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Research Article

Detection of antibiotic resistance profiles in mastitis producing *Escherichia coli* of buffalo and its environment

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Abstract

The present study investigated the antibiotic resistance patterns of *Escherichia coli* (*E. coli)* isolated from mastitis buffalo milk and environmental water samples collected from dairy surroundings, with an aim to understand the distribution of resistance profiles and associated genetic determinants. Out of 79 mastitis milk samples, *E. coli* was isolated from 52 samples, while 31 *E. coli* isolates were recovered from 54 water samples. Among these, 27 milk isolates and 14 water isolates were identified as ESBL/AmpC producers based on cultural, biochemical, and molecular characterization. Antibiotic susceptibility testing of *E. coli* isolates from mastitis milk revealed highest resistance to cefotaxime, followed by ampicillin, cotrimoxazole, imipenem, enrofloxacin, amoxyclav, tetracycline, chloramphenicol, and gentamicin. Similarly, isolates from water samples, exhibited highest resistance to cefotaxime followed by ampicillin, imipenem, cotrimoxazole, tetracycline, amoxyclav, enrofloxacin, chloramphenicol, and gentamicin. The ESBL/AmpC-producing isolates demonstrated particularly high resistance to ceftazidime, followed by cefoxitin and meropenem. PCR-based detection of resistance genes showed that 91.9% of all *E. coli* isolates carried AmpC gene, followed by SHV, NDM-1, TEM and OXA genes. AmpC was detected in all isolates exhibiting phenotypic resistance to third-generation cephalosporins, except for three water isolates. Interestingly, some isolates harboured AmpC gene but showed sensitivity or intermediate resistance to third-generation cephalosporins in AST, indicating a lack of phenotypic-genotypic concordance. Additionally, several AmpC-positive isolates didn't exhibit resistance to clavulanic acid. These findings highlight the occurrence of antimicrobial resistance, particularly β-lactamase–mediated resistance, in *E. coli* from buffalo mastitis cases and their environment, emphasizing the need for surveillance and prudent antimicrobial use in dairy ecosystems.

Keywords: Antimicrobial susceptibility testing (AST), Antibiotic resistance genes, Buffalo, ESBL-producing bacteria, Mastitis, Third-generation cephalosporins

Highlights

- High prevalence of ESBL/AmpC-producing *E. coli* in mastitis milk and water.
- Cefotaxime showed highest resistance in milk and water *E. coli* isolates.
- AmpC gene detected in most isolates with diverse β-lactamase gene profiles.
- Environmental water served as a reservoir for resistant *E. coli* strains.

INTRODUCTION

Among the various diseases affecting the productivity of dairy animals, mastitis is considered as one of the most expensive diseases in terms of production losses and cost of veterinary aid (Bardhan, 2013). Though mastitis has multiple etiologies, *E. coli* is considered as the second most important pathogen of mastitis and is responsible for environmental mastitis in dairy animals (Bansal et al., 1990; Char et al., 1993). The higher incidence of *E. coli* mastitis may be due to poor hygienic conditions in the farm management (Radostits et al., 2000).

Different antibiotics used as mastitis therapy are showing reduced effectiveness due to emerging antimicrobial resistance (AMR) phenomenon in the pathogenic bacteria such as *E. coli* (Sharun et al., 2021). In *Enterobacteriaceae* members, resistance to the β-lactam antibiotics is often associated with the production of extended spectrum β-lactamases (ESBLs) (Pitout & Laupland, 2008). The AMR due to metallo-β-lactamases and ESBLs is an emerging problem (Von Wintersdorff et al., 2016). The genes coding for ESBL, AmpC β-lactamase and metallo β-lactamase are often located on mobile genetic elements such as integrons, insertion sequences, transposons, and plasmids, favouring their dissemination. The antimicrobial resistance genes (ARGs) can be transmitted between the bacteria either vertically or horizontally (by conjugation, transformation, and transduction).

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The mastitis causing *E. coli* acts as reservoir for AMR genes and can play a role in the transmission of the AMR genes to other pathogenic and commensal bacteria in the farm environment (Aarestrup, 2015). Hence, AMR must be carefully monitored for prudent prescription of antibiotics and for implementation of appropriate AMR containment strategies. Despite the increasing reports of antimicrobial resistance in mastitis-associated *E. coli,* there is limited data integrating phenotypic resistance profiles with the molecular detection of key β-lactamase–encoding genes from both dairy animals and their immediate farm environment. The present study addresses this gap by providing comprehensive insights into the co-existence and dissemination of antimicrobial resistance determinants among *E. coli* isolates from clinical mastitis cases and water sources. Such information is essential for designing targeted intervention strategies and strengthening antimicrobial stewardship in dairy production systems. Therefore, this study was taken up to assess the antibiotic resistance profiles of the *E. coli* isolates and to detect selected antibiotic resistance genes (ARGs) in the *E. coli* isolated from dairy animals and environmental (water) samples.

MATERIALS AND METHODS

Sample collection: Milk samples of buffaloes with clinical and suspected subclinical mastitis were collected from the Veterinary Clinical Complex (VCC), NTR College of Veterinary Science, Gannavaram, Veterinary Dispensaries (VD)/ Veterinary Hospitals (VH)/ dairy farms/ sheds in Krishna district of Andhra Pradesh. Suspected subclinical mastitis cases were initially screened using the California Mastitis Test (CMT), and CMT-positive samples were included in the study. A total of 79 mastitis milk samples were collected.

Environmental water samples (n=54) were collected from the same farm environments corresponding to the animals from which milk samples were obtained. For cases presented at the VCC, water samples were collected from animal sheds located in the surrounding areas from where mastitis cases are routinely referred to the VCC. In the case of samples collected from Veterinary Dispensaries and dairy farms/sheds, farmers were requested to provide water samples from their respective animal housing units. The water samples collected represented drinking water sources used for buffaloes, and no sewage or wastewater samples were included in the study. Feed samples were not included as environmental samples in the present investigation.

Isolation and identification of *Escherichia coli*

Isolation of *E. coli* from mastitis milk samples- Mastitis milk samples (1 mL) were inoculated into 5 mL of Tryptone soya broth (TSB) and incubated at 37ºC for overnight. A loopful of culture from TSB was streaked on MacConkey agar (MCA) and incubated at 37ºC for 18-24 hours. Whereas for isolation of *E. coli* presumptively producing ESBL/AmpC, the mastitis milk samples were directly swabbed on MCA plate containing 1 mg/L of cefotaxime (CTX) and incubated at 37ºC for 18-24 hours (Ali et al., 2016). Typical bright pink colonies were picked up, sub-cultured and subjected to Gram's staining and standard biochemical tests like catalase, oxidase, triple sugar iron agar and IMViC tests.

Isolation of *E. coli* from water samples- Water samples were diluted with distilled water and filtered through 0.45 µm nylon membrane filters. After filtration, the top portion of nylon membrane removed from the filter was rolled on Tryptone Bile Glucuronic (TBX) agar and TBX agar plate containing 1 mg/L of cefotaxime (CTX) separately and incubated at 37ºC overnight for isolation of *E. coli* and *E. coli* presumptively producing ESBL/AmpC respectively (Byrne et al., 2022). The green colour colonies were picked up and sub-cultured for further biochemical characterization.

Molecular detection of *Escherichia coli*- The isolated *E. coli* culture was inoculated in TSB tubes and incubated at 37ºC overnight. The DNA from bacterial cell mass pellet was extracted by the high salt method of DNA extraction (Aravindakshan et al., 1997) with suitable modifications (Anand Kumar, 2009). Briefly, the culture was washed with phosphate buffered saline (PBS) and Tris-KCl-MgCl, (TKM) buffer, lysed with 10% SDS, and treated with 6 M NaCl. The nucleic acids were precipitated with absolute alcohol, washed with 75% ethanol, resuspended in sterile distilled water, and stored at –20°C until use. The *E. coli* isolated from mastitis milk samples and water samples that were confirmed in cultural and biochemical methods were subjected to reactivity with *E. coli* species-specific oligonucleotide primers and all the PCR reactions were carried out with a total volume of 25 µL of reaction in Proflex PCR system, Applied biosystems, under standardized cycling conditions. Then the PCR amplicons were analysed by electrophoresis on a 1.7% agarose gel containing 0.5 µg/mL of ethidium bromide in Tris Borate EDTA (TBE) buffer.

Antibiotic sensitivity test: The antibiotic sensitivity test (AST) was performed as per the modified disc

Table 1. Primary panel of antibiotic discs

S. No.	Antimicrobial agent	Symbol	Disc concentration (μg)
1	Ampicillin	AMP	10
2	Enrofloxacin	EX	5
3	Cotrimoxazole	COT	25
4	Cefotaxime	CTX	30
5	Amoxyclav	AMC	20/10
6	Imipenem	IMP	10
7	Gentamicin	GEN	10
8	Tetracycline	TE	30
9	Chloramphenicol	C	30

Table 2. Secondary panel of antibiotic discs

S. No.	Antimicrobial agent	Symbol	Disc concentration (μg)
1	Cefotaxime/clavulanic acid	CEC	30/10
2	Ceftazidime	CAZ	30
3	Ceftazidime/clavulanic acid	CAC	30/10
4	Cefoxitin	CX	30
5	Meropenem	MEM	10

producing ESBL/AmpC. The second panel consisted of selected β-lactam antibiotics with and without β-lactamase inhibitors and carbapenems, specifically included for the phenotypic screening and confirmation of extended-spectrum β-lactamase (ESBL), AmpC β-lactamase, and carbapenemase production, as recommended by standard guidelines. The zone of inhibition against each antibiotic disc after incubation was measured and noted.

Detection of genetic determinants of antibiotic resistance in *E. coli*: The selected genetic determinants of antibiotic resistance *viz.*, TEM, SHV, OXA, NDM-1 and AmpC in the *E. coli* isolates from mastitis milk and water samples were detected by testing the reactivity of *E. coli* DNA with target gene specific oligonucleotide primers in PCR test, under thermal cycler conditions as described in Table 3.

Table 3. Oligonucleotide primers and PCR conditions

Gene	Primer sequence (5'-3')	Annealing temp	Product size (bp)	References
Eco 2083	GCTTGACACTGAACATTGA	57ºC	662	Riffon et al., (2001)
E+co 2745	GGCACTTATCTCTTCCGCATT			
TEM	CATTTCCGTGTCGCCCTTATT	60ºC	800	Dallenne et al., (2010)
	CCGTTCATCCATAGTTGCCTGAC			
SHV	AGCCGCTTGAGCAAATTAAACA	60ºC	713	Dallenne et al., (2010)
	TCCCGCAGATAAATCACCAC			
+OXA	GGCACCAGATTCAACTTTCAAGG	60ºC	564	Dallenne et al., (2010)
	ACCCCAAGTTTCCTGTAAGTG			
NDM-1	GGGCAGTCGCTTCCAACGGTG	55ºC	475	Naeem et al., (2021)
	TAGTGCTCAGTGTCGGCAT			
AmpC	CCCCGCTTATAGAGCAACAA	58ºC for 10 cycles	634	Kar et al., (2015)
	TCAATGGTCGACTTCACACC	55ºC for 15 cycles		
		50ºC for 10 cycles		

diffusion method of Kirby-Bauer (Gefen et al., 2017). The AST was performed on Mueller-Hinton (MH) agar (HiMedia) with pH 7.4. Freshly grown *E. coli* culture suspended in sterile saline was adjusted to 0.5 McFarland standards and used as inoculum. The AST was performed using primary panel of antibiotic discs (listed in the Table 1) for all the *E. coli* isolates, including *E. coli* isolates that were presumptively identified for producing ESBL/AmpC. Further, AST with secondary panel of antibiotic discs (listed in Table 2) was done for *E. coli* isolates that were presumptively identified for

RESULTS

A total of 52 samples out of 79 mastitis milk samples (confirmed by California mastitis test) and 31 samples out of 54 water samples were confirmed as *E. coli* and a total of 27 mastitis milk samples and 14 water samples were identified as ESBL/AmpC producing *E. coli* based on cultural, biochemical, and molecular (PCR test) tests (Fig. 1).

Antibiotic sensitivity test (AST) with *E. coli* isolates: Results of ASTs for the *E. coli* isolates from mastitis

milk and water samples with primary panel of antibiotics discs are presented in Table 4.

The zone of inhibition of all the presumptive ESBL/AmpC producing isolates from mastitis milk and water samples in ASTs with Cefotaxime/clavulanic acid and Ceftazidime /clavulanic acid indicated that all these *E. coli* isolates are ESBL/AmpC producers by phenotypic disc diffusion test (Clinical and Laboratory Standards Institute [CLSI], VET 08, 2018 & CLSI, M100, 2020).

The AST results of presumptive ESBL/AmpC producing *E. coli* isolates from mastitis milk and water samples with secondary panel of antibiotics (ceftazidime, cefoxitin and meropenem) showed that, out of 27 isolates from mastitis milk samples that were presumptively identified for ESBL/AmpC, 25 isolates (92.9%) were found to be resistant to ceftazidime, 23 isolates (85.2%) were resistant to cefoxitin and

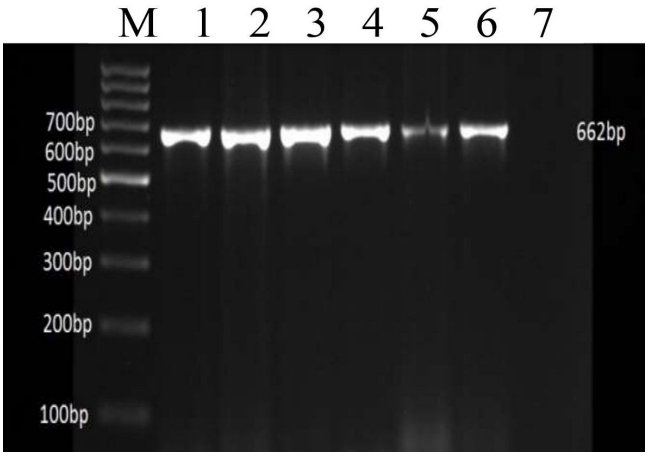


Fig. 1. Polymerase Chain reaction (PCR) test detecting *E. coli* isolated from representative mastitis milk samples and water samples (M: Marker, Lane 1 to 6: *E. coli* isolates, Lane 7: Negative control)

Table 4. Resistance (%) in *E. coli* isolates against primary panel of antibiotics

S. No	Antimicrobial agent	Resistance (%) in *E. coli* isolates			
		E. coli isolates		Presumptive ESBL/AmpC producing *E. coli* isolates	
		Mastitis milk samples	Water samples	Mastitis milk samples	Water samples
1	Ampicillin	63.5% (33/52)	87.1% (27/31)	77.8% (21/27)	100% (14/14)
2	Enrofloxacin	38.5% (20/52)	32.2% (10/31)	55.6% (15/27)	57.1% (8/14)
3	Cotrimoxazole	51.9% (27/52)	67.7% (21/31)	74.1% (20/27)	85.7% (12/14)
4	Cefotaxime	86.5% (45/52)	100% (31/31)	100% (27/27)	100% (14/14)
5	Amoxyclav	26.9% (14/52)	41.9% (13/31)	51.8% (14/27)	50% (7/14)
6	Imipenem	46.1% (24/52)	77.4% (24/31)	59.2% (16/27)	78.6% (11/14)
7	Gentamicin	11.5% (6/52)	9.7% (3/31)	25.9% (7/27)	21.4% (3/14)
8	Tetracycline	32.7% (17/52)	54.8% (17/31)	40.7% (11/27)	78.6% (11/14)
9	Chloramphenicol	17.3% (9/52)	25.8% (8/31)	29.6% (8/27)	35.7% (5/14)

Table 5. Percent (%) of *E. coli* isolates possessing selected antibiotic resistance genes

S. No	Antibiotic resistance gene	Percent (%) of isolates showing antibiotic resistance gene			
		E. coli isolates		Presumptive ESBL/AmpC producing *E. coli* isolates	
		Mastitis milk samples	Water samples	Mastitis milk samples	Water samples
1	TEM	44.2% (23/52)	41.9% (13/31)	44.4% (12/27)	50% (7/14)
2	SHV	65.4% (34/52)	71% (22/31)	51.8% (14/27)	78.6% (11/14)
3	OXA	21.1% (11/52)	9.6% (3/31)	18.5% (5/27)	21.4% (3/14)
4	NDM-1	55.8% (29/52)	58.1% (18/31)	51.8% (14/27)	57.1% (8/14)
5	AmpC	94.2% (49/52)	90.3% (28/31)	88.9% (24/27)	92.8% (13/14)

19 isolates (70.4%) were resistant to meropenem. Out of 14 *E. coli* isolates from water samples that were presumptively identified for ESBL/AmpC production, 12 isolates (85.7%) were found to be resistant to ceftazidime, 11 isolates (78.6%) were resistant to cefoxitin, and 7 isolates (50%) were resistant to meropenem.

Genetic determinants of antibiotic resistance in *E. coli* isolates: Results of the reactivity of oligonucleotide primers specific to TEM, SHV, OXA, NDM-1 and AmpC

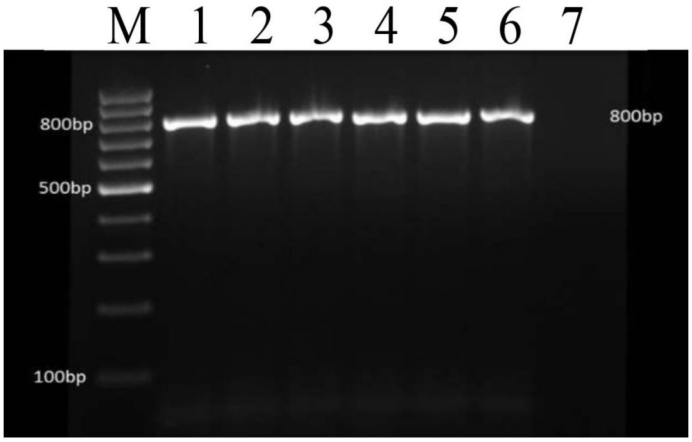


Fig. 2. Reactivity of oligonucleotide primers specific to TEM gene with representative *E. coli* isolates in PCR test (M: Marker, Lane 1 to 6: *E. coli* isolates, Lane 7: Negative control)

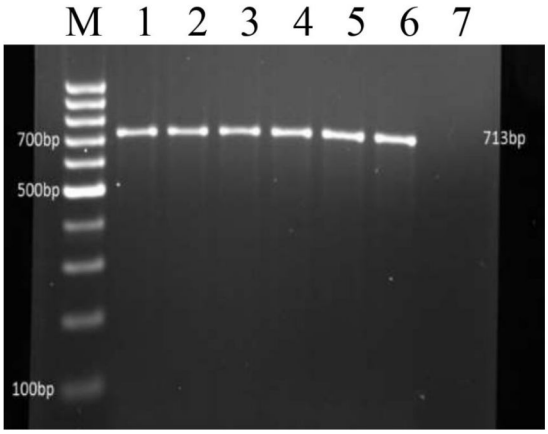


Fig. 3. Reactivity of oligonucleotide primers specific to SHV gene with representative *E. coli* isolates in PCR test (M: Marker, Lane 1 to 6: *E. coli* isolates, Lane 7: Negative control)

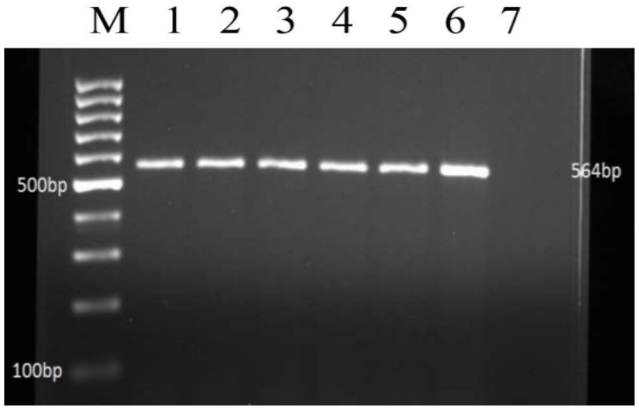


Fig. 4. Reactivity of oligonucleotide primers specific to OXA gene with *E. coli* isolates in PCR test (M: Marker, Lane 1 to 6: *E. coli* isolates, Lane 7: Negative control)

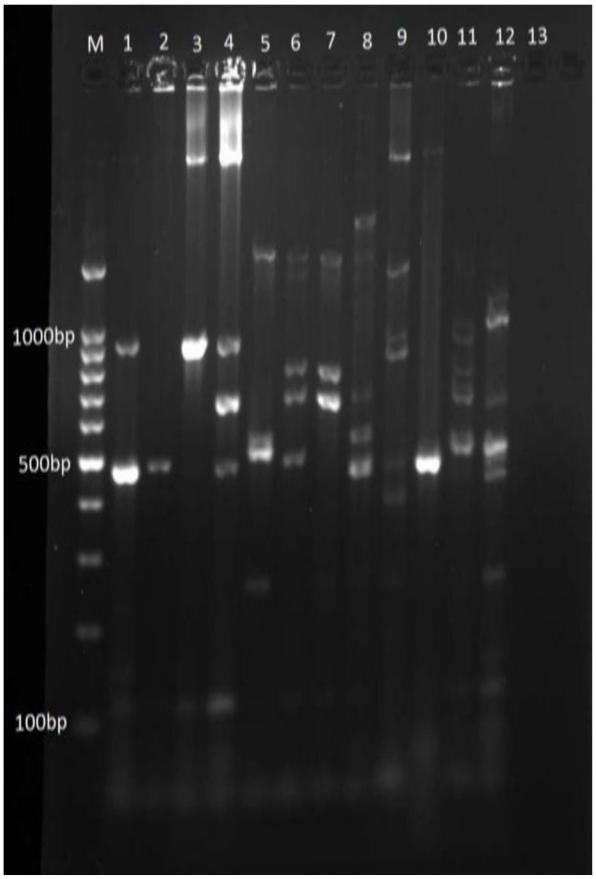


Fig. 5. Reactivity of oligonucleotide primers specific to NDM-1 gene with representative *E. coli* isolates in PCR test (M: Marker, Lane 1 to 12: *E. coli* isolates, Lane 13: Negative control)

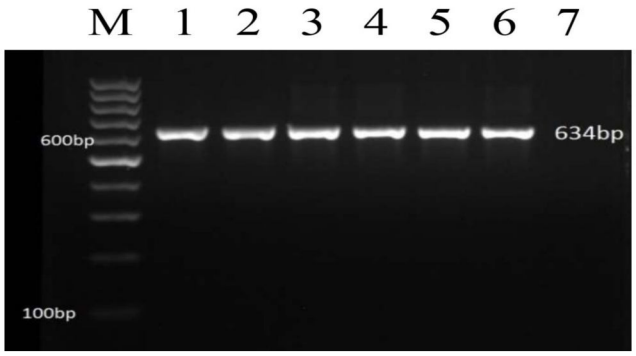


Fig. 6. Reactivity of oligonucleotide primers specific to AmpC gene with representative *E. coli* isolates in PCR test (M: Marker, Lane 1 to 6: *E. coli* isolates, Lane 7: Negative control)

genes of *E. coli* in PCR test are presented in Table 5. Specific PCR product of 800 bp, 713 bp, 564 bp, 475 bp and 634 bp size was yielded for TEM, SHV, OXA, NDM-1 and AmpC, respectively (Fig. 2, 3, 4, 5 & 6).

DISCUSSION

Increased AMR in bacteria is of great concern as it affects both human and animal health (Mahfouz et al., 2020; Perez et al., 2020). The present study is undertaken to investigate the antibiotic resistance patterns of *E. coli* isolates from mastitis milk samples of buffaloes and environment (water) samples in the vicinity of the same dairy animals.

A total of 52 (65.8%) milk samples and 31(57.4%) water samples collected in this study were confirmed as *E. coli* by cultural, biochemical and PCR tests. The present study findings indicated a higher prevalence rate of *E. coli* in mastitis milk samples than the earlier reports on the same from different states of India (Sumathi et al., 2008; Anand Kumar, 2009; Jeykumar et al., 2013; NaliniMohanty et al., 2013; Verma et al., 2018).

Increased resistance to third-generation cephalosporins in *E. coli* isolated from mastitis milk samples of dairy animals was also reported by Boireau et al. (2018), and Basant et al. (2022). However, the level of resistance to imipenem, enrofloxacin, amoxyclav and chloramphenicol, observed in the present study, was higher than those of earlier reports (Ibrahim et al., 2016; Abboud et al., 2021; Majumder et al., 2021; Babacan, 2022). The present study displayed higher rate of susceptibility to gentamicin (82.7%, n=43) which was in tune with the findings of Verma et al. (2018). The antibiogram of (31) *E. coli* isolates from water samples revealed that all the isolates showed resistance to more than one antibiotic. These resistance patterns may be due to the transmission of resistant bacteria and/or ARGs from animals and/or humans to farm environment settings. Findings of the present study are in conformity with Li et al. (2014), Hinthong et al. (2017), and Sobur et al. (2019), except the resistance to imipenem, which is higher than these reports.

All 27 *E. coli* isolates from mastitis milk, and 14 from water samples identified as presumptive ESBL/AmpC producers on cefotaxime-incorporated selective medium were confirmed as ESBL/AmpC producers using the combination disc diffusion method with cefotaxime and ceftazidime±clavulanic acid following CLSI guidelines (CLSI VET 08, 2018; CLSI M100, 2020). Previous studies by Das et al. (2017), and Nalband et al. (2020) reported higher prevalence rates of 46.91% and 48%, respectively, compared to this study. Conversely, Hu et al. (2006), Dahmen et al. (2013), and Freitag et al. (2017) observed lower prevalence (0.4–13%). The increased prevalence may be attributed to selective pressure from third and fourth-generation cephalosporin use in dairy animals (Gonggrijp et al., 2016). However, antimicrobial use data to support these findings is not available. The high resistance to imipenem among *E. coli* isolates, including presumptive ESBL/AmpC producers, is alarming, as carbapenem-resistant *Enterobacteriaceae* are classified as critical priority pathogens by the WHO (Tacconelli et al., 2018). Further phenotypic confirmation of carbapenemase production using commercial selective media is necessary.

ESBL/AmpC-producing *E. coli* isolates are increasingly identified in food-producing animals, raising concerns about their role as reservoirs contributing to the spread of antibiotic resistance (Ewers et al., 2012). Molecular techniques for detecting resistance genes have been invaluable in complementing phenotypic tests. β-lactamase genes like *blaTEM*, *blaSHV*, and *blaOXA* encode enzymes that confer resistance to first and second-generation cephalosporins. Mutations have expanded their activity to third and fourth-generation cephalosporins, leading to the evolution of ESBL variants (Bush et al., 1995). *blaNDM* genes confer resistance to nearly all antibiotics except colistin and tigecycline (Kumarasamy et al., 2010). AmpC β-lactamases, unlike ESBLs, are not inhibited by clavulanic acid and hydrolyze third-generation cephalosporins (Ewers et al., 2012; Dierikx et al., 2013).

PCR-based detection of resistance genes revealed that AmpC β-lactamase was most common, followed by *blaSHV*, *blaNDM-1*, *blaTEM*, and *blaOXA* in mastitis milk and water samples. *blaTEM* was detected in 44.2% (milk) and 41.9% (water) of *E. coli* isolates, consistent with Ghatak et al. (2013), and Abboud et al. (2021), who reported 80–100% frequencies, but higher than Das et al. (2017) at 12%. *blaSHV* was found in 65.4% (milk) and 71% (water) isolates, contrasting with Das et al. (2017), and Abboud et al. (2021), who found no *blaSHV*. ESBL/AmpC producers showed *blaTEM* in 48.1% (milk) and 42.8% (water) and *blaSHV* in 55.6% (milk) and 71.4% (water). Bandyopadhyay et al. (2014) found *blaTEM* in 100% and *blaSHV* in 66.67% of ESBL-producing *E. coli* from mastitis milk. The *blaOXA* gene was detected in 19.2% (milk) and 9.6% (water) of *E. coli* isolates, aligning with Abboud et al. (2021), who reported 16.67% positivity. Among ESBL/Amp C-producing *E. coli*, *blaOXA* was found in

22.2% of mastitis milk isolates and 14.3% of water isolates. In contrast, Kamaruzzaman et al. (2020) found no *blaOXA* in ESBL-producing *E. coli* from dairy farm environmental samples.

PCR testing for the *blaNDM-1* gene in *E. coli* isolates revealed polymorphism in 21 isolates, with amplicon sizes ranging from 475 bp to over 1500 bp, while 48 isolates produced the expected 475 bp product. *blaNDM-1* was detected in 55.8% of isolates from mastitis milk and 58.1% from water samples. Polymorphic PCR products may result from short sequence DNA repeats (SSRs) in prokaryotic genome that harbour short or long stretches of repeated nucleotide sequence motifs (Van Belkum et al., 1998) that would be amplified by binding to primers at a place other than ends thus yielding a PCR product of higher base pairs or due to presence of similar sequences in genome of certain bacterial isolates, but further analysis, including sequencing is needed for confirmation. This study identified a higher prevalence of *blaNDM-1* compared to Ghatak et al. (2013), who reported it in 12.5% of isolates. Among ESBL/AmpC producers, *blaNDM-1* was detected in 55.6% of milk and 57.1% of water isolates.

The AmpC β-lactamase gene was detected in 94.2% of *E. coli* isolates from mastitis milk and 93.5% from water samples. Abboud et al. (2021) reported a lower prevalence, while Saei et al. (2022) found no AmpC genes in ESBL/AmpC-producing *E. coli* from mastitis milk. Among ESBL/AmpC producers, AmpC was present in 92.6% of milk and 92.8% of water isolates. Interestingly, all *E. coli* isolates resistant to third-generation cephalosporins carried the AmpC gene except three from water. Additionally, seven isolates sensitive/intermediate to third-generation cephalosporins also carried the AmpC gene. Some AmpC-positive isolates were not resistant to clavulanic acid, supporting findings by Chen et al. (2005) that the presence of ARGs does not always confer phenotypic resistance. Variations in sensitivity patterns may be attributed to differing sample numbers and collection locations, with fewer water samples often collected compared to mastitis milk samples.

Compared to the earlier published reports by different researchers on AMR pattern in *E. coli* isolates, the variation in the number/percentage of *E. coli* isolates that were found to be sensitive/resistant to different antibiotics (including the presence of selected antibiotic resistance genes) in this study may be due to different geographical region from where the mastitis milk samples and water samples were collected in this study and also due to the different sample size of mastitis milk samples and water samples for isolation of *E. coli*. In the present study, the samples were collected only from part of the Krishna District of Andhra Pradesh (AP). Further studies are required with a greater number of mastitis milk samples and water samples for investigation of AMR pattern in *E. coli* isolates in other parts of AP.

In conclusion, the present study demonstrates a high prevalence of multidrug-resistant and ESBL/AmpC-producing *E. coli* in mastitis milk and nearby water sources, indicating a strong environmental linkage in the dissemination of antimicrobial resistance. The widespread detection of AmpC, ESBL, and NDM-1 genes highlights the alarming burden of β-lactam and carbapenem resistance in dairy farm ecosystems. These findings emphasize the need for judicious antimicrobial use, improved farm hygiene, and continuous AMR surveillance to mitigate risks to animal and public health.

Conflict of interest: The authors do not have any conflict of interest in this study.

Author's contribution: NR: Executed the lab work, completed analysis, writing original draft; PA: Designed the lab work and methodology.

Data availability statement: Data set generated during research will be available from the corresponding author upon reasonable request.

Ethical approval: Not applicable.

Use of artificial intelligence tools: No AI tools were used for the preparation of manuscript. Both authors reviewed and edited the manuscript as needed and take full responsibility for the content of publication.

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