, particularly in feed and water hygiene, to reduce the risk of bacterial contamination and the spread of pathogenic *E. coli*. This study highlights the importance of regular monitoring and the implementation of stringent hygiene measures in livestock farms to safeguard both animal and human health.**

Keywords: **Enterotoxigenic *E. coli*, Antibiotic resistance, Fecal contamination, Environmental monitoring and Microbial prevalence**

INTRODUCTION

Enterotoxigenic *Escherichia coli* (ETEC) is a strain of *Escherichia coli* known for its pathogenic effects[1]. It plays a significant role in causing diarrhoeal illness among small and larger animals within and among different farms, extending to the community at large[2]. ETEC is a bacterial infection that causes diarrhea in calves and animals in general, mostly appearing in the early days of birth in smaller calves and animals[3]. The disease-causing *Escherichia coli* have distinctive virulence traits that allow them to colonize the small intestine and produce an enterotoxin, leading to an excessive amount of fluid being discharged into the intestinal lumen[4]. Newborn calves take in these enterotoxins after birth from contaminated environments and materials, as they are introduced into the environment by diseased animals within the farm environment[3]. Despite some natural resistance to enterotoxigenic *Escherichia coli*, it is often not strong enough to prevent calves from being produced in contemporary husbandry practices. ETEC strains need to possess specific virulence traits that are crucial for causing intestinal illness and initiating diarrhea[2], [5]. One of these traits is the ability to produce enterotoxins, which are substances that mediate fluid discharge, as the name suggests. Upon colonizing the intestine, ETEC produces heat-labile (LT) and heat-stable (ST) enterotoxins, which act on intestinal enterocytes, leading to secretory diarrhea[6], [7]. ETEC can create two forms of enterotoxins: the large-molecular-weight LT protein, which shares structural and functional similarities with cholera toxin, and the small-molecular-weight ST protein, which exists in two subtypes: STa and STb. ST is produced by ETEC strains isolated from pigs and humans but not from calves[4], [5].

The main source of ETEC is infected animals, and their manure is the major cause of environmental contamination with this bacteria[8]. When infected, animals excrete a large number of bacteria, creating a

"multiplier effect". For example, diarrheic calves can pass 1,000 ml or more of diarrheal feces containing 10 million ETEC/ml within 12 hours, and even after recovery, they can continue to shed the bacteria for several months[9]. Healthy calves that are infected but do not show symptoms can also excrete significant numbers of ETEC[10].

Enterotoxigenic *Escherichia coli* (ETEC) continues to be a major public health concern[11]. It is the third most common cause of moderate-to-severe diarrhea (MSD) in children under five years of age and in visitors[10]. In livestock settings, enterotoxigenic *Escherichia coli* (ETEC) stands out as the predominant cause of *E. coli*-induced diarrhea, leading to high economic losses[7], [12]. ETEC is characterized by its production of two crucial virulence factors. First, enterotoxins instigate the release of fluids in the intestines, causing diarrhea[13]. Second, adhesins play an important role in facilitating the binding and colonization of the intestinal epithelium, helping bacteria establish infection and causing disease[10], [14].

This study aimed to assess the prevalence and diversity of enterotoxigenic *E. coli* in soil, feed, fecal, and water samples from two different cow farms in Chittorgarh city, Rajasthan, India, to compare the prevalence and distribution of ETEC among soil, fecal, and water samples and address the following research questions:

i. Are there significant differences in the prevalence and concentration of ETEC in samples from different cow farms?
ii. What environmental and farm management practices are associated with higher or lower levels of ETEC contamination in soil, feed, fecal, and water samples?
iii. Is there a correlation between ETEC levels in soil, feed, fecal, and water samples within individual cow farms?

MATERIALS AND METHODS

Location of the Study

Phase one of the study involved collecting samples from two different cow farms located in Chittorgarh city, Rajasthan, India. The geographic coordinates of the location are between $23^0 32^I$ and $25^0 13^I$ north latitudes and between $74^0 12^I$ and $75^0 49^I$ east longitudes. Phase two of the study took place at the microbiology laboratory of the Department of Life Science, Mewar University in Gangrar, Chittorgarh, India.

Various equipment, materials, and apparatuses were used during the study, including water samples, feed samples, soil, normal and diarrheal fecal samples, 15 ml sterile tubes, laminar cabinets, autoclaves, incubators, disposable petri plates, electrophoresis setup kits, conical flasks, electronic weighing balances, measuring cylinders, micropipettes, pipette tips, hand gloves, glass slides, microscopes, and a variety of reagents and media, such as MacConkey agar medium, glucose phosphate agar, EMB agar, saline solution, TAE buffer, ethidium bromide, 70% ethanol, distilled water, alpha naphthol, methyl red, tryptophan, urea, hydrogen peroxide, and 40% KOH.

Sample collection

The following samples were collected from two different livestock farms: 5 soil samples, drinking water samples from 4 different troughs, 5 feed samples, 4 diarrhoeal faecal samples, and 5 normal faecal samples. Disposable plastic gloves were used to collect fecal samples, with the gloves being changed between samples. While it was not possible to associate fecal samples from the pen floor with a specific steer, efforts were made to collect samples from different areas within each pen to minimize the possibility of collecting multiple samples from the same animal. The soil samples were collected via a spatula sterilized with 70% ethanol to prevent contamination. All the samples were appropriately labelled, cooled, and transported to the laboratory for analysis.

Figure 1: Samples collected

The equipment, materials, and apparatus used were washed, cleaned, and ensured to be aseptic. The culture vessels were sterilized by autoclaving[15] at a temperature of 121°C for 15 minutes on 15 PSI for proper sterilization. The laminar cabinet was opened, cleaned, and ensured to be aseptic before starting the experiment.

Physicochemical analysis of the water samples

The collected water samples were subjected to physicochemical tests for pH and total dissolved solids (TDS) using a pH meter and TDS meter, respectively. The results obtained for the test are given in Table 1.

Table 1: Physicochemical values of the water samples

S/N	SAMPLE ID	TDS (ppm)	PH
1	FA_1	751 ± 2%	7.4 ± 0.05
2	FA_2	732 ± 2%	7.3 ± 0.05
3	FA_3	731 ± 2%	7.1 ± 0.05
4	FB_1	768 ± 2%	7.4 ± 0.05
5	FB_2	761 ± 2%	7.1 ± 0.05
6	FB_3	754 ± 2%	7.4 ± 0.05

**TDS (Total Dissolved Solid)*

Sample Preparation and Analysis

A small portion of each sample was transferred into a 15 ml sterile tube via a spatula (for solid samples). Saline solution was added at the appropriate level to dilute the samples. The solid samples were filtered through filter paper to remove the residue and larger particles.

Serial dilutions of the samples

The sample was diluted in three tubes at concentrations of 10^{-1}, 10^{-2}, and 10^{-3} to achieve the desired bacterial concentration. Two types of agar, MacConkey and EMB (Eosin Methylene Blue), were prepared for culturing *E. coli* according to standard methods[16]. The agars were prepared by mixing 55.07 g and 33.09 g per 1000 ml of distilled water. The media were then microwaved, autoclaved, and poured into Petri plates in a laminar flow cabinet to solidify without forming bubbles.

Inoculation and incubation

Bacterial samples from each collection were serially diluted and then inoculated via the pouring plate method, following the standard procedure of the American Society for Microbiology. After inoculation, the plates were placed in a laboratory incubator set at 37°C overnight incubation[17]. After 24 h, the samples were analysed for bacterial development, and the colonies were counted via an electronic colony counter machine. A single colony from each sample was selected and cultured in nutrient broth to obtain a pure culture for further analysis. The samples were then incubated overnight again. The samples were subsequently inoculated onto MacConkey and EMB agar via the pour plate method and incubated overnight according to standard procedures.

Gram Staining Analysis of the Bacterial Cultures

After the pure culture was obtained, all the cultured samples were subjected to a Gram staining procedure to identify the bacterial stain on the basis of the standard procedure[16]. The results obtained are presented in the Results section (chapter four).

BIOCHEMICAL TESTS

The samples were subjected to biochemical tests[18] to reveal a specific bacterial strain (*E. coli*) in the samples for further analysis. The tests carried out are explained below:

Catalase Test

Using a sterile inoculation loop, a small amount of the bacterial colony from an existing culture plate was collected, and a drop of hydrogen peroxide was placed onto a clean glass slide. The bacterial colony was smeared into a drop of hydrogen peroxide via an inoculating loop. Immediate bubbling was observed in some samples, indicating a positive catalase reaction, as the catalase enzyme in the bacteria broke down hydrogen peroxide into water and oxygen gas, while some did not. In the absence of bubbling, a negative catalase reaction was indicated.

Figure 4: Catalase test

Methyl red test

A glucose phosphate broth was prepared according to standard guidelines[19], and the bacterial culture was inoculated in a sterile loop. The cultures were incubated at 37°C for 24 hours. After incubation, 3 drops of methyl red indicator were added to each broth culture. The color change was observed in each broth culture.

Figure 6: Methyl red test

Voges–Proskauer test

Using a sterile inoculation loop, the MR-VP broth was inoculated with the bacterial culture. The culture was incubated at 37°C for 24 hours. After incubation, 0.6 ml of α-naphthol (VP reagent A) was added to the broth culture, 0.2 ml of potassium hydroxide (VP reagent B) was added to the broth culture, and the tube was gently shaken to mix the reagents[19]. The tube was allowed to stand for up to 60 minutes, and the color change was observed.

Indole Ring Test

A tryptone broth was prepared and inoculated with bacterial culture using a sterile loop. The culture was incubated at 37°C for 24 hours. After incubation, 0.5 ml of Kovac's reagent was added to the broth culture, the tube was gently shaken, and the color change at the top layer was observed[16].

MOLECULAR ANALYSIS

DNA Extraction

The genomic DNA from the three diarrhoeal samples was extracted via a commercial DNA extraction kit[20]. The samples were first lysed to release the DNA, followed by purification steps to obtain clean genomic DNA.

PCR amplification

A PCR mixture containing the extracted DNA, specific primers for enterotoxigenic *E. coli* (ETEC) virulence genes (*elt* and *est*), Taq DNA polymerase, dNTPs, and a buffer solution was prepared. PCR amplification was performed via a thermocycler with the following conditions: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute; and a final extension at 72°C for 10 minutes[21].

Gel Electrophoresis

The PCR products were run on a 1.5% agarose gel stained with ethidium bromide, a DNA ladder was loaded alongside the samples to determine the size of the PCR products, and the gel was visualized under UV light to confirm the presence of the expected size bands for the virulence genes[20].

Sequencing

The PCR products were purified via a commercial PCR purification kit to remove excess primers and nucleotides, and the purified PCR products were sequenced to confirm the presence of enterotoxigenic *E. coli*-specific sequences[21].

Sequence analysis

The obtained sequences were compared with known sequences of enterotoxigenic *E. coli* virulence genes in the NCBI database via BLAST (Basic Local Alignment Search Tool)[20], which confirmed that the sequences matched the *elt* and *est* genes, indicating the presence of enterotoxigenic *E. coli* in the diarrheal samples.

ANTIBIOTIC RESISTANCE OF ETEC STRAIN OF *E. coli*

Two different types of antibiotics at different concentrations were used on two strains of ETEC from three diarrhoeal samples. The antibiotics used were amoxicillin and gentamicin, which were prepared at concentrations of 60 and 40 µg/mL and 30 and 20 µg/mL, respectively. The bacterial samples were inoculated via the spread plate method, and antibiotics were introduced into the culture plates via the plate well method. The antibacterial resistance was analysed by measuring the zone of inhibition on each plate, and the results are presented in the Results section.

RESULT

Table 2: Results of the bacterial Gram-stain analysis of the samples

Sample Type	Farms	Total Samples Collected	Gram-positive (%)	Gram-negative (%)
Soil	Farm A	3	1 (33.33)	2 (66.66)
	Farm B	3	1 (33.33)	2 (66.66)
Water	Farm A	3	0 (0.00)	3 (100)
	Farm B	3	1 (33.33)	2 (66.66)
Feed	Farm A	3	1 (33.33)	2 (66.66)
	Farm B	3	0 (0.00)	3 (100)
Diarrheal sample	Farm A	3	0 (0.00)	3 (100)
	Farm B	3	0 (0.00)	3 (100)
Normal Faecal	Farm A	3	1 (33.33)	2 (66.66)
	Farm B	3	0 (0.00)	3 (100)
Total	2	n=30	5 (16.66)	25 (83.33)

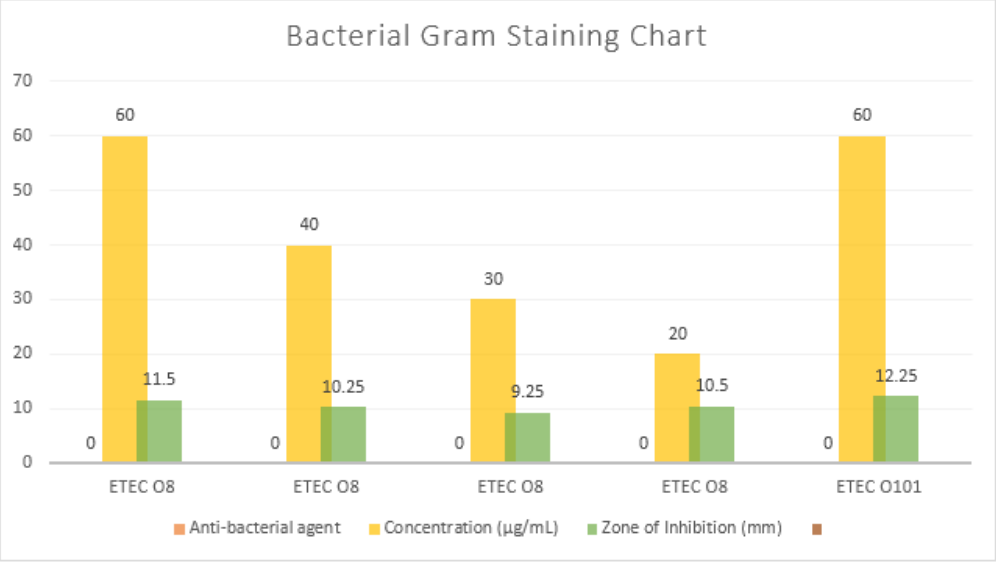


Figure 1: Chart of bacterial Gram staining

Table 3: Results of Biochemical Tests of the Samples

Sample Type	Farm	Total Samples Collected	Indole Test (%)		Methyl Red Test (%)		Voges-Proskauer (%)		Citrate Test (%)		Catalase Test (%)	
			Positive Test (%)	Negative Test (%)	Positive Test (%)	Negative Test (%)	Positive Test (%)	Negative Test (%)	Positive Test (%)	Negative Test (%)	Positive Test (%)	Negative Test (%)
Soil	Farm A	3	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	1 (33.33)	2 (66.66)
	Farm B	3	0 (0.00)	3 (100)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	0 (0.00)	3 (100)	3 (100)	0 (0.00)
Water	Farm A	3	1 (33.33)	2 (66.66)	0 (0.00)	3 (100)	0 (0.00)	3 (100)	0 (0.00)	3 (100)	3 (100)	0 (0.00)
	Farm B	3	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	1 (33.33)	2 (66.66)
Feed	Farm A	3	0 (0.00)	3 (100)	0 (0.00)	3 (100)	1 (33.33)	2 (66.66)	0 (0.00)	3 (100)	3 (100)	0 (0.00)
	Farm B	3	0 (0.00)	3 (100)	0 (0.00)	3 (100)	0 (0.00)	3 (100)	0 (0.00)	3 (100)	3 (100)	0 (0.00)
Diarrheal sample	Farm A	3	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	2 (66.66)	1 (33.33)
	Farm B	3	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	1 (33.33)	2 (66.66)
Normal Faecal	Farm A	3	0 (0.00)	3 (100)	0 (0.00)	3 (100)	0 (0.00)	3 (100)	0 (0.00)	3 (100)	0 (0.00)	3 (100)
	Farm B	3	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	1 (33.33)	2 (66.66)

Table 4: Results of *E. coli* counts from each sample**.**

Sample Type	Farm	Total Samples Collected	*E. coli* Positive (%)	*E. coli* Negative (%)
Soil	Farm A	3	2 (66.66)	1 (33.33)
	Farm B	3	0 (0.00)	3 (100)
Water	Farm A	3	0 (0.00)	3 (100)
	Farm B	3	2 (66.66)	1 (33.33)
Feed	Farm A	3	0 (0.00)	3 (100)
	Farm B	3	0 (0.00)	3 (100)
Diarrheal sample	Farm A	3	1 (33.33)	2 (66.66)
	Farm B	3	2 (66.66)	1 (33.33)
Normal Faecal	Farm A	3	0 (0.00)	3 (100)
	Farm B	3	2 (66.66)	1 (33.33)
Total	2	n=30	9 (30)	21 (70)

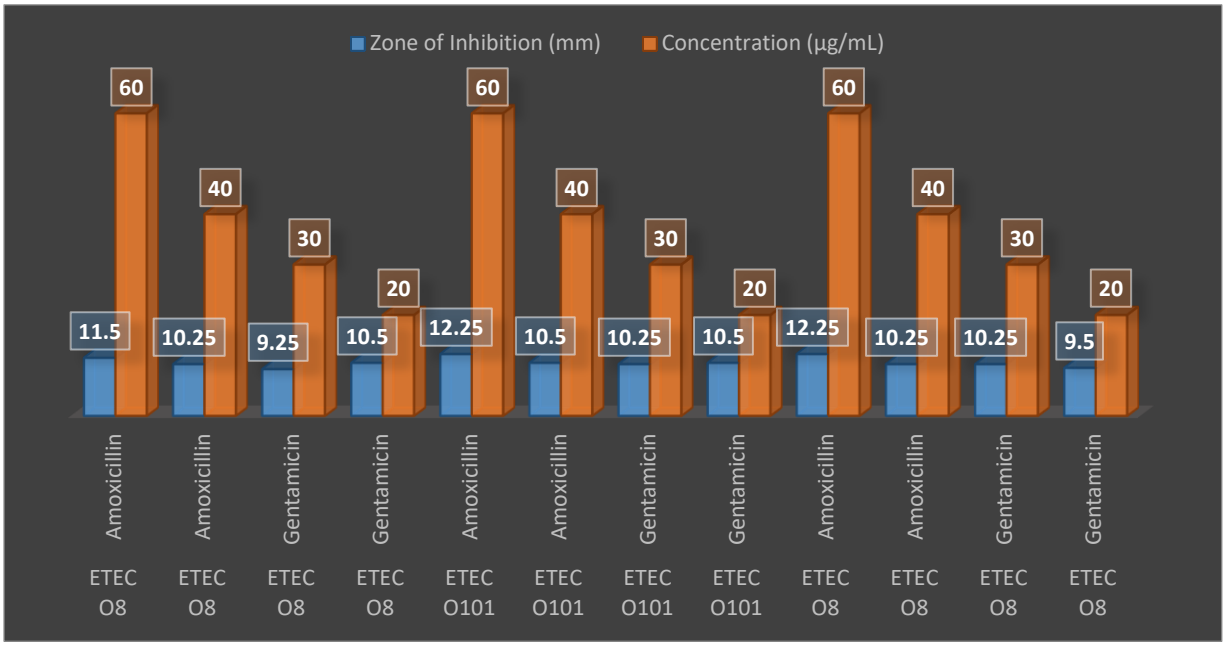
Table 5: Results of the Molecular Analysis of the Samples

Sample Type	Farm	Total Samples Collected	Positive ETEC Genes Detected (%)	Negative ETEC Genes Detected (%)

Soil	Farm A	3	0 (0.00)	3 (100)
	Farm B	3	0 (0.00)	3 (100)
Water	Farm A	3	0 (0.00)	3 (100)
	Farm B	3	0 (0.00)	3 (100)
Feed	Farm A	3	0 (0.00)	3 (100)
	Farm B	3	0 (0.00)	3 (100)
Diarrheal sample	Farm A	3	1 (33.33)	2 (66.66)
	Farm B	3	2 (66.66)	1 (33.33)
Normal Faecal	Farm A	3	0 (0.00)	3 (100)
	Farm B	3	0 (0.00)	3 (100)
Total	2	n=30	3 (10)	27 (90)

Table 6: Result of the Antibacterial Resistance of ETEC Isolated from Diarrheal Samples

Sample ID	ETEC Strain	Antibacterial Agent	Concentration (µg/mL)	Zone of Inhibition (mm)
001	ETEC O8	Amoxicillin	60	11.50 ± 0.5
			40	10.25 ± 0.5
		Gentamicin	30	9.25 ± 0.5
			20	10.50 ± 0.5
002	ETEC O101	Amoxicillin	60	12.25 ± 0.5
			40	10.50 ± 0.5
		Gentamicin	30	10.25 ± 0.5
			20	10.50 ± 0.5
003	ETEC O8	Amoxicillin	60	12.25 ± 0.5
			40	10.25 ± 0.5
		Gentamicin	30	10.25 ± 0.5
			20	9.50 ± 0.5



The results of the Gram staining of the samples taken from two distinct cow ranches are shown in Table 2. According to the results of the soil sample collection for farm A, one sample was gram-positive (S2), and the other two were gram-negative (S1, S3). In contrast, Farm B displayed a pattern similar to that of Farm A, with one gram-positive (S2) and two gram-negative (S1, S3) pattern. One feed sample (F1) and two feed samples (F2, F3) from farm A and all three feed samples (F1, F2, F3) from farm B were reported to be

gram-negative. Gram-negative bacteria are consistently found in diarrhoeal faecal samples from Farm A, although there is significant variance in one gram-positive sample from Farm B. Both Farm A and B samples (NF1, NF2, and NF3) were discovered to be gram-negative as opposed to normal faecal samples (Table 2).

Table 4 displays the results of the *E. coli* outbreak from the samples that were taken from two different livestock ranches. The results of the three soil samples from each farm that were analysed revealed that, whereas Farm B had a negative result for *E. coli* in every sample that was analysed, Farm A had two positive samples and one negative sample. There was a substantial difference in the amount of *E. coli* detected in the water samples between the two farms, with Farm B having the highest percentage. In the present study, there was a substantial difference in the prevalence of fecal *E. coli* (both diarrheal and normal), with Farm B exhibiting a greater number and occurrence of bacteria in the samples but still having an almost low occurrence, suggesting a low level of colonization.

Table 5 displays the isolates of enterotoxigenic *E. coli* from 3 (10%) of the 30 samples that were collected. In both research farms, ETEC was found in the diarrhoeal faeces of at least one animal, leading to a 10% herd-level infection.

Three diarrhoeal samples of enterotoxigenic *Escherichia coli* (ETEC) strains were tested for antibacterial resistance to two drugs (Table 6) at different concentrations: amoxicillin and gentamicin. The zone of inhibition results revealed that the three samples responded differently to the antibiotics. Sample 002 showed the highest susceptibility, with 12.25, 10.25, and 10.25, 9.50 mm for amoxicillin and gentamicin at 60, 40, and 30, 20 concentrations, followed by Sample 003, with 12.25, 10.25, and 10.50, 10.25 mm for amoxicillin and gentamicin at 60, 40, and 30, 20 concentrations. However, Sample 002 showed the highest resistance, with 11.50, 10.25, 9.25, and 10.50 mm for amoxicillin and gentamicin at concentrations of 60, 40, 30, and 20, respectively.

DISCUSSION

Similar soil bacterial populations are suggested by the distributions of gram-positive and gram-negative bacteria in the soil samples from both farms (Table 2). With respect to the water samples, all three samples from both farms were gram-negative. The water samples from both farms contained only gram-negative bacteria, suggesting that their microbial habitats or levels of water contamination were identical. One gram-positive (F1) and two gram-negative (F2, F3) feed samples were reported in the samples from Farm A, whereas all three feed samples (F1, F2, F3) were gram-negative in the samples from Farm B. The feed samples from Farm B were completely gram-negative, whereas the feed samples from Farm A contained a mixture of gram-positive and gram-negative bacteria.

This can be a result of variations in feed handling or storage procedures[22]. Additionally, differences in the diarrhoeal faecal samples across the various farms were detected. Although one gram-positive sample (D1) and two gram-negative samples (D2, D3) were reported from farm B, all three samples (D1, D2, and D3) from farm A were gram-negative. Gram-negative bacteria are consistently found in diarrhoeal samples from Farm A, whereas one gram-positive sample from Farm B exhibits some variance. This could suggest that the bacterial infections affecting the animals are different[23]. Both the farm A and B samples (NF1, NF2, and NF3) were discovered to be gram-negative as opposed to the normal fecal samples (Table 2). The normal fecal samples from both farms consistently included gram-negative bacteria, suggesting that the gut microbiota of healthy animals was identical.

The majority of samples from both farms contained high concentrations of gram-negative bacteria, which may be important for understanding the microbial ecosystem and possibly harmful microorganisms[24], [25]. It is interesting to note the variation in gram-positive bacteria between the feeds from the two farms; perhaps more research could determine whether this variation is related to variations in feed types, storage conditions, or farming methods.

In samples taken from two distinct farms, the present investigation revealed and verified the presence of *E. coli* (see Table 4). Three soil samples from each farm were studied, and the results revealed that Farm B had negative results for *E. coli* in every sample examined, whereas Farm A had two positive samples and one negative sample. Prior studies have indicated a separate relationship between soil contamination and the existence of specific animal species or any animal at all within the complex[26], [27]. The ingestion of highly polluted soil may act as a mediator for both direct and indirect transmission[13]. A lesser amount of *E. coli* was found in the soil, which contrasts with earlier US research that reported more *E. coli*. This larger percentage makes sense because dirt has a

greater likelihood of carrying germs due to its mixture of bedding, wastewater from various animal sources, and feces[1], [12], [28].

There was a substantial difference in the percentage of *E. coli* in the water samples between the two farms, with Farm B having a greater percentage (refer to Table 4). The notable differences across the farms could be attributed to various factors, including the source of the water[28]. Manure is a common fertilizer used by farms in pasture areas, but it can contaminate water, soil, and feed[29]. Therefore, there are serious consequences for both human and environmental health when *E. coli* is found in soil, feed, and water samples[30]. Even while *E. coli* bacteria may still spread across farms through oral–fecal transmission via contaminated feed and water[7], [31], the study's discovery of the germs in these samples indicates that the occurrence of bacteria in these samples was considerable. The regular cleaning of feed troughs and water sources may help lower the likelihood of *E. coli* maintenance on farms[6], [32].

In this investigation, we found that the two farms had different degrees of fecal *E. coli* prevalence, with Farm B exhibiting a greater incidence than Farm A. Overall, nevertheless, the prevalence was not very high, suggesting a limited degree of colonization. According to our research, the prevalence of fecal *E. coli* is currently lower than that reported earlier from dairy cattle in Ohio (9.4%)[33] and Europe (41%)[34]. This figure is comparable to the 4.6% reported prevalence in cow-calf enterprises in the United States[35]. These discrepancies might be explained by variances in the way beta-lactam antibiotics are used, as well as by other management techniques that could affect the development and spread of *E. coli* on farms[34].

Increased pollution in the home environment is closely associated with animal feces found in the courtyard. The presence of animal excrement in the compound, regardless of species, was independently correlated with the levels of *E. coli* in the soil[36]. Increased soil pollution, pond contamination, and supplementary food contamination have all been linked to animal excrement. Given the connection to food contamination, it is possible that caregivers cannot clean their hands after handling animal waste before cooking[33], [36]. When making food, caregivers handle dung cakes with their bare hands, according to a prior study conducted in Bangladesh[33]. Dug-out cakes are moist to handle, but they are sun-dried before being used as fuel, which should considerably reduce the number of pathogens[36].

Table 5 displays the isolates of enterotoxigenic *E. coli* from 3 (10%) of the 30 samples that were collected. The fact that this bacteria was found only in samples of diarrhoeal faeces (10) suggests that cattle, or bovines, may be a significant reservoir for this particular strain of ETEC and a possible source of contamination[37]. While enterotoxigenic *E. coli* strains were rare on all of the farms studied, Farm B had the highest prevalence, accounting for 6.67% of all the isolates (3/30) (Table 5). Among the diarrhoeal faeces samples from this farm, the ETEC O8 strain was the most often detected strain. This suggests that environmental pollution may have led to cross-contamination among animal feces, water, feed, and soil[38]. This highlights the importance of implementing hygienic standards, particularly because pathogenic *E. coli* are frequently found in cattle feces (STEC and ETEC)[37].

Ten percent of the herd was affected when ETEC was found in diarrhoeal faecal samples from at least one animal on each of the two research farms. This incidence is lower than that reported in earlier US research, which revealed that 20% (5/25 farms) were from Ohio[39], 85% (18/21) were from Washington, and 4% (3/80) were from dairy herds in Pennsylvania[37]. This comparison should be used with caution, however, as the criteria employed in each study to determine the number of farms, samples, and sampling farms may vary. The high temperature during sample collection and other farm management-related factors may have contributed to the limited spread of *E. coli* across the current study farms[40].

Compared with the dry season, the ETEC was found along all the pathways considerably more frequently and at higher concentrations during the rainy season[40]. This seasonal change may have an impact on the ETEC from sample data; this conclusion contradicts the prior finding of Thaden[41], which indicates that there is no relationship between ETEC incidence and weather.6.

The different zones of inhibition of the three ETEC strains point to variations in their innate or acquired resistance mechanisms (Table 6). Sample 002, which has the largest zone of inhibition and is therefore most sensitive to gentamicin and amoxicillin, implies that this strain may not have any functional resistance mechanisms[42]. This may indicate that the resistance genes normally linked to ETEC are absent from

Sample 002 or that their expression is relatively low. Sample 003 has an intermediate level of resistance, as indicated by its moderately sized zone of inhibition. This could be caused by mutations that provide some protection against antibiotics[37], but not as much as shown in 002, or by incomplete resistance mechanisms. Sample 001 exhibited the greatest degree of resistance and the lowest zone of inhibition. This implies that Sample 001 most likely possesses potent resistance mechanisms, such as the synthesis of beta-lactamases (for resistance to amoxicillin), efflux pumps, or changes in antibiotic targets that decrease gentamicin effectiveness[42], [43]. This resistance may have developed as a result of horizontal gene transfer or mutations that were gradually chosen over time as a result of earlier antibiotic exposure[37].

The observed differential resistance highlights how difficult it is to cure infections caused by ETEC[36]. Owing to the high level of resistance of Sample 001, traditional antibiotic treatments with amoxicillin and gentamicin may be less effective, and if such strains are present in an illness, treatment failure may result. These medicines may still be useful in treating infections caused by ETEC strains that are comparable to Sample 002 and, to a lesser extent, Sample 003.

This research emphasized that, considering the documented resistance variations, cautious antibiotic selection is essential for treating ETEC infections[44]. Effective infection management may require ongoing surveillance of resistance trends and the application of combination medicines, particularly when highly resistant strains such as Sample 001 are present[45]. The development of novel treatment approaches to combat resistant ETEC strains may benefit greatly from additional investigations into the genetic foundation of this resistance.

CONCLUSION

The study highlights the importance of monitoring and managing bacterial contamination in livestock farms to prevent the spread of pathogens like E. coli and ETEC. The findings suggest that variations in farm management practices, environmental factors, and seasonal changes can influence the prevalence and distribution of these bacteria. Implementing proper hygiene and sanitation measures, along with regular monitoring, can help mitigate the risks associated with bacterial contamination in livestock farms

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Ethics statement

The study did not have ethical approval because the study did not involve any animal welfare or abuse.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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