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Molecular Characterization and Epidemiology of Extended-spectrum beta-lactamase (ESBL)-producing Escherichia coliat the Human–Animal Interface in Cattle Farms: Insights into Resistance Profiles and Genetic Exchange

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Research Article

Keywords: Extended-spectrum beta-lactamase (ESBL), E. coli, phylogrouping, ERIC-PCR, integrons

Posted Date: August 19th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10649732/v1

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Additional Declarations: No competing interests reported.

Abstract

Good health and wellbeing of society is not possible without accepting the challenges affecting global scenario like antimicrobial resistance and Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* at the human-animal-environment interface are one of the important reservoirs of antimicrobial resistance in dairy ecosystems, that directly affect society. This study investigated the prevalence, resistance profiles, clonal diversity, and integron-associated resistance genes of ESBL-producing *E. coli* from Indian dairy farms. Samples from animals, humans, and water bodies (n = 75) were screened for ESBL-producing *E. coli* and major resistance genes, followed by phylogrouping, ERIC-PCR, sequencing, and integron analysis. Out of 75 samples, 60 were positive for *E. coli*, giving an overall prevalence of 80% (95% CI: 70.94–89.06), with highest prevalence in humans (96%), followed by animals (84%) and environmental samples (60%) ($p < 0.01$). Resistance was highest to cefuroxime, ampicillin, tetracycline, ciprofloxacin, gentamicin, and carbapenems, and 26.6% of isolates had a MAR index > 0.2. ESBL-producing *E. coli* were most frequent in animals (47.62%), followed by humans and environmental samples (33.33% each). ERIC-PCR showed substantial genetic diversity, with 6 clusters in human isolates, 6 in animal isolates, and 3 in environmental isolates. The most resistant isolates were concentrated in specific human and animal clusters, while environmental cluster 3 showed the greatest diversity. A hyper-resistant human isolate (HEC3) resisted 17 antimicrobials and co-harboredblaTEM, blaCTX-M-1, blaNDM, and tet(B). Across sources, blaTEM was common, blaCTX-M predominated in environmental isolates, blaSHV was found only in humans, and blaNDM occurred in all three sources. Class 1 integrons were detected in human and animal isolates, class 2 integrons only in animals, and no integrons were found in environmental isolates. These findings highlight the concern by the circulation of multidrug-resistant ESBL-producing *E. coli* and mobile resistance elements across the dairy One Health interface.

1. Introduction

Antimicrobial resistance (AMR) has become one the biggest health challenge in today's world. In 2016 a report on AMR predicted that the death cause by AMR could reach 10 million by 2050 (O'Neill, 2016) and could potentially lead to around 10 million deaths each year and a $100 trillion economic cost by 2050 if current trends continue (Mim, 2024). Bacterial AMR was found to be responsible for about 1.27 million deaths around the world in 2019 (World Health Organization, 2023). The growing global problem of antimicrobial resistance requires a thorough understanding of its complicated epidemiology, especially in ecosystems where human, animals and the environment are all connected Livestock systems, particularly cattle farms, are increasingly acknowledged as substantial reservoirs for antimicrobial-resistant bacteria and their corresponding genetic determinants (Maciel-Guerra et al., 2022). This necessitates a robust surveillance framework to address the public health implications of antimicrobial-resistant pathogens and their underlying resistance mechanisms within these agricultural settings (Daoud&Rolain, 2023). The high levels of antimicrobial resistance in these settings can be traced back to the selective pressure of antibiotic classes used in both animal and human medicine. This is because resistant pathogens and genes can move through food chains or by coming into contact with livestock

(Jaleta, 2024). The complex interplay among humans, animals, and the environment constitutes a multifactorial nexus for the dissemination of antimicrobial resistance determinants (Tyrrell, 2025). This integrated approach is crucial for determining the degree to which antimicrobial usage in one sector affects resistance levels in another, thus guiding targeted interventions (Trinchera et al., 2025). The interspecies transmission and environmental dissemination underscore the necessity for a One Health approach to elucidate the intricate network of resistance (Pires et al., 2024). Thus, One Health perspective is essential for examining the emergence and transmission of antimicrobial resistance particularly Extended-spectrum beta-lactamase (ESBL) producing commensal and pathogenic Escherichia coli (Aila&Aish, 2025; Hickman et al., 2021) ESBL producing *E. coli* is among the important indicator bacterium for the spread of antimicrobial resistance because it is found everywhere and can exchange genes between different hosts with significant morbidity, mortality, and substantial economic losses (Suwono, 2021). Moreover, *E. coli* is a major pathogen involved in urinary tract infections (UTIs) and causes approximately 80% of humans UTI's with 404.61 million cases officially recorded worldwide in 2019 (Yang et al., 2022). ESBL-producing and carbapenem-resistant *E. coli*, as members of the *Enterobacteriaceae*, were included in the "Critical" priority category of the WHO 2017 Global Priority Pathogens List, underscoring the urgent need for novel antimicrobial therapies (WHO, 2017). Consequently, examining the dynamics of *E. coli* resistance profiles and genetic exchange at human-animal interfaces on cattle farms can provides significant insights into essential transmission pathways (Klaas, 2026). Thus, the present study was designed and conducted to unveiling ecological dynamics of ESBL-producing *E. coli* in human animal interface with their resistance profiles and genetic exchange capabilities.

2. Material and Methods

2.1. Sample Collection

A total of 75 samples were collected from 25 cattle farms, including cattle, farm workers, and the surrounding farm environment. The studied was after obtaining due ethical approval from Institutional Animal Ethics Committee (IAEC) of SVPUAT, Meerut, India as protocol number IAEC/SVPUAT/2026/193 dated 05/01/2026 Farm selection was based on purposive sampling to ensure representation of both small and large farm settings. To keep One Health in view, three samples were taken from each site. Animal faecal samples were collected immediately post-defecation utilizing sterile applicators to avoid external contamination. Farm workers voluntarily provided human samples of faecal matter for the study's purpose. These were elucidated and written consents were obtained. To evaluate the environmental interface, water samples were obtained from ponds or other nearby water bodies directly associated with each farm, utilizing sterile 50 mL containers. A total of 75 samples were collected which included cattle faeces (n = 25), farm worker stool (n = 25), and farm-associated water (n = 25). To make sure that the microbes stayed alive, all samples were transported to laboratory on ice and processed within 24 hours of being collected.

2.2. Isolation and confirmation of *E. coli*

Faecal and water samples were initially enriched in Tryptone Soya Broth (TSB) and incubated at 37°C for 24 hours. After enrichment the samples were inoculated on MacConkey Lactose Agar (MLA) and incubated at 37°C for 24 hours. Sub-culturing of presumptive lactose-fermenting colonies onto Eosin Methylene Blue (EMB) agar was performed, and colonies displaying distinctive metallic green sheen were identified as presumptive *E. coli*. The species level identification was performed by Polymerase Chain Reaction (PCR) targeting the uidA gene (Anbazhagan et al., 2011).

2.3. Antibiotic susceptibility test (AST) and confirmation of ESBL-producing *E. coli*

The Kirby-Bauer disk diffusion method (Hudzicki, 2009) was performed following Clinical and Laboratory Standards Institute guidelines (CLSI, 2021). A panel of 16 antibiotics and antibacterials namely Ampicillin, Amoxyclav, Cefalexin, Cefuroxime, Cefixime, Ceftriaxone, Ceftazidime, Cefepime, Ertapenem, Meropenem, Aztreonam, Tetracycline, Gentamicin, Chloramphenicol, Ciprofloxacin, Co-trimoxazole and Nitrofurantoin was tested. The results were interpreted as per the CLSI breakpoints (CLSI, 2021). *E. coli* ATCC 25922 was used as quality control strain. The isolates that were resistant to three or more classes of antimicrobials were considered as multidrug resistant (MDR). Confirmation of ESBL production was performed by combined disk test according to CLSI guidelines (CLSI 2021). The assay was performed with disks of cefotaxime (CTX, 30 mcg), ceftazidime (CAZ, 30 mcg), cefotaxime-clavulanic acid (CEC, 30/10 mcg), ceftazidime-clavulanic acid (CAC, 30/10 mcg), phenylboronic acid (400 µg) and EDTA (292 µg). The isolate showing the difference of more than 5 mm in the diameter of the inhibition zone between CEC and CTX and/or between CAC and CAZ considered ESBL positive.

2.4. Molecular detection of Antibiotic Resistance Genes (ARGs) and sequence analysis

The resistant phenotypes were screened for their corresponding ARGs by PCR. Briefly, rapid DNA extraction was performed from bacterial cell suspension. Total DNA (2 µl) was used as a template in the PCR reaction for the detection of ARGs which included blaTEM, blaSHV, blaCTX-M group1, blaIMP, blaVIM (Dallenne et al. 2010) blaNDM (Suranadee et al., 2022), and tet(A), tet(B) tet(C) (Nawaz et al. 2009). The final volume of the PCR mixture was 25 µl containing 12.5µl of 1X GoTaq® Green Master Mix (Promega Corp., WI, USA) 0.2–0.4 µM of forward and reverse primer of each gene, and approximately 50 ng of genomic DNA for the amplification of each gene. Amplified products were analysed on 1.5% agarose gel with ethidium bromide and visualized using a gel documentation system. The PCR amplicons of blaTEM, blaNDM and blaCTX-M group genes were sequenced by the sanger di-deoxy method to determine the allelic variant of the enzymes. The obtained nucleotide sequences were compared with the gene sequences of the GenBank data base using the blast algorithm to determine their relatedness to other nucleotide sequences.

2.5. Molecular Detection of integrase genes and class 1 integron gene cassettes

Extracted DNA by boiling method was used as the templates for integron identification. Identification of three classes of integrons was carried out for all isolates using specific primer pairs for intI1, intI2 (Hossain et al. 2019) and intI3 genes (Goldstein et al. 2001). Amplified products were analysed on 1.5% agarose gel with ethidium bromide and visualized using a gel documentation system.

2.6. Molecular characterization of *E. coli*

2.6.1. Phylogrouping

Clermont Triplex PCR was used to categorize *E. coli* isolates into phylogroups A, B1, B2 and D using the primers chuA, yjaA and TspE4.C2 (Clermont et al. 2000)

2.6.2. Clonal relatedness by enterobacterial repetitive intergenic consensus PCR (ERIC PCR)

The ERIC-PCR was performed to determine the clonal relatedness of *E. coli* isolates by using ERIC1R-ATG TAA GCT CCT GGG GAT TCAC and ERIC2-AAG TAA GTG ACT GGG GTG AGCG primers (Versalovic et al. 1991). Briefly, the PCR reaction of 12.5µl of 1X GoTaq® Green Master Mix (Promega Corp., WI, USA) 0.2µM of forward and reverse primer and approximately 50 ng of genomic DNA for the amplification cycling conditions were 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 48°C for 2.5 min, extension at 72°C for 1.5 min, and a final extension at 72°C for 10 min. The amplified products were visualized in 1.2% agarose gel with 3 kb ladder after running at 60 V for 3 hrs. The gel was photographed and the dendrogram was constructed in GelClust using the UPGMA clustering method and dice distance coefficient (Khakabimamaghani et al. 2013)

3. Results

3.1. Prevalence of *E. coli* in Human-Animal-Environment Interface

Out of the 75 samples screened, 60 were positive for *E. coli*, presenting an overall prevalence of 80% (95% CI: 70.94–89.06%). The prevalence ($p < 0.01$) rate of *E. coli* was found to be much higher in human samples than in samples from animals or the environment. The positivity rate of *E. coli* in human samples was 96% (95% CI: 88.32–100%), followed by 84% (95% CI: 69.63–98.37%) in animals and 60% (95% CI: 40.79–79.21%) in environmental samples. All 60 isolates were positive for uidA gene. The prevalence of isolates is given in Table 1

Table 1
Prevalence of *E. coli* in Human, Animal and Environmental Samples

Source	Samples Tested (n)	Positive (n)	Prevalence (%)	Confidence Interval (95%)
Human	25	*24	96%	88.32–100%
Animal	25	21	84%	69.63–98.37%
Environment	25	15	60%	40.79–79.21%
Total	75	60	80%	70.94–89.06%

***$\chi^2 = 10.50$, df = 2, $p < 0.01$**, indicating a significant difference in prevalence among the three sources.

3.2 Antibiotic sensitivity pattern

The AMR profile of the *E. coli* isolates from the three different sources which are human, animal and environment have shown variability across resistance towards the antibiotic classes (Fig. 1). Overall the isolates from animals showed higher resistance compared to human and environment isolates.

Animal isolates showed noticeable resistance to β-lactams, particularly cefuroxime (52.38%), along with moderate resistance to ampicillin (47.62%) and amoxicillin-clavulanate (19.05%). Elevated resistance was also observed against tetracycline (33.33%), ciprofloxacin (28.57%), and gentamicin (23.81%), Notably, resistance to carbapenems such as ertapenem and meropenem remained relatively lower (14.29%).

Human isolates demonstrated comparatively lower resistance levels, with the highest resistance observed against ampicillin (33.33%) and tetracycline (29.17%). Moderate resistance was recorded for cefuroxime (25.00%), ciprofloxacin (25.00%), and gentamicin (20.83%). Resistance to third-generation cephalosporins such as ceftriaxone and ceftazidime was relatively low (8.33%), and minimal resistance was observed against carbapenems (4.17%), indicating retained susceptibility to last-resort antibiotics in human-derived isolates.

Environmental isolates exhibited a distinct resistance pattern, with relatively high resistance to cephalosporins, including cefotaxime (33.33%), ceftazidime (33.33%), and cefuroxime (33.33%). Additionally, resistance to ciprofloxacin (33.33%) and tetracycline (26.67%) was notable. Moderate resistance was observed for chloramphenicol (13.33%) and gentamicin (13.33%), whereas lower resistance levels were recorded for carbapenem (6.67%).

Additionally, 26.6% (n = 16) of total isolates (n = 60) had a MAR index greater than 0.2, including 12.5% (n = 3) of human isolates, 38.09% (n = 8) of animal and 33.33% (n = 5) of environment isolates. Animal isolates exhibited the highest MAR indices, followed by environment and human isolates.

3.3. Prevalence of ESBL producing *E. coli*

The prevalence of ESBL producing *E. coli* was analysed across three distinct sample categories. The highest prevalence of ESBL producing *E. coli* was observed in animal samples, which recorded a rate of 47.62% (n = 10). In contrast, both human and environmental samples exhibited lower prevalence rates of 33.33% (n = 8, 5).

3.4. Antimicrobial Resistant Genes in *E. coli* isolates

As shown in Fig. 2, PCR-based detection of antimicrobial resistance genes (ARGs) revealed the distribution of β-lactamase and tetracycline resistance determinants across human, animal, and environmental originated *E. coli* isolates. Among the ESBL marker genes, blaCTX-M was the most predominant, particularly in environmental isolates (80%), followed by human (37.5%) and animal isolates (20%). The blaTEM gene also showed substantial prevalence, with the highest occurrence in environmental samples (60%), followed by human (37.5%) and animal isolates (30%). In contrast, the SHV gene was detected only in human isolates (12.5%) and was absent in both animal and environmental samples, indicating its comparatively lower distribution.

Regarding carbapenem resistance genes, blaNDM was identified across all mileus, with the highest prevalence in animal isolates (30%), followed by environmental (20%) and human samples (12.5%). However, blaIMP and blaVIM genes were not detected in any of the isolates, suggesting their limited or absent role in resistance within the studied population.

For tetracycline resistance determinants, tetA was more frequently observed than tetB, with the highest prevalence in animal isolates (40%), followed by human and environmental samples (25% and 20%, respectively). The tetB gene showed moderate presence in animal (20%) and human isolates (12.5%), but was absent in environmental samples.

The PCR amplicons of blaCTX-M and blaNDMwere sequenced to determine their variants. Nucleotide blast search analysis revealed that the ESBL producing isolates harbored blaCTX-M-3; blaCTX-M-15 and blaNDM-5gene variants. The blaNDM-5 gene sequences and its NDM-5 protein revealed > 99% similarity with NDM-5 sequences available in the GenBank database of NCBI. The obtained nucleotide sequences were submitted to NCBI with accession numbers PZ535890, PZ535891, PZ535892, PZ535893, PZ531025, PZ531026, PZ529513, PZ482464, PZ482465, PZ482466.

3.5. Integrase gene and gene cassettes

The integrase 1 (intI1) gene was detected in 37.5% (n = 3) and 30% (n = 3) of the tested isolates from human and animals, respectively and integrase 2 (IntI2) gene was detected in only 10% (n = 1) of animal isolate. Integrase 3 (IntI3) gene could not be amplified in any strain. None of integrase gene could be amplified from environment isolates. Three (50%) of the IntI1 positive isolates carried empty gene cassette and Integron Finder analysis identified complete class 1 integron in three *E. coli* isolates (HEC3, AEC19, and HEC4). The gene cassette arrays exhibited considerable variation in both composition and complexity among the isolates. Isolate HEC3 possessed the most complex cassette array, comprising five antimicrobial resistance genes: aac(6')-Ib, ant(3")-I, qacE, blaOXA-1-like, and dfrA12. In contrast,

isolate AEC19 harboured a single-gene cassette consisting of dfrA1-like, which is associated with trimethoprim resistance, suggesting a comparatively simple integron cassette architecture. Isolate HEC4 contained a three-gene cassette array comprising blaOXA-1-like, sat2, and dfrA1-like.

3.6. Association between AMR phenotype and resistance genes

In human isolates, blaCTX-M-1 showed strong positive correlations with third-generation cephalosporin, including cefotaxime ($r \approx 0.8–0.9$), ceftriaxone ($r \approx 0.8$), and ceftazidime ($r \approx 0.6–0.7$), as well as with ampicillin ($r \approx 0.7–0.8$), highlighting its dominant role in ESBL-mediated resistance, while blaTEM demonstrated moderate positive correlations with β-lactams ($r \approx 0.4–0.6$). The carbapenemase gene blaNDM was positively associated with carbapenem resistance, particularly ertapenem ($r \approx 0.7–0.8$). Tetracycline resistance genes tetA and tetB showed strong correlations with tetracycline phenotypes ($r \approx 0.7–0.9$), whereas blaSHV displayed occasional negative correlations with cephalosporin ($r \approx -0.4$ to -0.5).

In environmental isolates, blaCTX-M-1 maintained positive correlations with cefuroxime and ceftazidime ($r \approx 0.6–0.7$) and with ampicillin/amoxicillin-clavulanate ($r \approx 0.5–0.6$), while blaTEM showed moderate correlations with both β-lactams and ciprofloxacin ($r \approx 0.4–0.5$), suggesting co-selection. The blaNDM gene correlated positively with ertapenem resistance ($r \approx 0.6–0.7$), and tetA remained strongly associated with tetracycline resistance ($r \approx 0.7–0.8$).

However, in animal isolates, strong positive correlations were again observed for blaCTX-M-1 with cefotaxime, ceftriaxone, and ceftazidime ($r \approx 0.8–0.9$) and with ampicillin ($r \approx 0.7–0.8$), while blaTEM showed moderate associations ($r \approx 0.5–0.6$). The blaNDM gene exhibited strong correlation with carbapenem resistance (ertapenem; $r \approx 0.8–0.9$), and tetracycline resistance genes (tetA, tetB) were highly correlated with tetracycline phenotypes ($r \approx 0.8–0.9$). Additionally, moderate correlations were observed between aminoglycoside resistance (gentamicin) and multiple ARGs ($r \approx 0.4–0.6$), indicating co-resistance patterns.

significant ($p < 0.05$) positive and negative correlations, respectively. The intensity of color and the size of the circle represent the strength of the phi correlation coefficient. White squares show a non-significant correlation.

3.7. Molecular characterization

3.7.1. Phylogroup analysis

Phylogroup B1 was the dominant lineage among both human (70.8%) and animal (71.4%) isolates, indicating its predominance in host-associated populations. Conversely, environmental isolates exhibited a markedly different phylogenetic profile, with phylogroup A being the most abundant (46.7%), followed by phylogroups B1 (33.3%) and D (20.0%). Phylogroup D was observed more frequently in environmental isolates than in human (12.5%) and animal (4.8%) isolates (Fig. 4).

3.7.2. Diversity Analysis

3.7.2.1. Human

Diversity analysis of human *E. coli* isolates (n = 24) by ERIC-PCR showed that the isolates dispersed into 6 major clusters along with 2 independent outer lineages (HEC2 and HEC14) on the basis of different genetic patterns (Fig. 5). The isolates belonging to cluster 4 and cluster 5 demonstrated the most severe multi-resistance profiles. Notably, isolate HEC3 within cluster 5 emerged as a hyper-resistant strain, displaying phenotypic resistance to 17 antimicrobial agents and co-carrying an advanced resistome comprising blaTEM, blaCTX-M-1, blaNDM, and tet(B). Among the ARGs screened across the human population, blaCTX-M-1, blaTEM, tet(A), blaNDM, tet(B), and blaSHV were detected. Class 1 integrons (int1) were identified in human isolates HEC1, HEC3, and HEC6, correlating strongly with the co-selection of extended-spectrum beta-lactamase (ESBL) and carbapenemase genes.

3.7.2.2. Animal

Similarly, in case of animal originated *E. coli* isolates (n = 21), ERIC-PCR revealed that the isolates dispersed into 6 clusters on the basis of different genetic patterns (Fig. 6). The structural profiling of the clusters showed that cluster 2 and cluster 3 possessed the highest concentration of critical resistance determinants. Specifically, isolates AEC20 and AEC21 (cluster 2) and AEC19 (cluster 3) exhibited extensive MDR patterns. Among the ARGs screened, tet(A), blaTEM, blaNDM, tet(B), and blaCTX-M-1 were identified across the isolates. Mobile genetic element analysis showed that Class 1 integrons (Int1) were identified in isolates AEC7, AEC19, and AEC20, whereas a Class 2 integron (Int2) was detected only in AEC19.

3.7.2.3. Environment

Diversity analysis of environmental *E. coli* isolates (n = 15) by ERIC-PCR showed that the isolates dispersed into 3 clusters on the basis of different genetic patterns (Fig. 7). The isolates of cluster 1 and cluster 2 mostly exhibited narrow resistance or entirely susceptible profiles, with the exception of EEC13 which harboured both blaTEM and blaCTX-M-1 genes. Conversely, cluster 3 displayed high genomic and phenotypic diversity, containing the highly multidrug-resistant (MDR) isolate EEC14 (phylogroup D) which concurrently co-harboured blaTEM, blaCTX-M-1, and blaNDM. Among the antimicrobial resistance genes (ARGs) screened, blaCTX-M-1, blaTEM, blaNDM, and tet(A) were detected. No integrons were detected among any of the environmental isolates.

4. Discussion

The study reveals high prevalence of ESBL and carbapenemase genes in the *E. coli* from human animal and environmental resources with notable multidrug resistance patterns and evidence of gene exchange across these interfaces. The emergence and dissemination of antimicrobial resistant Escherichia coli

across human-animal interfaces represents a critical public health concern within the One Health framework (McInnes et al., 2020; Wieler, 2014) This investigation examined the prevalence of antibiotic-resistant *E. coli*, characterization of resistance mechanisms, and genetic exchange dynamics among cattle farm workers, livestock, and environmental reservoirs. Our findings underscore the complexity of resistance development and transmission within integrated farming ecosystems.

Our study documented an overall *E. coli* prevalence of 80% across 75 samples collected from 25 cattle farms, with significant variation across sources. Human-derived samples exhibited the highest positivity rate (96%), followed by animal samples (84%) and environmental samples (60%). This differential distribution suggests distinct epidemiological pathways and exposure levels across these interfaces (Das et al., 2020; Nugawela et al., 2022) The elevated prevalence in human samples may reflect dietary consumption of contaminated animal products, occupational exposure during animal handling, or fecal shedding from clinically in apparent colonization.

The antimicrobial resistance profiles demonstrated substantial heterogeneity across sample sources. Animal-derived isolates exhibited pronounced resistance to β-lactam antibiotics, with cefuroxime resistance observed in 52.38% of isolates, alongside notable resistance to tetracycline (33.33%) and fluoroquinolones (28.57%) (Hickman et al., 2021). Human isolates demonstrated comparatively lower resistance frequencies, though ampicillin (33.33%) and tetracycline (29.2%) resistance remained considerable. Environmental samples displayed a distinct resistance architecture, with elevated resistance to third-generation cephalosporins (33.33% for both ceftazidime and cefotaxime) and quinolones (33.33%), indicating these ecological niches serve as persistent reservoirs of clinically relevant resistance determinants (Malik et al., 2025). The observed resistance hierarchy with animal isolates demonstrating the highest burden of resistance, followed by environmental and human isolates —reflects the selective pressure exerted by antimicrobial usage in livestock production (Wang et al., 2023).

This pattern aligns with epidemiological observations from intensively managed agricultural systems where antimicrobials are used therapeutically and, in some contexts, for growth promotion (Trinchera et al., 2025; Van Boeckel et al., 2015) The retention of carbapenem susceptibility (ertapenem and meropenem resistance ≤ 14.29% across all sources) provides some reassurance regarding the preservation of last-resort therapeutic options, though continued vigilance is warranted (Poulou et al., 2014).

Extended-spectrum β-lactamase (ESBL) production was detected in 47.62% of animal-derived isolates, compared to 33.33% in both human and environmental samples. This higher prevalence in livestock reflects increased selection pressure from broad-spectrum antimicrobial usage in veterinary practice (Brower et al., 2017; Tseng et al., 2023) ESBL-producing organisms present particular clinical challenges due to their resistance to third-generation cephalosporins, compounds frequently reserved for serious infections (Peirano&Pitout, 2019). The zoonotic potential of ESBL-producing *E. coli* is underscored by their presence in human samples, confirming interspecies transmission pathways (Chowdhury et al.,

2022; Nossair et al., 2022) While carbapenem resistance genes (blaNDM, blaIMP, blaVIM) were identified, actual phenotypic carbapenem resistance remained limited (≤ 14.29%), suggesting that gene possession does not necessarily translate to clinically significant resistance in all isolates (Zwaluw et al., 2015). However, the detection of blaNDM across all three source types—with highest prevalence in animal isolates (30%)—indicates established circulation of these critical resistance determinants (Bush & Jacoby, 2009). The absence of blaIMP and blaVIM in all screened isolates suggests these particular mechanisms have not yet become established in this cattle farming population, though their detection elsewhere necessitates continued surveillance (Liu et al., 2022).

Analysis of antimicrobial resistance genes revealed differential distribution patterns consistent with selective pressure profiles. The blaCTX-M gene, encoding extended-spectrum β-lactamases active against third-generation cephalosporins, demonstrated highest prevalence in environmental isolates (80%), followed by human (37.5%) and animal isolates (20%) (Bevan et al., 2017; Suranadee et al., 2022) This distribution pattern suggests environmental persistence and potential amplification of ESBL-encoding organisms in farm-associated water systems (Gelalcha et al., 2022). The blaTEM gene, encoding a classical β-lactamase, was detected in 60% of environmental, 37.5% of human, and 30% of animal isolates, indicating broad distribution across interfaces (Dallenne et al., 2010).

The blaSHV gene, detected exclusively in human isolates (12.5%) and absent from animal and environmental sources, suggests limited establishment in livestock production systems despite its clinical significance in nosocomial settings (Aila&Aish, 2025). The blaNDM gene distribution predominantly in animal isolates (30%) with lower frequencies in environmental (20%) and human samples (12.5%) parallels observed phenotypic carbapenem resistance patterns and indicates potential animal-to-environment-to-human transmission pathways (Weber et al., 2021).

Tetracycline resistance genes showed a distinct prevalence pattern, with tetA detected in 40% of animal, 25% of human, and 20% of environmental isolates. The higher prevalence in livestock correlates with widespread tetracycline usage in veterinary medicine for therapeutic and growth-promoting purposes (Msemakweli et al., 2024; Pires et al., 2024) The tetB gene, encoding an alternative tetracycline efflux mechanism, appeared less frequently and was entirely absent from environmental samples. The disparity between tetA and tetB prevalence suggests selective advantages for specific resistance mechanisms within distinct ecological contexts (Ahmed et al., 2020).

Correlation analysis demonstrated strong associations between specific resistance genes and phenotypic profiles. In animal isolates, blaCTX-M-1 showed exceptionally strong positive correlations ($r \approx 0.8–0.9$) with resistance to cefotaxime, ceftriaxone, and ceftazidime, confirming the functional significance of this enzyme in β-lactam resistance (Koster et al., 2021; Magiorakos et al., 2011) Similar patterns were observed in human isolates ($r \approx 0.8–0.9$), indicating mechanistic consistency across host populations (Sonola et al., 2021). Environmental isolates exhibited comparatively weaker correlations ($r \approx 0.6–0.7$), potentially reflecting lower expression levels, fitness costs associated with resistance maintenance, or environmental conditions unfavorable for resistance selection (Klaas et al.,2025).

The blaNDMcarbapenemase gene demonstrated strong correlation with ertapenem resistance in animal isolates (r ≈ 0.8–0.9), confirming its enzymatic activity within these organisms (Waade et al., 2021). Tetracycline resistance genes (tetA and tetB) showed consistently high correlations with tetracycline phenotypes across all sources (r ≈ 0.7–0.9), indicating reliable genotype-phenotype predictions for these determinants (Arabzadeh et al., 2022; Gāliņa et al., 2021)

Notably, blaSHV displayed occasional negative correlations (r ≈ − 0.4 to − 0.5) with certain cephalosporins, suggesting epistatic interactions or context-dependent expression (Mutua et al., 2020). The presence of multiple resistance genes within individual isolates indicates horizontal gene transfer and co-selection mechanisms (McArthur et al., 2013). These complex resistance profiles substantially constrain therapeutic options and represent formidable challenges to antimicrobial stewardship (O'Neill, 2016).

ERIC-PCR analysis revealed substantial clonal diversity within each sample source category (Versalovic et al., 1991). Human isolates segregated into six major clusters with independent lineages, animal isolates formed six clusters, and environmental isolates organized into three clusters. This clonal heterogeneity suggests multiple independent acquisition events of resistance determinants rather than single-clone outbreaks (Khakabimamaghani et al., 2013).

Phylogroup analysis identified phylogroup B1 as predominant in human and animal isolates, with phylogroup A dominant in environmental samples (Hossain et al., 2019; Goldstein et al., 2001). This phylogroup distribution reflects previously characterized patterns wherein commensal phylogroup B1 strains commonly acquire and harbor resistance genes (Skurnik et al 2016). The detection of class 1 integrons (intI1) among human and animal isolates highlights the potential role of these mobile genetic elements in the capture, accumulation, and expression of multiple antimicrobial-resistance gene cassettes, thereby facilitating multidrug resistance (Partridge et al., 2018). The identification of class 2 integron (intI2) in select animal isolates suggests broader diversity of mobilization mechanisms, though class 1 integrons predominated.

The absence of integrons in environmental isolates, despite substantial ARG prevalence, suggests either different mobilization mechanisms (plasmids, transposons) or reduced environmental persistence of integron-carrying organisms. This distinction warrants further investigation through plasmid characterization studies.

The similarity of resistance patterns across human, animal, and environmental samples provides circumstantial evidence for inter-interface transmission (Black et al., 2005). Specifically, the shared phylogroups, overlapping resistance gene profiles, and comparable resistance frequencies suggest bidirectional or multidirectional exchange of resistant organisms or genetic determinants (Zhao et al., 2024). Human-derived isolates exhibiting resistance genes characteristic of animal reservoirs support food chain transmission or occupational exposure pathways. Conversely, the presence of ESBL-producing *E. coli* in environmental samples suggests potential retrograde movement from human-animal interfaces into water systems, with subsequent exposure risks to agricultural communities.

Our findings substantiate concerns regarding cattle farming as a reservoir and potential amplifier of antimicrobial resistance (Montso et al., 2019). The high prevalence of ESBL-producing and multidrug-resistant *E. coli* in animal and environmental samples, coupled with detection in human samples, indicates established circulation of clinically problematic organisms (Schmiege et al., 2021). The presence of carbapenem resistance genes, even with limited phenotypic expression, represents an emerging threat requiring preemptive surveillance and mitigation strategies (Levy & Marshall, 2004).

The concentration of resistance genes in phylogroup B1 isolates is particularly concerning, as these commensal strains commonly colonize human gastrointestinal tracts and can serve as gene reservoirs for pathogenic *E. coli* variants or facilitate horizontal transfer to other *Enterobacteriaceae* species (Poirel et al., 2018) The cross-sectional design precludes assessment of temporal dynamics or seasonal variation in resistance frequencies. Longitudinal studies incorporating temporal sampling would elucidate acquisition and transmission dynamics. Thus, educational interventions targeting farm workers regarding hygiene practices, appropriate antimicrobial use in veterinary medicine, and biosecurity measures represent priority interventions (Kouam et al., 2018). Further, current investigation employed culture-based isolation methodology, which may underestimate environmental or phenotypically diverse populations not readily culturable under laboratory conditions (Holmes et al., 2015).

Declarations

Conflict of interests:

The authors declare that they have no competing interests.

Ethical statement:

Samples were collected with the consent of animal owners and followed IAEC guidelines.

Funding:

This work received financial support from the institutional support for the doctoral research.

Author Contribution

S.U and T.R conducted main trial of studiesV.V and H.G were involved in support in laboratory, data compilation and processingV. J and A. R. conceptualize the work and drafted work outlineR.K and A.K provided overall supervision and mentorshipS.C and A.K written the manuscript

Acknowledgement

Authors acknowledge Vice chancellor and Director Research of Sardar Vallabhbhai Patel University for all support and facilities provided during study period.

Data availability statement:

Data set generated during research will be available from the corresponding author on reasonable request.

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Figures

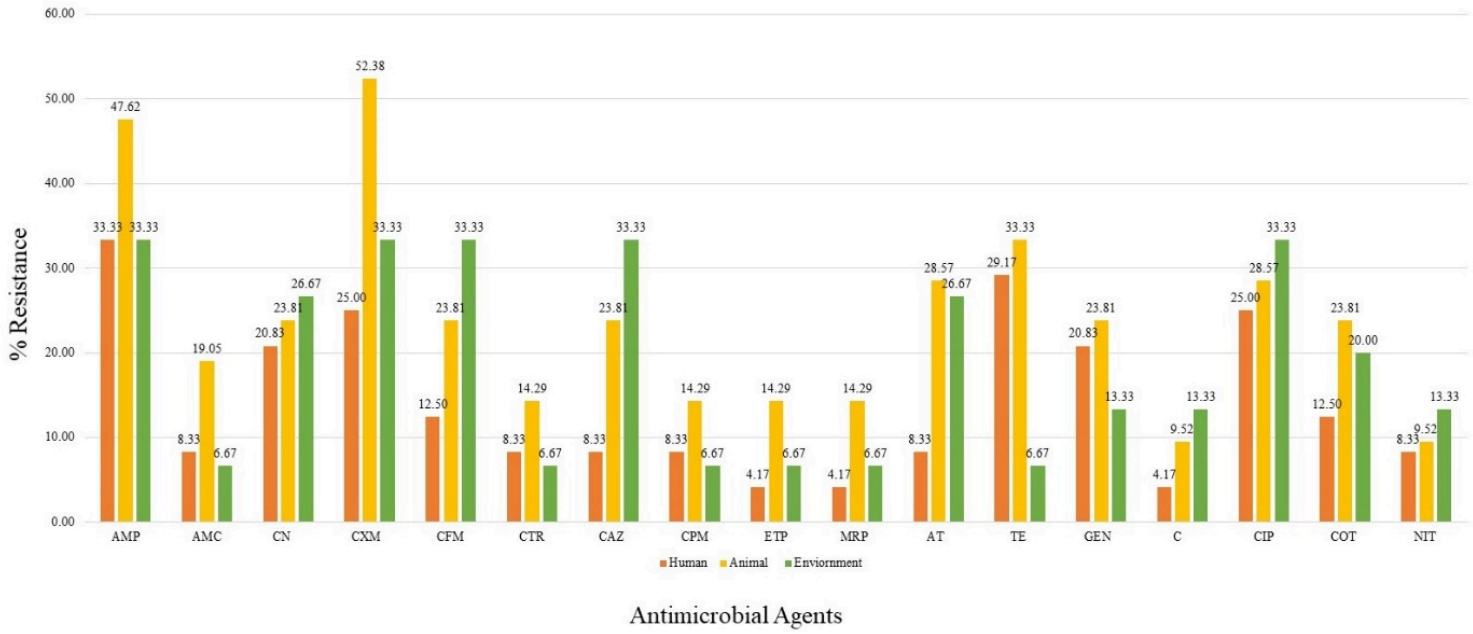


Figure 1

Antimicrobial resistance profile of *E. coli* isolates.

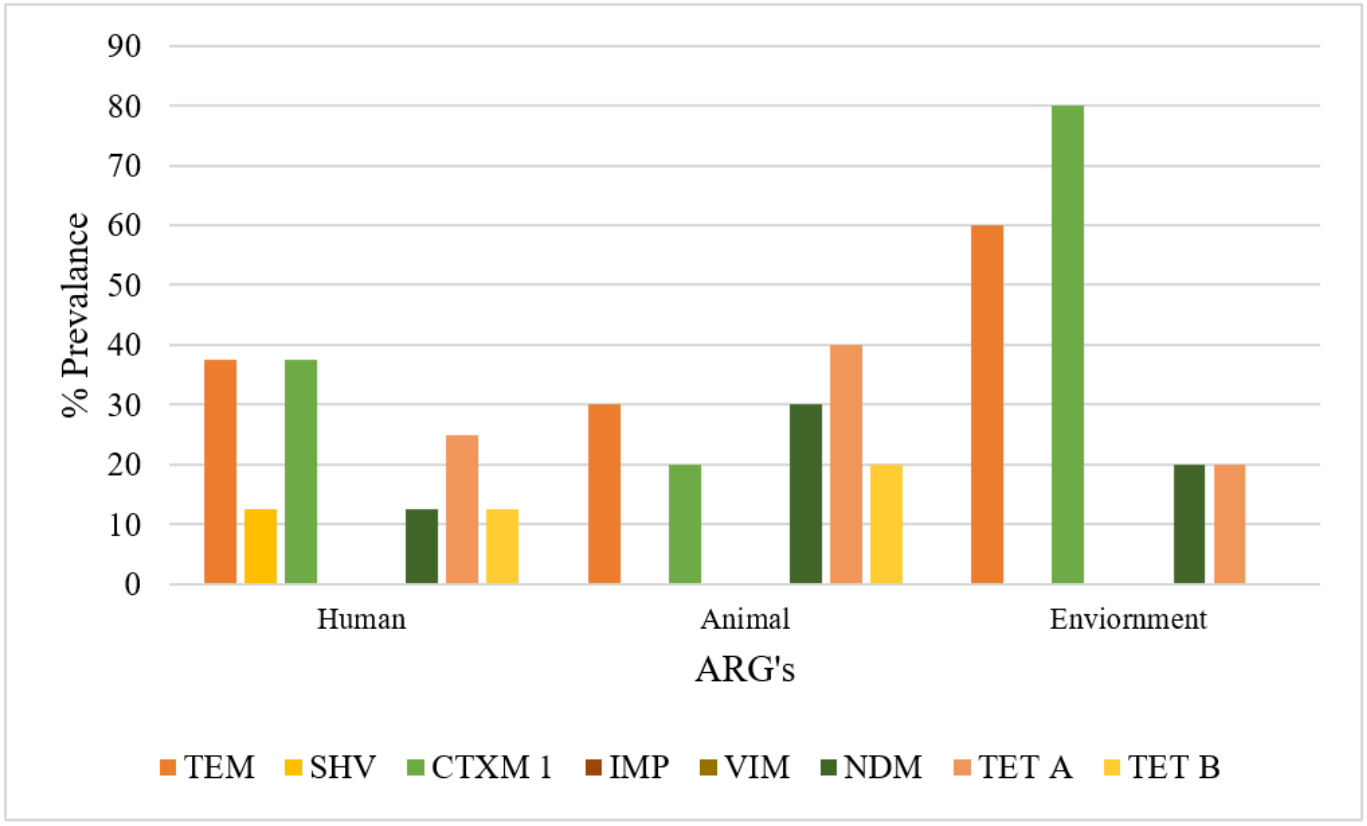


Figure 2

Antimicrobial Resistance Genes across different milieus.

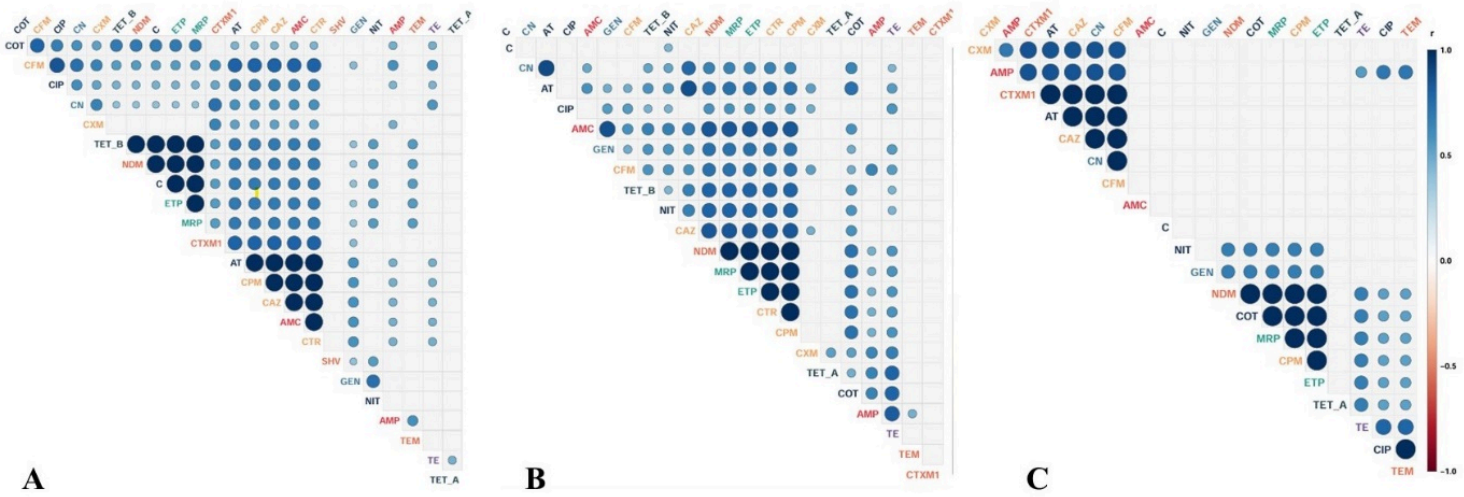


Figure 3

Correlation matrix illustrating the significant correlation (phi coefficient) between antimicrobial resistance phenotypes (AMP, Ampicillin;AMC, Amoxyclav; CN, Cefalexin;CXM, Cefuroxime;CFM, Cefixime;CTR, Ceftriaxone;CAZ, Ceftazidime;CPM, Cefepime;ETP, Ertapenem;MRP, Meropenem;AT,

Aztreonam;TE, Tetracycline;GEN, Gentamycin;C, Chloramphenicol;CI, Ciprofloxacin;COT, Co-trimoxazole and NIT, Nitrofurantoin), antimicrobial resistance genes (ARGs). Blue and the red circles show significant ($p < 0.05$) positive and negative correlations, respectively. The intensity of color and the size of the circle represent the strength of the phi correlation coefficient. White squares show a non-significant correlation.

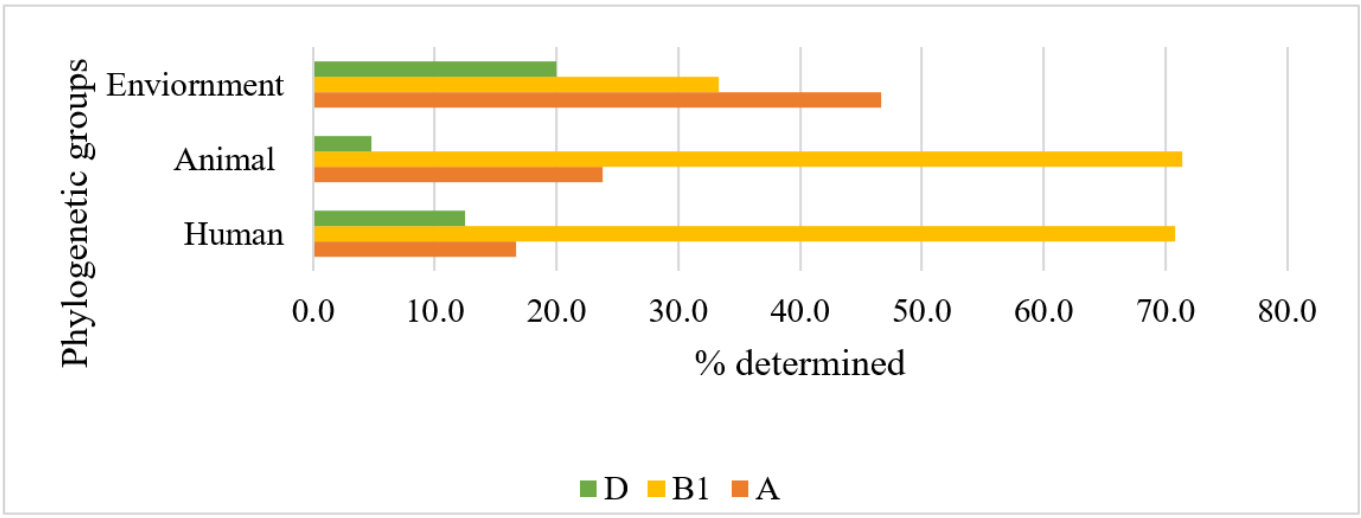
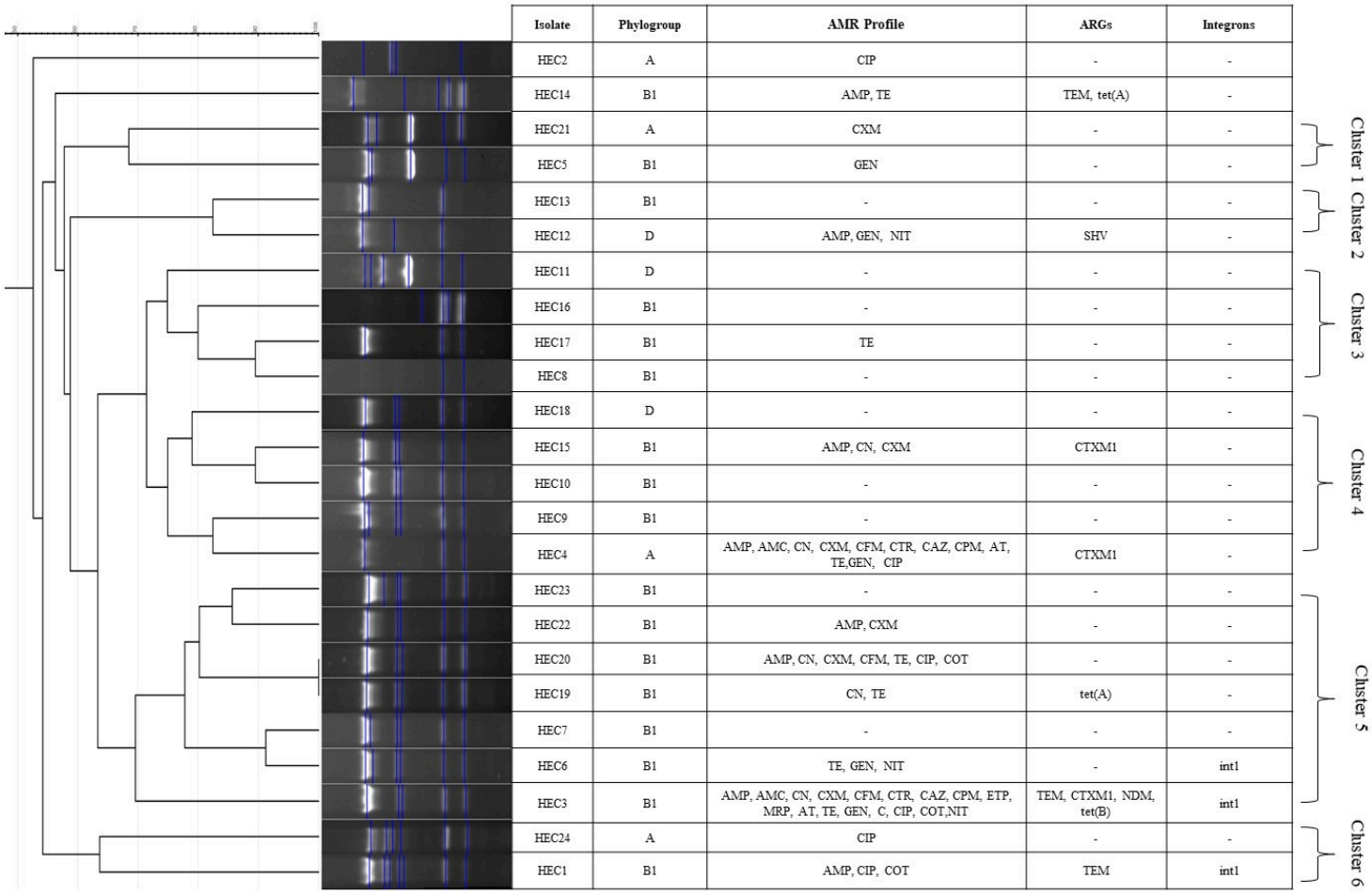


Figure 4

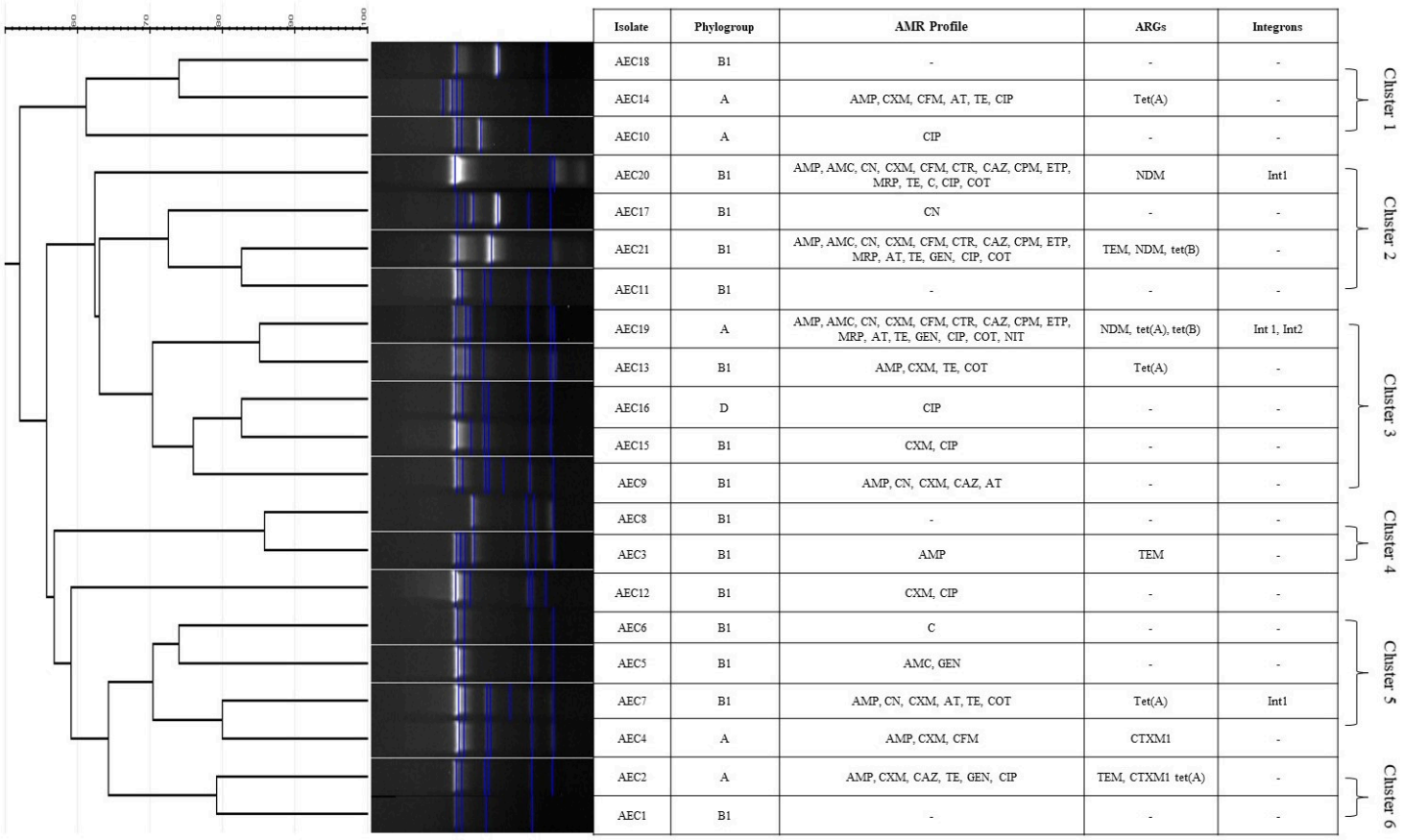
Percentage of different phylogenetic groups determined across different groups of *E. coli* isolates.



Isolate	Phylogroup	AMR Profile	ARGs	Integrons	
HEC2	A	CIP	-	-	
HEC14	B1	AMP, TE	TEM, tet(A)	-	
HEC21	A	CXM	-	-	Cluster 1
HEC5	B1	GEN	-	-	
HEC13	B1	-	-	-	Cluster 2
HEC12	D	AMP, GEN, NIT	SHV	-	
HEC11	D	-	-	-	Cluster 3
HEC16	B1	-	-	-	
HEC17	B1	TE	-	-	
HEC8	B1	-	-	-	
HEC18	D	-	-	-	Cluster 4
HEC15	B1	AMP, CN, CXM	CTXM1	-	
HEC10	B1	-	-	-	
HEC9	B1	-	-	-	
HEC4	A	AMP, AMC, CN, CXM, CFM, CTR, CAZ, CPM, AT, TE,GEN, CIP	CTXM1	-	
HEC23	B1	-	-	-	Cluster 5
HEC22	B1	AMP, CXM	-	-	
HEC20	B1	AMP, CN, CXM, CFM, TE, CIP, COT	-	-	
HEC19	B1	CN, TE	tet(A)	-	
HEC7	B1	-	-	-	
HEC6	B1	TE, GEN, NIT	-	int1	
HEC3	B1	AMP, AMC, CN, CXM, CFM, CTR, CAZ, CPM, ETP, MRP, AT, TE, GEN, C, CIP, COT,NIT	TEM, CTXM1, NDM, tet(B)	int1	
HEC24	A	CIP	-	-	Cluster 6
HEC1	B1	AMP, CIP, COT	TEM	int1	

Figure 5

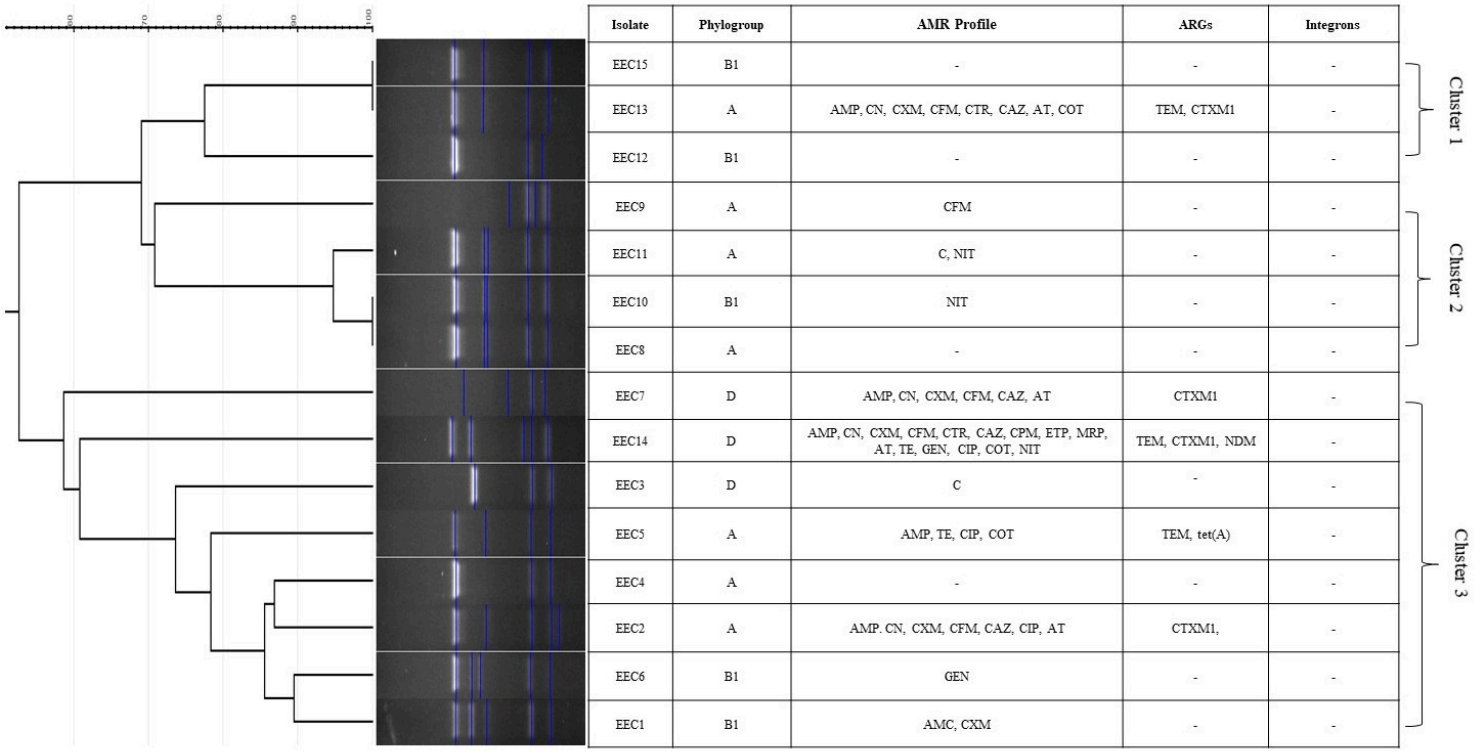
Dendrogram of ERIC-PCR banding pattern representing the genetic relationship among the carbapenem resistant *E. coli* along with their phenotypic, genotypic characteristics and antimicrobial agents in human isolates.



Isolate	Phylogroup	AMR Profile	ARGs	Integrons	
AEC18	B1	-	-	-	
AEC14	A	AMP, CXM, CFM, AT, TE, CIP	Tet(A)	-	Cluster 1
AEC10	A	CIP	-	-	
AEC20	B1	AMP, AMC, CN, CXM, CFM, CTR, CAZ, CPM, ETP, MRP, TE, C, CIP, COT	NDM	Int1	
AEC17	B1	CN	-	-	
AEC21	B1	AMP, AMC, CN, CXM, CFM, CTR, CAZ, CPM, ETP, MRP, AT, TE, GEN, CIP, COT	TEM, NDM, tet(B)	-	Cluster 2
AEC11	B1	-	-	-	
AEC19	A	AMP, AMC, CN, CXM, CFM, CTR, CAZ, CPM, ETP, MRP, AT, TE, GEN, CIP, COT, NIT	NDM, tet(A), tet(B)	Int 1, Int2	
AEC13	B1	AMP, CXM, TE, COT	Tet(A)	-	
AEC16	D	CIP	-	-	Cluster 3
AEC15	B1	CXM, CIP	-	-	
AEC9	B1	AMP, CN, CXM, CAZ, AT	-	-	
AEC8	B1	-	-	-	Cluster 4
AEC3	B1	AMP	TEM	-	
AEC12	B1	CXM, CIP	-	-	
AEC6	B1	C	-	-	
AEC5	B1	AMC, GEN	-	-	Cluster 5
AEC7	B1	AMP, CN, CXM, AT, TE, COT	Tet(A)	Int1	
AEC4	A	AMP, CXM, CFM	CTXM1	-	
AEC2	A	AMP, CXM, CAZ, TE, GEN, CIP	TEM, CTXM1 tet(A)	-	Cluster 6
AEC1	B1	-	-	-	

Figure 6

Dendrogram of ERIC-PCR banding pattern representing the genetic relationship among the carbapenem resistant *E. coli* along with their phenotypic, genotypic characteristics and antimicrobial agents in animal isolates.



Isolate	Phylogroup	AMR Profile	ARGs	Integrons	
EEC15	B1	-	-	-	Cluster 1
EEC13	A	AMP, CN, CXM, CFM, CTR, CAZ, AT, COT	TEM, CTXM1	-	
EEC12	B1	-	-	-	
EEC9	A	CFM	-	-	Cluster 2
EEC11	A	C, NIT	-	-	
EEC10	B1	NIT	-	-	
EEC8	A	-	-	-	
EEC7	D	AMP, CN, CXM, CFM, CAZ, AT	CTXM1	-	Cluster 3
EEC14	D	AMP, CN, CXM, CFM, CTR, CAZ, CPM, ETP, MRP, AT, TE, GEN, CIP, COT, NIT	TEM, CTXM1, NDM	-	
EEC3	D	C	-	-	
EEC5	A	AMP, TE, CIP, COT	TEM, tet(A)	-	
EEC4	A	-	-	-	
EEC2	A	AMP. CN, CXM, CFM, CAZ, CIP, AT	CTXM1,	-	
EEC6	B1	GEN	-	-	
EEC1	B1	AMC, CXM	-	-	

Figure 7

Dendrogram of ERIC-PCR banding pattern representing the genetic relationship among the carbapenem resistant *E. coli* along with their phenotypic, genotypic characteristics and antimicrobial agents in environment isolates.