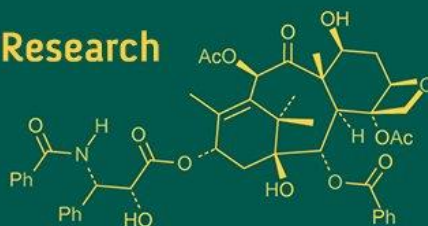


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(6): 952-955
www.biochemjournal.com
Received: 13-03-2026
Accepted: 16-04-2026

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Isolation, characterization and antimicrobial resistance profiling of mastitogenic *Escherichia coli* from dairy cows

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6l.8797>

Abstract

Bovine mastitis is a major infectious disease of dairy animals that causes substantial economic losses through reduced milk production, treatment costs, and premature culling. Among environmental pathogens, *Escherichia coli* is an important cause of clinical mastitis and is increasingly associated with antimicrobial resistance. The present study aimed to isolate, characterize, and determine the antimicrobial susceptibility profile of *E. coli* recovered from clinical bovine mastitis cases. A total of 54 milk samples were collected from clinically affected bovines and subjected to bacteriological examination. Fourteen isolates exhibited characteristic cultural morphology suggestive of *E. coli*, of which 12 were confirmed through biochemical characterization. The prevalence of *E. coli* was 22.2% among clinical mastitis samples. Antimicrobial susceptibility testing revealed high resistance to sulphonamides (93.75%), amoxicillin (87.50%), erythromycin (81.25%), and tetracycline (75.00%). Conversely, ceftiofur (87.50%), ceftriaxone (81.25%), ciprofloxacin (81.25%), and gentamicin (75.00%) showed high efficacy. These findings emphasize the need for routine antimicrobial susceptibility testing to ensure effective treatment and support antimicrobial stewardship in dairy herds.

Keywords: Bovine mastitis, *Escherichia coli*, clinical mastitis, antimicrobial resistance, antibiotic susceptibility testing, and dairy cattle

Introduction

Bovine mastitis, an inflammatory condition of mammary tissue is one of the important infectious diseases, significantly hampers the sustainability and productivity of dairy sector (Fesseha *et al.*, 2021; Kour *et al.*, 2023) ^[10, 17]. It incurred substantial economic losses reflected as decreased quantity and quality of milk, increased culling rate and veterinary services (Sharun *et al.*, 2021; Morales-Ubaldo *et al.*, 2023) ^[27, 23]. Being one of the costliest diseases of dairy sector, mastitis accounts for 20-30 billion USD annual economic loss (direct/indirect losses) worldwide (Gayathri *et al.*, 2024; Bose and Siva Kumar, 2025) ^[12, 4]. As per a study, the economic loss/cow due to gram negative pathogen mastitis (211.03 USD) was significantly higher than gram positive mastitis (133.73 USD) (Fu *et al.*, 2022) ^[11]. As per Bansal and Gupta (2009) ^[2], the financial loss incurred due to clinical and subclinical mastitis in India is approximately INR 7165.51 crores. Mastitis in dairy animals caused by wide variety of pathogens including contagious (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium pyogens* and *Mycoplasma* spp.), environmental (*E. coli*, *K. pneumoniae*, *S. dysagalactiae* and *S. uberis*) and opportunistic (Coagulase negative Staphylococci) agents (Ndahetuye *et al.*, 2019) ^[25]. Environmental mastitis pathogens (mainly *E. coli* and *K. pneumoniae*) predominantly present in animal surroundings and gained access through teat canal during or one to two hours of milking (Hogan and Smith, 2012; Cobirka *et al.*, 2020) ^[14, 7]. *E. coli* predominantly associated with acute mastitis, characterized by abnormal changes in milk and udder accompanied by systemic signs (fever, anorexia and depression) (Bobbo *et al.*, 2017) ^[3]. Based on the presence or absence of apparent clinical signs, bovine mastitis is classified into two types: clinical mastitis (CM) and sub-clinical mastitis (SCM) (Abebe *et al.*, 2016) ^[1].

CM characterized by physical and biochemical changes in milk, pathological changes in mammary tissue and systemic signs. In contrast, no evident clinical signs were observed in cases of subclinical mastitis (Jesse *et al.*, 2023). As per Kour and Co-worker (2023), 70-80% share of production losses are attributed to subclinical mastitis. The high incidence of mastitis is closely related to high lactation yield of animal with approximately 15-20% cow population affected annually (Haider *et al.*, 2023) [13]. In a meta-analysis study of bovine mastitis, the pooled prevalence of CM and SCM is 15% and 42% in world and respectively and 18% and 45% in India respectively (Krishnamoorthy *et al.*, 2021) [18]. Despite *E. coli* being a major cause of clinical bovine mastitis, information on the current antimicrobial susceptibility patterns of mastitis-associated isolates remains limited. The emergence of antimicrobial-resistant *E. coli* strains has further complicated treatment outcomes, highlighting the need for continuous surveillance. Therefore, isolation and characterization of *E. coli* from clinical mastitis cases, along with antimicrobial susceptibility testing, are essential to identify effective therapeutic agents, monitor resistance trends, and support evidence-based mastitis control and antimicrobial stewardship programs. The aim of the study was to investigate the bacteriological characteristics and antibiotic resistance patterns of *E. coli* associated with clinical bovine mastitis for supporting effective mastitis management and antimicrobial stewardship.

Material and methods

Sample Collection and Isolation of *E. coli*

Milk samples were aseptically collected from bovine quarters clinically affected with mastitis presented at the Referral Veterinary Polyclinic and Teaching Veterinary Clinical Complex (RVP-TVCC), ICAR-Indian Veterinary Research Institute (ICAR-IVRI), Izatnagar, Bareilly, Uttar Pradesh, India. Samples were transported to the laboratory under refrigerated conditions for bacteriological examination. For isolation of *E. coli*, milk samples were centrifuged at $2,670 \times g$ for 10 min and the sediment was inoculated into Brain Heart Infusion (BHI) broth followed by incubation at 37 °C for 24-48 h. Enriched cultures were streaked onto MacConkey Lactose Agar (MLA) and incubated at 37 °C for 24-48 h. Presumptive lactose-fermenting colonies were further sub-cultured onto Eosin Methylene Blue (EMB) agar for selective isolation of *E. coli*. The isolates were subsequently subjected to biochemical.

Biochemical Characterization of *E. coli*

Presumptive *E. coli* isolates were biochemically characterized using the KB010 HiE. coli™ Identification Kit (HiMedia Laboratories, India). The kit comprised four carbohydrate utilization tests (glucose, sorbitol, sucrose, and lactose) and eight conventional biochemical tests including methyl red, Voges-Proskauer, citrate utilization, indole production, β -glucuronidase activity, nitrate reduction, ONPG, and lysine utilization. Pure bacterial cultures were inoculated into Brain Heart Infusion broth and incubated until turbidity matched the 0.5 McFarland standard. Subsequently, 50 μ L of inoculum was dispensed into each well of the kit and incubated at 35 ± 2 °C for 18-24 h. Test results were interpreted according to the manufacturer's instructions.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of confirmed *E. coli* isolates was performed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) following standard guidelines. Bacterial suspensions equivalent to a 0.5 McFarland standard were prepared and uniformly spread on MHA plates. Antibiotic discs were placed aseptically on the inoculated agar surface, and plates were incubated at 35 ± 2 °C for 16-18 h. The diameter of inhibition zones was measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotic exhibiting the highest susceptibility against the isolates was selected for subsequent *in vivo* studies.

Results

Isolation and Biochemical characterization of *Escherichia coli*

A total of 54 milk samples were collected from clinical cases of bovine mastitis. Following enrichment and selective culturing, 25 (46.3%) samples produced characteristic pink lactose-fermenting colonies on MacConkey Lactose Agar (MLA). These isolates were subsequently streaked onto Eosin Methylene Blue (EMB) agar, where 14 isolates exhibited the characteristic green metallic sheen suggestive of *E. coli*. Biochemical characterization using the KB010 HiE. coli Identification Kit revealed that 12 isolates showed positive reactions in 9 out of 12 biochemical tests, whereas the remaining two isolates exhibited positive reactions in 6 out of 12 tests.

Antimicrobial Susceptibility Profile of *E. coli* Isolates

The antimicrobial susceptibility pattern of the 12 confirmed *E. coli* isolates is presented in Table X. The isolates exhibited a high level of resistance to sulphonamides (93.75%), followed by amoxicillin (87.50%), erythromycin (81.25%), and tetracycline (75.00%). Intermediate susceptibility was observed for co-trimoxazole (31.25%), amoxicillin-clavulanate (31.25%), and ampicillin (25.00%). Among the tested antimicrobials, the isolates showed the highest susceptibility to ceftiofur (87.50%), followed by ciprofloxacin (81.25%), ceftriaxone (81.25%), gentamicin (75.00%), and enrofloxacin (68.75%). Based on the antimicrobial susceptibility profile and its widespread therapeutic use against bovine coliform mastitis, ceftriaxone was selected as the reference antibiotic for subsequent evaluation in the *E. coli*-induced murine mastitis model.

Table 1: Antibiotic susceptibility testing profiling against *E. coli* isolated from clinical mastitis

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Amoxicillin	6.25	6.25	87.50
Ampicillin	43.75	25.00	31.25
Ceftiofur	87.50	6.25	6.25
Ceftriaxone	81.25	6.20	12.50
Ciprofloxacin	81.25	6.25	12.50
Co-trimoxazole	31.25	31.25	37.50
Enrofloxacin	68.75	18.75	12.50
Erythromycin	6.25	12.50	81.25
Gentamicin	75.00	18.75	6.25
Sulfonamide	0.00	6.25	93.75
Tetracycline	6.25	18.75	75.00
Amoxicillin-clavulanate	43.75	31.25	25.00

Discussion

In the current study, pathogenic *E. coli* was isolated from the clinical cases of mastitis presented to RVP-TVCC and cattle and buffalo farm, IVRI. On screening 54 mastitis milk samples, 12 (22.2%) samples were found positive for *E. coli* by culture and biochemical test. In a study conducted in Maya city, Ethiopia, the prevalence rate of *E. coli* mastitis was 22.6% which is in agreement to our study finding (Dahesa *et al.*, 2025) [8]. Researcher reported similar finding of *E. coli* prevalence (22.45%) in an investigation done at Romania dairy farm from 2018-2022 (Druega *et al.*, 2025) [9]. A nine years (2016-2024) study in Eastern Iran also documented the comparable prevalence (19.92%) of *E. coli* mastitis cases, closely aligning with our findings (Moradi *et al.*, 2025) [22]. The positivity of *E. coli* in current study was consistent with Xu and co-workers (2022) [29], who reported that the prevalence of *E. coli* mastitis was 26.99% in certain parts of Jiangsu province, China. In Contrary to our finding, Rana and co-worker (2022) [26] reported *E. coli* prevalence (30.43%) which is higher than our study prevalence. In a meta-analysis study from 1995-2019, the pooled prevalence of *S. aureus* and *E. coli* was 41% and 15% respectively in India (Krishnamoorthy *et al.*, 2021) [18]. Manasa and co-workers (2019) [21] reported that 13 (16.6%) out of 78 samples were found positive for *E. coli* in clinical cases of bovine mastitis. In another study conducted between 2005-2016, the prevalence of mastitis due to *E. coli* was 14% (Krishnamoorthy *et al.*, 2017) [18]. A study undertaken in Free State Province of South Africa reported that, the prevalence of SCM was 39.1% with majority of cases (88.23%) attributed to *E. coli* infection (Khasapane *et al.*, 2025) [16]. The variation in prevalence of *E. coli* mastitis could be attributed to unhygienic practices in the farm as well as suboptimal managemental conditions.

Twelve confirmed isolates of *E. coli* from clinical cases of bovine mastitis were used for ABST. Findings suggested high degree of resistance to sulphonamides (93.75%), amoxicillin (87.5%), erythromycin (81.25%) and ampicillin (75.00%) followed by co-trimoxazole (37.50%) and ampicillin (31.25%). The *E. coli* strains were quiet susceptible to ceftiofur (87.50%), ceftriaxone (81.25%), ciprofloxacin (81.25%) followed by gentamicin (75.00%). In a study carried out in North America between 2011-22 reported that *E. coli* exhibited low level of resistance to ceftiofur (2.8%) and cefoperazone (3.4%) with intermediate level for ampicillin (9.2%) (Sweeney *et al.*, 2024) [28]. The 6.25% resistant level by *E. coli* strains for ceftiofur was slightly higher than reported (2.8%) by Sweeney and co-workers (2024) [28]. Contrary to our findings, Khasapane and co-worker (2025) [16] reported alarming level of resistant in *E. coli* with degree of resistance 71.6%, 70% and 30% for penicillins, ciprofloxacin and gentamicin respectively. In contrast to our findings, antimicrobials like streptomycin (17.7%), tetracycline (15.93%) and ampicillin (11.5%) showed lower degree of resistant against *E. coli*, whereas none *E. coli* isolate exhibited resistant to gentamicin and ciprofloxacin (Majumder *et al.*, 2021) [20]. In a study in Brazil, the resistant to ceftriaxone was slightly lower (7.3%) than our study (Campos *et al.*, 2022) [5]. In a study conducted in Bangladesh, the susceptibility rate (>70%) of ceftriaxone and ciprofloxacin was in agreement with our finding (Rana *et al.*, 2022) [26]. In contrast to our finding, My *et al.* (2023) [24] reported low level of resistance (32%) in mastitogenic *E. coli* isolates to tetracycline. The resistant to

ceftiofur (4%) was slightly lower than our findings, which might be due to small sample size of our study (My *et al.*, 2023) [24]. As per a study, the sensitivity rate (89.5%) of ceftiofur against *E. coli* strains is in alignment with our findings (Zhao *et al.*, 2024) [29]. The findings of Li and co-worker (2022) were also in agreement with current study showing almost similar level of resistant (52.5%) to ampicillin. Rana *et al.* (2022) [26] documented somewhat higher level of resistance in mastitogenic *E. coli* to ampicillin (58.82%) and ceftriaxone (26.47%). The findings of our study revealed alarming data of antimicrobial resistance among mastitogenic *E. coli* isolates, with only 6/14 antibiotics exhibited >75% sensitivity rate. Majumder and co-worker (2021) [20] documented that, out of 32 isolates of mastitogenic *E. coli*, 19 isolates showed multi drug resistance, while 13 isolates exhibited resistant to single antibiotic. Chowdhury and co-worker (2025) [6] also highlighted the severity of resistance, reported 31.9% multi drug resistance and 8.9% extensively drug resistance *E. coli* strains.

Conclusion

The present study confirmed the occurrence of *Escherichia coli* in clinical bovine mastitis through cultural and biochemical characterization. The isolates exhibited high resistance to commonly used antibiotics, particularly sulphonamides, amoxicillin, erythromycin, and tetracycline. In contrast, ceftiofur, ceftriaxone, ciprofloxacin, and gentamicin demonstrated high antimicrobial efficacy against the isolates. These findings emphasize the importance of routine antimicrobial susceptibility testing for effective therapeutic decision-making and prudent antimicrobial use in bovine mastitis management.

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