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Antimicrobial resistance profiling of *Escherichia coli* isolates from dairy farms of Thrissur, Kerala: A One Health perspective[#]

M. Fathima[1], Deepthi Vijay[1*], B. Sunil[1], Deepa Jolly[1], Binsy Mathew[1] and V.L. Gleeja[2]

[1]Department of Veterinary Public Health, [2]Department of Statistics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala, India.

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Abstract

The growing concern of antimicrobial resistance (AMR) poses a significant threat to global public health. The indiscriminate use of antimicrobials in veterinary medicine is considered as one of the major contributer to this challenge. The present study was undertaken to determine the occurrence of Escherichia coli (E. coli) in dairy farms of Thrissur, Kerala. Out of 128 samples collected from various sources including raw milk, water, equipment swabs and handler hand swabs from 32 dairy farms in Thrissur, 38.28 percent tested positive for E. coli using culture techniques. The highest occurrence of E. coli was found in milk and equipment swab samples, with 14 out of 32 samples (43.75%) testing positive for E. coli in both. The occurrence of E. coli in water samples collected from 32 dairy farms was 34.37 per cent and occurrence in handler's hand swab samples was 31.25 per cent. All the 49 isolates were tested for susceptibility against 13 antibiotics. The highest resistance was observedagainst tetracycline (26.53%) followed by ampicillin (24.49%) and ciprofloxacin (20.41%) while all the isolates were sensitive to chloramphenicol. Multidrug resistance was detected in 12 isolates (24.49%). Three isolates (6.12%) were phenotypically identified as Extended Spectrum Beta Lactamase (ESBL) producers. The results underscore the importance of stringent hygiene practices in dairy farms to mitigate microbial contamination and safeguard human and animal health. The findings also call for the necessity of targeted strategies and policy-level interventions to combat AMR.

Keywords: *Dairy farm, E. coli, ESBL, multidrug resistance*

Antimicrobial resistance (AMR) develops when microorganismsincluding bacteria, viruses, fungi and parasites evolve to resist medications that were once effective, making standard treatments ineffective. According to the reports of O'neill (2014), AMR is considered as silent pandemic projected to result in 10 million deaths worldwide by 2050, in addition to serious secondary impacts on global health and massive economic loss. The World Health Organisation (WHO) has acknowledged AMR as one of the top global public health and development threatsand recommends surveillance of AMR as a priority strategy to address AMR (WHO, 2023). The AMR crisis is largely attributed to the misuse and overuse of antimicrobials in both human health and veterinary sector. Antimicrobials are widely administered in animals to treat infections and prevent diseases, potentially leading to AMR, if not used judiciously. The global veterinary antimicrobial consumption is expected to rise by 11.5 percent, from 93,309 tonnes in 2017 to 104,079 tonnes of active ingredients by 2030, mirroring the upward trend observed in human antimicrobial

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**Corresponding author: deepthivijay@kvasu.ac.in Ph. 9847774527*

use, and India will remain as one among the top ten countries as a geographical hotspot for antimicrobial usage in 2030 (Tiseo *et al*., 2020).

An organism that has been particularly associated with multitude of AMR surveillance programs is *Escherichia coli (E. coli)* (Anjum *et al*., 2021). Although *E. coli* exists as a commensal in the gastrointestinal tract of warm-blooded animals, pathogenic strains of *E. coli* can lead to infections in both humans and animals. There has been a significant rise in resistance among *E. coli* strains, particularly those producing Extended Spectrum Beta-Lactamases (ESBL). The resistance conferred to *E. coli* can enter the human food chain through contaminated milk, meat or water and early detection of such resistant strains including ESBL-producing strains is crucial to prevent the spread of resistance from dairy farms to humans and associated environment (Kamaruzzaman *et al*., 2020). Over the years, there has been a significant growth in the dairy sector of Kerala and a study regarding AMR management in the local context can provide essential insights. With the understanding of the interconnection between human, animal, and environmental health, the 'One Health' approach is important in addressing AMR holistically. By fostering collaboration across these domains, we can safeguard the efficacy of antibiotics and implement sustainable strategies to mitigate resistance (Rhouma *et al*., 2022). The present study assesses the occurrence of *E. coli* from different sources of the farm *viz.*, raw milk, water, equipment and handler's hand along with antimicrobial resistance profiling.

Materials and methods

Sample collection

For the study, 32 dairy farms across Thrissur district of Kerala were randomly selected from a list provided by the Animal Husbandry Department of Kerala. During the period between May and July 2024, four types of samples consisting of pooled raw milk (n=32), animal drinking water (n=32), equipment swab (n=32) and handler's hand swab (n=32) were collected from each farm, resulting in a total of 128 samples. From each farm, milk samples were collected from the milk can. The animal drinking water was collected from the pipes or the water storage tank present in the farm. The swabs of one feeding equipment per farm were collected, and the handler's hand swab was collected post milking in the farm. All the aseptically collected samples were brought to the laboratory under refrigerated condition and processed for the analysis within 24 h of collection at Quality Control Laboratory, Department of Veterinary Pubic Health, College of Veterinary and Animal Sciences, Mannuthy.

Isolation of E. coli

All the samples were inoculated on Tryptone Soya Broth (TSB) and incubated at 37ºC for 24 h and further plated on to MacConkey agar (MCA) and incubated at 37°C for 24 h. The characteristic lactose fermenting pink colonies surrounded by bile precipitate obtained on MCA were streaked onto Eosine Methylene Blue agar (EMB) and were incubated at 37°C for 18- 24 h. The colonies with distinctive metallic green sheen on EMB, characteristic of *E. coli* were identified by standard biochemical tests such as Gram's staining, indole, methyl red, Voges-Proskauer and citrate (IMViC) tests, urease, triple sugar iron (TSI) agar, catalase and oxidase tests, as described by Barrow and Feltham (1993).

Antimicrobial susceptibility test

Antimicrobial susceptibility testing was carried out by using Kirby-Bauer disc diffusion method as described by Bauer *et al*. (1966). Three to four isolated colonies were selected from a pure culture and transferred into sterile nutrient broth and incubated at 37°C for 3-4 h and the turbidity of the culture was adjusted to 0.5M MacFarland standard. From the broth, the cultures were inoculated on Mueller-Hinton agar plates using sterile swabs. On these agar plates, 13 commonly used antibiotic discs (HiMedia) were placed aseptically at an appropriate distance with the help of sterile forceps and incubated aerobically at 37°C for 18-20h. The antibiotic discs used for the test were amoxycillin/ clavulanic acid (AMC, 20/10µg or 30µg), ampicillin (AMP,10µg), azithromycin (AZM, 15µg), aztreonam (AT, 30µg), cefepime (CPM, 30µg), cefotaxime (CTX, 30µg), ceftriaxone (CTR, 30µg), chloramphenicol (C, 30µg), ciprofloxacin (CIP, 5µg), cotrimoxazole (COT, 25µg), gentamicin (GEN, 10µg), meropenem (MRP, 10µg) and tetracycline (TE, 30µg).

Screening for ESBL production

For detection of ESBL production, modified disc diffusion synergy testing was performed with cefotaxime with and without clavulanic acid (CTX, 30µg and CEC, 30/10µg) and cefepime with and without clavulanic acid (CPM, 30µg and CFC, 30/10µg) according to the method described by Shoorashetty *et al*. (2011). Increase in inhibition zone of ≥ 5mm in cephalosporin with clavulanic acid against cephalosporin alone in the isolate was considered to be positive for ESBL production by phenotypic confirmation.

Results and discussion

Occurrence of E. coli from various sources of dairy farm

The *E. coli* colonies on MCA after incubation at 37°C for 24 h, appeared as lactose fermenting, dry, flat

Fig. 1. Pink colour lactose fermenting colonies of *E. coli* on MacConkey Agar

Fig. 2. Greenish metallic sheen colonies of *E. coli* on Eosin Methylene Blue agar

and pink colonies due to precipitation of bile salts present in the media (Fig. 1). On EMB agar, dark colonies with distinctive metallic green sheen in deflected light (Fig. 2) were identified as *E. coli*.

Of the 128 samples tested, 49 samples (38.28%) were positive for *E. coli*. The occurrence of *E. coli* varied among different sources of dairy farms and was the highest in milk samples and equipment swabs (Fig. 3). From the total 32 pooled milk samples and 32 equipment swab samples collected, 14 isolates (43.75%) each from both sources were positive for *E. coli.* Further, *E. coli* was positive in water samples of 11 farms (34.37%) from a total of 32 farms studied. From the 32 handler's hand swab samples collected from 32 farms, 10 samples (31.25%) were positive for *E. coli*.

The findings of the present study aligned with the study conducted by Lakshmi and Jayavardhanan (2016), in which 27 per cent of isolates from mastitic milk samples of cattle in organised farms in Kerala were *E. coli*. However, in the study conducted by Sandeep *et al*. (2023) in Thrissur, only eight isolates (16%) out of 50 samples collected from cattle including mastitis milk and diarrhoeic samples were positive for *E. coli.*

The occurence of *E. coli* varied across different sources and different regions. In the current study, the most important sources of *E. coli* were milk and equipment in the farm. In the study conducted by Sethulekshmi (2016), the prevalence of *E. coli* in milk samples collected from Kozhikode, Thrissur and Alappuzha districts of Kerala was 85.92 per cent. However, similar findings as in the present study were reported by Vanitha *et al*. (2018) in which *E. coli* was isolated from 27.78 per cent of pooled and 26.52 per cent of individual raw milk samples collected from milk co-operative societies of Thrissur district, Kerala. While there is a paucity of reports exploring the prevalence of *E. coli* from varied sources within dairy farms of Kerala, studies from other regions have reported the widespread occurrence of *E. coli* in dairy farm environment. In a study conducted by Jindal *et al*. (2021), in dairy farms of Punjab, the prevalence of *E. coli* among 153 raw pooled

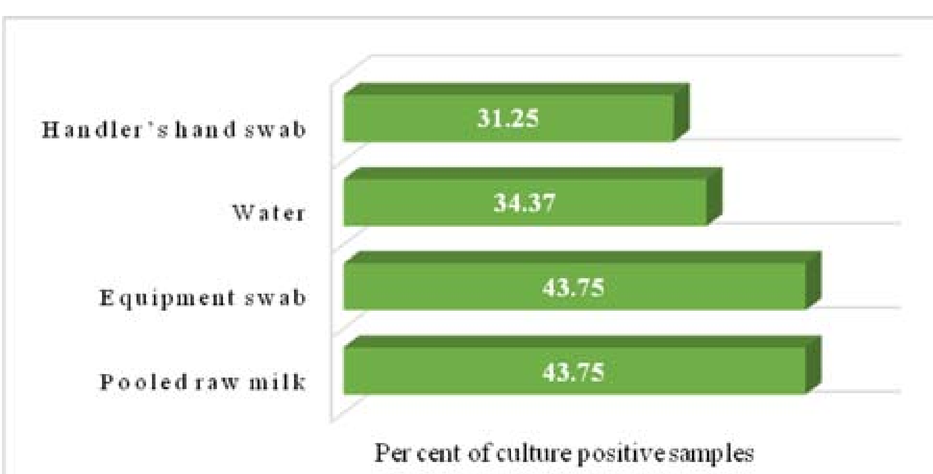

Fig. 3. Occurrence of *E. coli* from different sources in dairy farms

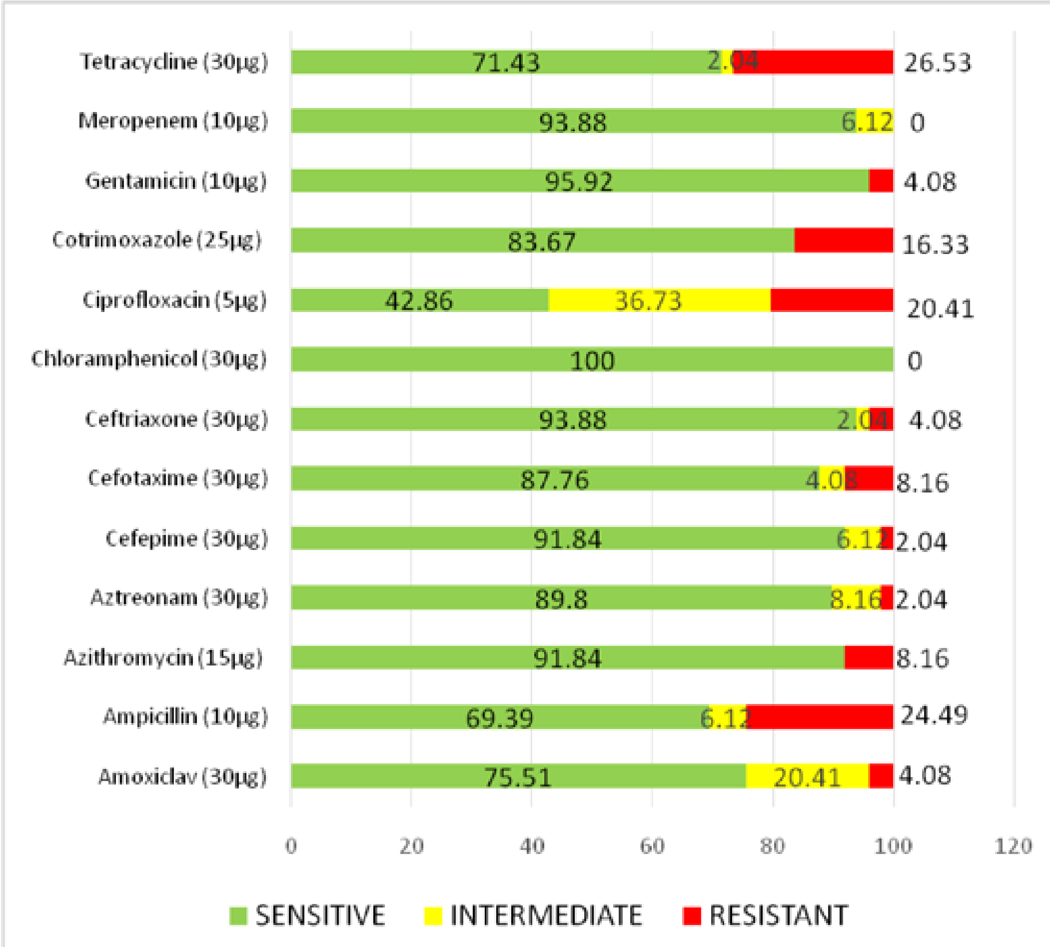

Fig. 4. Antibiotic resistance profile of *E. coli* from dairy farms in Thrissur

milk samples and animal drinking water samples was 60 per cent and 26.7 per cent, respectively. The presence of *E. coli* in dairy farm environment, irrespective of the source, has far-reaching implications on both animal and public health. The occurrence of *E. coli* in milk underscores potential lapses in farm hygiene practices and becomes crucial particularly when pathogenic strains are involved leading to foodborne disease outbreaks. Functioning as a versatile pathogen, it is associated with diarrhoeal diseases and urinary tract infections in personnels (Allocati *et al*., 2013) and contributes to the etiology of mastitis in dairy cattle, significantly impacting milk production and udder health (Goulart and Mellata, 2022).

Antibiotic resistance profiling of E. coli isolates

The 49 isolates of *E. coli* were subjected to antibiotic sensitivity testing using 13 commonly used antibiotics. The highest resistance was observed against tetracycline (26.53%) followed by ampicillin (24.49%) and ciprofloxacin (20.41%) (Fig. 4). Intermediate susceptibility was observed in 36.73 per cent of samples against ciprofloxacin and in 20.41 per cent of samples against amoxiclav. All the isolates were susceptible to chloramphenicol and 95.92 per cent samples were susceptible to gentamicin.

The sensitivity and resistance towards antibiotics varied among the source of isolates. The number of isolates from each source which were susceptible, intermediate susceptible and resistant to the antibiotics are shown in Table 1. Among the 14 isolates of milk samples, high resistance against ampicillin (35.71%) followed by ciprofloxacin (21.43%) and tetracycline (21.43%) could be observed. The resistance observed in water samples were higher against ampicillin, cotrimoxazole and tetracycline, with 27.27 per cent of the isolates resistant to each of these antibiotics. Among equipment swab samples the maximum number of samples showed resistance against the antibiotic tetracycline (42.86%) followed by resistance against cotrimoxazole (28.57%). Resistance against ciprofloxacin was seen in 40 per cent of *E. coli* isolates of handler's hand swab samples.

The overuse and misuse of frequently prescribed antibiotics are key factors driving the development of antibiotic resistance and as reported by Raosaheb *et al*. (2020), penicillins and tetracyclines are among the most

Table 1. Antibiotic resistance profile of *E. coli* from various sources in dairy farms

Sl. No.	Antibiotic	Milk			Water			Equipment			Hand		
		S	I	R	S	I	R	S	I	R	S	I	R
1	AMC	10	3	1	10	-	1	10	4	-	7	3	-
2	AMP	7	2	5	8	-	3	10	1	3	9	-	1
3	AZM	13	-	1	10	-	1	12	-	2	10	-	-
4	AT	12	1	1	11	-	-	12	2	-	9	1	-
5	CPM	12	2	-	11	-	-	13	1	-	9	1	-
6	CTX	12	1	1	11	-	-	11	1	2	9	-	1
7	CTR	13	-	1	11	-	-	13	1	-	9	-	1
8	C	14	-	-	11	-	-	14	-	-	10	-	-
9	CIP	8	3	3	7	4	-	2	9	3	4	2	4
10	COT	14	-	-	8	-	3	10	-	4	9	-	1
11	GEN	14	-	-	11	-	-	12	-	2	10	-	-
12	MRP	14	-	-	10	1	-	12	2	-	10	-	-
13	TE	10	1	3	8	-	3	8	-	6	9	-	1

S- Susceptible, I-Intermediate susceptible, R-Resistant

AMC- amoxycillin/clavulanic acid (20/10µg or 30µg), AMP - ampicillin(10µg), AZM - azithromycin (15µg), AT - aztreonam (30µg), CPM - cefepime (30µg), CTX - cefotaxime (30µg), CTR - ceftriaxone (30µg), C - chloramphenicol (30µg), CIP - ciprofloxacin (5µg), cotrimoxazole- COT (25µg), GEN - gentamicin (10µg), MRP - meropenem (10µg), TE - tetracycline (30µg)

commonly used antibiotics in dairy cattle in India. The findings of the present study resonated with previous research conducted in other regions of India. For instance,the highest resistance was observed against amoxicillin (100%) and tetracycline (37.03%), while complete sensitivity was shown to azithromycin and chloramphenicol in the study conducted by Singh *et al*. (2018) on *E. coli* isolates from milk samples collected from dairy farms in Ludhiana. Oxytetracycline (61.5%) resistance was reported by Jindal *et al*. (2021) in milk samples from dairy farms of Punjab.

Among the 49 positive isolates, 25 isolates were not resistant to any of the antibiotics studied while 12 isolates (24.49%) exhibited multidrug resistance (MDR), including one isolate resistant to six antibiotics, three isolates resistant to four antibiotics and eight isolates resistant to three antibiotics. The highest MDR was observed among the equipment swab samples with 35.71 per cent of the positive isolates being resistant to three or more antibiotics, as shown in Fig. 5. A higher MDR prevalence in *E. coli* has been previously reported from Kerala (Joseph and Kalyanikutty, 2022) and Murugesan *et al*. (2022) in Karnataka (35.71%) among livestock samples.

Phenotypic assessmentof ESBL production among E. coli isolates

Modified double disc synergy testing was performed to identify ESBL producers. The cephalosporins used for the test were cefotaxime (30µg) and cefepime (30µg), both with and without clavulanic acid (Fig. 6). Cefepime was used to improve the detection

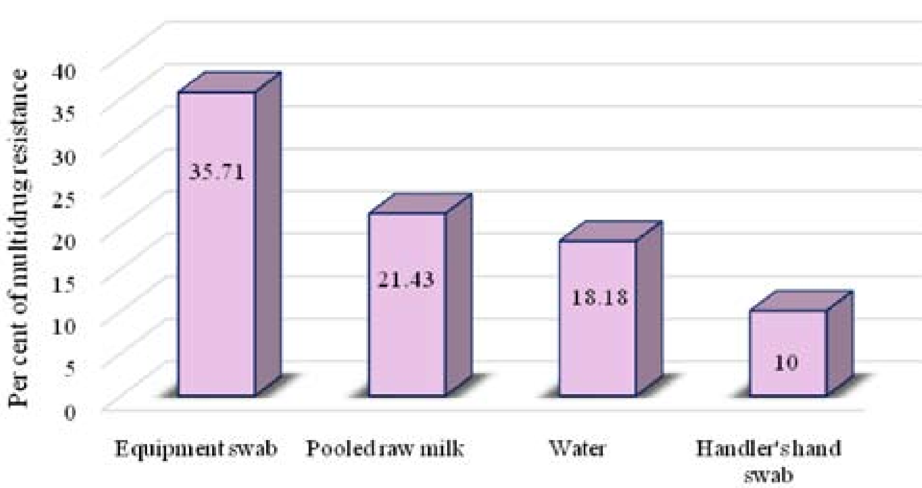

Fig. 5. Total per cent of multidrug resistance obtained from different sources

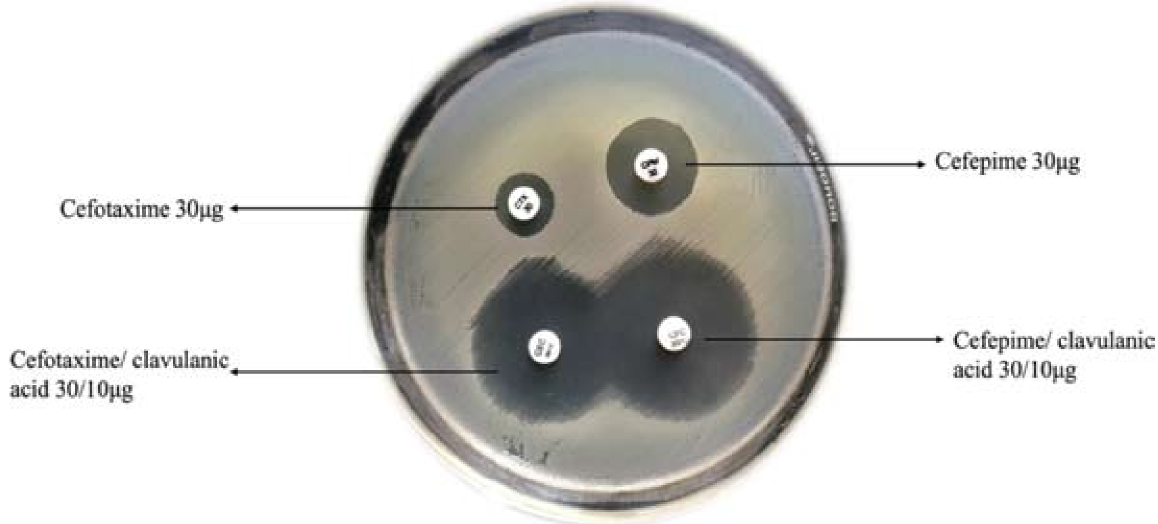

Fig. 6. Increase in zone of inhibition against cefotaxime with clavulanic acid and cefepime with clavulanic acid on Muller Hinton agar

of ESBL producers and according to Kaur *et al*. (2016), cefepime was the best cephalosporin in detecting ESBL in presence of AmpC production.

Among the 49 isolates, three isolates (6.12%) including one milk, one equipment swab and one handler's hand swab were positive for ESBL production. The findings were almost similar to the study conducted by Kar *et al*. (2015) in Odisha where only two isolates out of 64 positive *E. coli* isolates tested positive for ESBL production. The prevalence of ESBL producing *E. coli* among animals in India was reported as 9 per cent in the meta analysis study conducted by Kuralayanapalya *et al.* (2019). However a higher ESBL *E. coli* prevalence (12.3%) was reported from dairy farm environments of Haryana (Kamboj *et al*., 2024). Similar findings have been documented internationally, as Kamaruzzaman *et al*. (2020) reported that 4.8 per cent of *E. coli* isolates from milk and the dairy farm environment in Malaysia exhibited ESBL production. The overuse and misuse of antibiotics in farm environments could contribute to the persistence and development of ESBL-producing *E. coli*, limiting treatment options and posing a public health challenge.

Conclusion

The study concludes that the overall occurrence of *E. coli* from different sources in dairy farms in Thrissur was 38.28 per cent. The presence of *E. coli* in dairy farm environment is indicatitive of faecal contamination and unhygienic management practices. The detection of multidrug resistance among isolates, including phenotypically confirmed ESBL-producing isolates, underscores the growing threat of AMR within farm environments. The high prevalence of resistance to commonly used antibiotics such as tetracycline and ampicillin calls for prudent control measures in the use of antibiotics in dairy farming. Regular pathogen surveillance and responsible use of antimicrobials, along with proper upgradation and implementation of biosecurity measures are essential in mitigating the spread of resistant pathogens. Future research should focus on exploring alternative strategies to reduce the reliance on antibiotics through a 'one health' approach and curtail the development of resistant bacterial strains, safeguarding both animal and public health.

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Conflict of interest

The authors declare that they have no conflict of interest.

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