Research Article

Extended Spectrum Beta-Lactamase and AmpC Producing Enterobacteriaceae in Broiler Chicken and Goat in Palghar district, Maharashtra

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ABSTRACT

Background: The increasing global demand for food and animal products has led to the unregulated use of antimicrobials in agriculture, leading to the emergence of extended-spectrum beta-lactamases (ESBLs) in Enterobacteriaceae, which are often multidrug- resistant and pose significant health risks.

Aims/ objective: To assess the prevalence of ESBL and AmpC- producing Enterobacteriaceae in chicken and goat dropping, meat and ceacum sources.

Methods: Sixty chicken and goat meat samples were collected from three poultry shops in Talasari, India, between October 2023 and April 2024. Enterobacteriaceae isolates were identified, tested for resistance to cephalosporins, and assessed for antimicrobial susceptibility.

Results: Of the 60 samples, 76% contained Enterobacteriaceae, including *Escherichia coli*, *Klebsiella* spp., and Proteus spp., whereas 23% contained other bacteria. Among the Enterobacteriaceae isolates, 63.04% were monomicrobial, and 26.33% were polymicrobial, often involving *E. coli* with *Klebsiella* spp. or Proteus spp. *E. coli* showed high resistance to ampicillin (53%), imipenem (53%), meropenem (46%), aztreonam (46%), and cefotaxime (46%). Klebsiella spp. had notable resistance to ampicillin (53%) and cefepime (38%). Among the 46 *E. coli* isolates, 17% were ESBL producers, and 13% were AmpC producers, with DDST detecting 57% and the E test detecting 87% of the ESBL isolates.

Conclusion: This study revealed that *E. coli* from broiler farms is associated with increased rates of antibiotic resistance in Enterobacteriaceae-producing Enterobacteriaceae. The regular detection, monitoring, and rotation of antimicrobial drugs are recommended to reduce this risk. The emergence of colistin resistance in broiler chickens is alarming.

Keywords: Enterobacteriaceae, *E. coli*, ESBL, AmpC, Colistin, Broiler chicken.

INTRODUCTION

Antimicrobial drug resistance is a growing threat to human medicine, with a significant increase in environmental resistance, particularly among enterobacteria such as *Escherichia coli*, which produce extended-spectrum beta-lactamases (ESBLs).[1] ESBLs are enzymes that degrade most beta-lactam antibiotics, although they are inhibited by clavulanic acid. These resistance genes are often found on plasmids, which are transferable between bacteria, facilitating the spread of resistance traits.[2]

Chromosomal AmpC genes are present in many enterobacteria, but plasmid-bound versions can spread antimicrobial resistance among bacteria. These plasmid-bound types, though not always pathogenic, can cause opportunistic infections, particularly with ESBL-producing *E. coli*, which are linked to urinary and systemic infections.[3] *E. coli* infections can be nosocomial, community-acquired, or foodborne, with CTX-M ESBLs becoming more common over the past decade than TEM and SHV types. Antimicrobial resistance, driven by ESBLs and plasmid-encoded cephamycinases (pAmpC), is a major concern in both human and veterinary medicine, with CTX-M being the most widespread and increasing ESBL type.[4]

ESBLs confer resistance to most beta-lactam antibiotics, including third- and fourth-generation cephalosporins, severely limiting treatment options. This resistance often extends to last-resort antibiotics such as carbapenems. Initially, common in hospital settings, ESBL-producing *Klebsiella pneumoniae* infections now also frequently occur in the community, particularly as urinary tract infections caused by *Escherichia coli*.[5] ESBL-producing strains are often multidrug resistant, complicating further treatment. These bacteria, which are found in both humans and food animals, serve as reservoirs of resistance genes, with the food chain and direct livestock contact being significant pathways for the spread of resistance.[6]

The increase in ESBL and AmpC - producing Enterobacteriaceae in food-producing animals poses a risk of community infections, where standard antibiotics may fail.[7] The emergence of plasmid-encoded colistin resistance (mcr-1 gene) further threatens treatment options, as colistin is a last-resort antibiotic. *Salmonella enterica* serovar Infantis, which is common in broilers and

their meat, is increasingly linked to multidrug-resistant (MDR) strains, complicating infection control and treatment.[8] The present study aimed to assess the prevalence of ESBL and AmpC producing Enterobacteriaceae in chicken and goat dropping, meat and ceacum sources.

MATERIALS AND METHODS

This study was conducted after obtaining ethical approval from the institutional review board of Vedantaa Institute of Medical Sciences, Dahanu, Maharashtra, India.

Sample collection

In the present study, a total of 60 samples from the droppings, meats and caeca of chickens and goats were collected from 3 retail poultry meat shops in Talasari, Palghar district, Maharashtra**.**

Sample preparation

All samples were routinely cultured on MacConkey and blood agar plates. These plates were routinely incubated at 37°C aerobically, and after overnight incubation, they were checked for bacterial growth.

Species identification

Suspected gram-negative organisms were identified by colony characteristics, staining characteristics, pigment production, motility, oxidase reactions, citrate utilization, indole and gas production and sugar fermentation reactions. Triple sugar iron agar was used for H_2S production and sugar fermentation**.**

DNA extraction

The bacterial colonies were cultured in nutrient broth for 24 hours at 37°C with occasional shaking to ensure proper aeration. Next, a microcentrifuge spine at 1000 rpm for 5 minutes was used to pellet the cells and remove debris. The cell suspension was then transferred to a new tube and centrifuged at 4000 rpm for 10 minutes to further concentrate the bacterial cells. To isolate genomic DNA, the cell pellet was re-suspended in 50 µL of water and heated to 102–110°C for 15 minutes, which lysed the cells and released the DNA. After boiling, the sample was quickly cooled on ice and centrifuged at 5000 rpm for 1 min to separate the DNA from the cellular debris. The supernatant containing the DNA was then mixed with cold 95% ethanol and stored at -20°C until polymerase chain reaction (PCR) analysis was performed.

Polymerase chain reaction (PCR) assay

The PCR mixture was prepared in a 0.2-ml tube and contained 50 µL of distilled water, 1x PCR buffer, MgCl2, Taq polymerase, dNTPs, and primers (as specified in Table 1). A 20 µL volume of this PCR mixture was created, and 10 µL of DNA template was added. This mixture was then placed in the Insta Q96 (Himedia) thermal cycler for amplification. The thermal cycling process included initial denaturation at 92°C for 30 seconds, followed by a denaturation step at 92°C for 10 seconds, annealing at 56°C for 10 seconds, and extension at 72°C for 10 seconds.

Table 1: Primers and probes used for the detection of target genes of extended-spectrum beta-lactamase- and AmpC-producing Enterobacteriaceae. [9]

Organism	Target		Oligonucleotides (5'-3')
E. coli	RfaH	F	TACGCCCGCCGTTGAC
	RfaH	R	AGCCAGCAGGCGCAAA
	RfaH	CY5	AACAGGACGAATACTGACGCGCCA
Klebsiella spp.	CopG	F	CGAAGAAGACGGCATGGAAT
	CopG	R	CGCAGATCCGGAGGTCATTA
	CopG	YY	TCAACGTCAGCTACGCAAGGAGTG
	Pgi	F3	GAAGGTGAAGATGTTCATAATCACG
	Pgi	R2	CGTGAAATCACGCCGTTCAG
	Pgi	R3	GCGTGAAATTAAGCCGTTCAG
	Pgi	YY	CATACAGGGCAATCAGCGCGCC
P. mirabilis	Hns	F	GCACGTTTAGCACGACCAGTT
	Hns	R	TGCCGATGGTATTGATCCAA
	Hns	FAM	CGCCAGCAGCTTCAAGCAGGTCA

Maintenance and preservation of culture strains

Organisms grown in appropriate media for 18 hours were preserved on a nutrient agar slant at 2–8°C in a refrigerator, and this culture was used within two weeks for routine laboratory work. For long-term preservation, strains were stored in brain heart infusion broth with 20% glycerol and stored frozen without significant loss of viability at -20°C until further study**.**

Antimicrobial susceptibility testing

The isolates were screened for ESBL production via disc diffusion of cefotaxime (CTX), ceftazidime (CAZ),

ceftriaxone (CTR) and Aztreonam (AT), which were placed on inoculated plates containing Muller–Hinton agar according to the CLSI recommendations. Isolates showing an inhibition zone size of ≥22 mm with ceftazidime (30 µg), ≥25 mm with ceftriaxone (30 µg), ≥ 27 mm with cefotaxime (30 µg), and ≥ 27 mm with aztreonam (30 µg) were suspected for ESBL production.[10]

Double disk diffusion method (DDDT)

In this test, a disc of ceftazidime (30 µg), cefotaxime (30 µg) alone and a disc of ceftazidime and cefotaxime in combination with clavulanic acid (30/10 µg) were used for each isolate. Both discs were placed 25 mm apart centered on a lawn culture of the test isolate on a Muller Hinton agar plate and incubated overnight at 37°C. A ≥5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was designated ESBL positive. Double disc diffusion test. When there was an increase of ≥ 5 mm in the inhibition zone diameter around the combination disk of ceftazidime + clavulanic acid versus the inhibition zone diameter around the ceftazidime disk alone, ESBL production was confirmed.

ESBL E-Test

The E-test ESBL strips were obtained from Liofilchem (Italy) and included: E-test strips containing cefotaxime (MIC test range, 0. 25–16 µM) at one end with cefotaxime plus clavulanic acid (MIC test range, 0. 016–1 µg/L) at the other end and another strip containing cefepime (MIC test range, 0. 25–16 µg/L) and cefepime plus clavulanic acid (MIC test range, 0. 064–4 µg/L) at the other end was used. A reduction in the MIC of cefotaxime or cefepime by three doubling dilutions in the presence of clavulanic acid (i.e., an MIC ratio of ≥8) was interpreted as confirmation of ESBL production. Deformation of ellipses or the presence of keyhole zones 'phantom' were also considered indications of ESBL production even if the MIC ratio was <8 or could not be read.

RESULTS

Enterobacteria Isolates

Among the 60 samples, 46 (76%) contained Enterobactriceae, and 14 (23%) contained Enterobactriceae. Among all the organism isolates, the most common isolate was *Escherichia coli* 46 (73.01%), followed by Klebsiella spp. 10 (20.63%) and Proteus spp. 4 (6.3%). The common monomicrobial isolate was *Escherichia coli* 29 (63.4%). The polymicrobial growth of the 17 samples was most common in combination with *Escherichia coli* and Klebsiella spp. 13 (21.66%), followed by *Preoteus* spp. and *Escherichia coli* 4 (6.66%) (Figure 1).

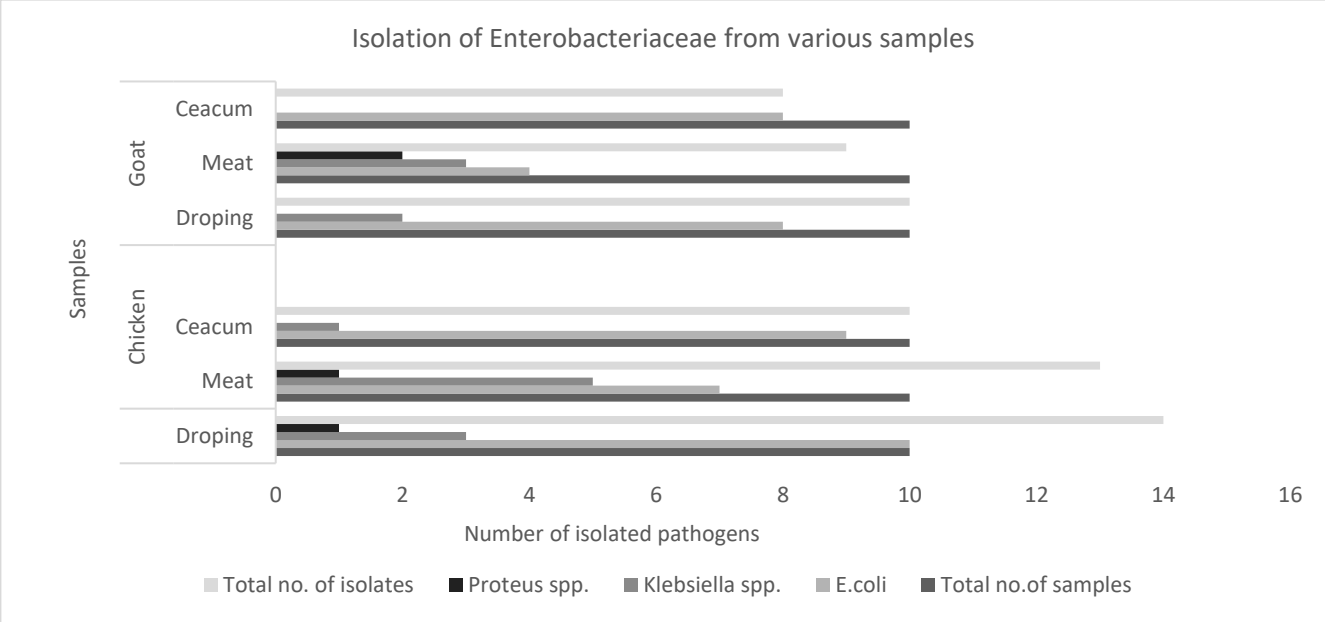

Figure 1: Isolation of pathogens from dropping, meat and ceacum samples of goats and chickens

Monomicrobial and polymicrobial isolation

Among all the monomicrobial isolates, the most common isolate was *E. coli* (29, 63.04%).

Escherichia coli was the predominant pathogen identified in polymicrobial infections, accounting for 29 cases. This prevalence underscores the significant role of *E. coli* as a common etiological agent in the studied infections. In these cases, *Escherichia coli* was present as one of the pathogens, suggesting that it is a frequent contributor to polymicrobial infections. The high number of infections involving *E. coli* may reflect its importance in both hospital and community-acquired infections, particularly in settings such as urinary tract infections and gastrointestinal diseases.

The combination of *Escherichia coli* and Klebsiella spp. was identified in 13 patients, indicating that these two pathogens frequently co-occur in polymicrobial infections. This combination may be influenced by shared environmental factors or similar pathogenic mechanisms that facilitate their concurrent presence. The prevalence of

this pairing suggests that infections involving both *E. coli* and *Klebsiella* spp. may require targeted antibiotic therapy to address the specific challenges posed by both organisms. **Proteus** species and ***Escherichia coli*** were found together in 4 patients with polymicrobial infections. Although less frequent than the other combinations, this pairing is nonetheless significant. Proteus species, such as *Proteus mirabilis*, are known to be involved in urinary tract infections, and their co-occurrence with *E. coli* might indicate a complex infection scenario that necessitates a broader approach to treatment.

Antibiotic resistance pattern

Figure 2 shows that ampicillin has a high resistance rate of 53% for both *Escherichia coli* and *Klebsiella spp.*, indicating that these bacteria are significantly resistant to this beta-lactam antibiotic. This resistance is consistent with the known ability of these organisms to produce beta-lactamases, which can hydrolyze ampicillin and render it ineffective. *Proteus spp.*, however, do not have a reported resistance rate for ampicillin in this dataset. **Colistin,** a last-resort antibiotic for multidrug-resistant gram-negative bacteria, has a low resistance rate of 8% in *Escherichia coli* and complete susceptibility in *Klebsiella spp.* These findings suggest that colistin remains largely effective against *Escherichia coli* and is a viable treatment option, whereas *Klebsiella spp.* maintain high susceptibility, which is favorable for therapeutic outcomes. The absence of data for *Proteus spp.* in this context may be due to variability in resistance patterns or lack of testing. **Cefoxitin** resistance is observed in 13% of *Escherichia coli* strains, whereas *Klebsiella spp.* and *Proteus spp.* show no resistance. Cefoxitin is a cephamycin antibiotic that targets gram-negative bacteria, and the observed resistance in *Escherichia coli* may be attributed to the production of cephalosporinases that hydrolyze the antibiotic.

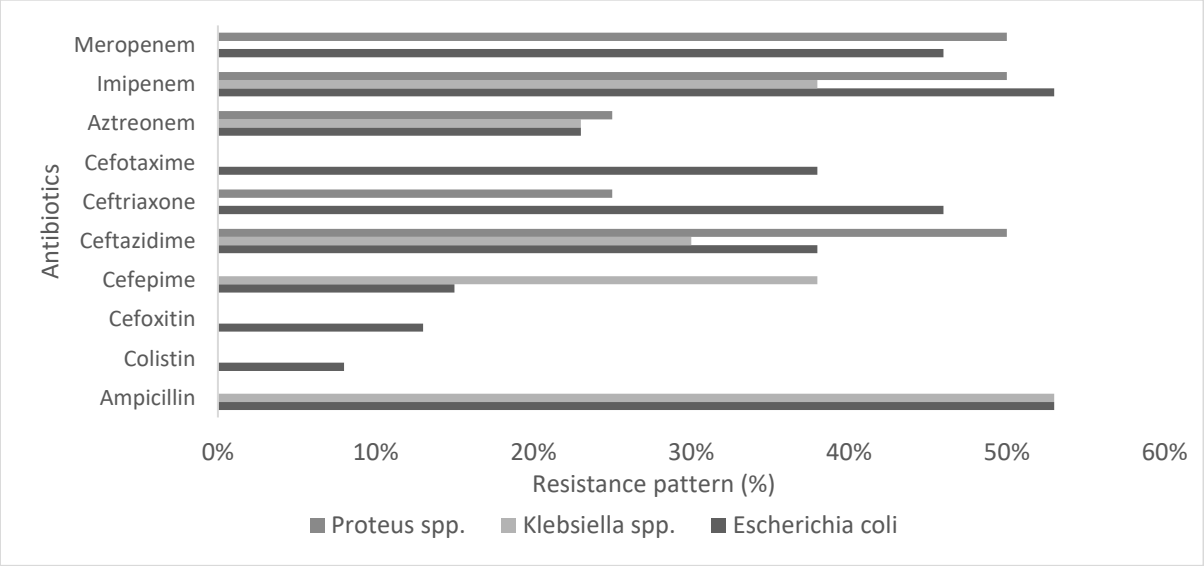

Figure 2: The antibiotic resistance rates of *Escherichia coli*, *Klebsiella* spp. and Proteus spp. isolates from dropping, meat and ceacum samples from goats and chickens.

Cefepime, a fourth-generation cephalosporin, has varying resistance rates, with 15% in *Escherichia coli*, 38% in *Klebsiella spp.*, and no resistance in *Proteus spp.* The relatively high resistance rate in *Klebsiella spp.* suggests that this species may possess extended-spectrum beta-lactamases (ESBLs) that compromise the efficacy of cefepime. **Ceftazidime,** another cephalosporin, has a higher resistance rate in *Escherichia coli* (38%) than in *Klebsiella spp.* (30%) and a notably high resistance rate of 50% in *Proteus spp.* The elevated resistance in *Proteus spp.* could be due to the production of beta-lactamases or altered permeability, impacting the effectiveness of the drug. **Ceftriaxone** has a resistance rate of 46% in *Escherichia coli* and 25% in *Proteus spp.*, with *Klebsiella spp.* showing no resistance. These data indicate a significant level of resistance in *Escherichia coli*, potentially due to similar mechanisms affecting ceftazidime. **Cefotaxime** has a resistance rate of 38% in *Escherichia coli* but not in *Klebsiella spp.* or *Proteus spp.* The observed resistance in *Escherichia coli* highlights the presence of beta-lactamases that can inactivate cefotaxime. **Aztreonam,** a monobactam, has a resistance rate of 23% in both *Escherichia coli* and *Klebsiella spp.* and 25% in *Proteus spp.* This moderate level of resistance across all three species indicates the presence of mechanisms that can inactivate or prevent the action of aztreonam, although it remains a potential option for treatment. **Imipenem,** a carbapenem antibiotic, has a high resistance rate of 53% in *Escherichia coli*, 38% in *Klebsiella spp.*, and 50% in *Proteus spp.* This substantial resistance across all species reflects the growing issue of carbapenem-resistant bacteria, which are often linked to the production of carbapenemases. **Meropenem,** another carbapenem, has resistance rates of 46% in *Escherichia coli*, none in *Klebsiella spp.*, and 50% in *Proteus spp.* The high resistance in *Proteus spp.* and *Escherichia coli* underscores the challenge of treating infections caused by these resistant strains, emphasizing the need for continued surveillance and alternative therapeutic strategies.

Prevalence of ESBL and AmpC producing Enterobacteriaceae in Broiler Chickens and Goats

The species analyzed included *Escherichia coli*, *Klebsiella spp.*, and *Proteus spp.*, with a focus on the occurrence of these beta-lactamase enzymes, which are critical in conferring resistance to commonly used antibiotics. ***Escherichia coli*** was the most prevalent species among the isolates, with a total of 46 samples analyzed. Among these isolates, 8 (17%) were identified as ESBL producers, indicating a significant presence of ESBLs within this species. ESBLs are known to hydrolyze a broad spectrum of beta-lactam antibiotics, including penicillins and cephalosporins, which complicates treatment options for infections caused by these bacteria. Additionally, 6 isolates (13%) were AmpC beta-lactamase producers. AmpC enzymes are typically chromosomally encoded and can hydrolyze cephalosporins and, in some cases, penicillins. The presence of both ESBL and AmpC producers in *E. coli* underscores the dual resistance mechanisms that may be involved in this species, contributing to its ability to resist multiple classes of beta-lactam antibiotics. In contrast, *Klebsiella spp.*, with a total of 13 isolates, did not produce any ESBL or AmpC beta-lactamases. This absence of ESBL and AmpC enzymes in the analyzed *Klebsiella spp.* isolates suggests that, in this dataset, these strains do not contribute to the beta-lactam resistance observed in other species. Importantly, however, *Klebsiella spp.* are known to harbor ESBLs and other resistance mechanisms in different contexts, so this finding may be specific to the current dataset or location.

Similarly, 4 *Proteus spp.* isolates did not produce ESBLs or AmpC beta-lactamases. This lack of ESBL and AmpC production in *Proteus spp.* isolates suggests a lower prevalence of these resistance mechanisms within this particular set of samples. However, as with *Klebsiella spp.*, the absence of these enzymes in this dataset does not preclude the possibility of their presence in other environments or populations.

DISCUSSION

The prevalence of Enterobacteriaceae bacteria has been examined across different studies to understand trends and variations. Zhao et al. (2001) reported a high prevalence of 85.71%, indicating a significant dominance of Enterobacteriaceae in their samples. This finding contrasts with that of Chavhan et al. (2004), who reported a lower prevalence of 57.10%. The present study (2024) reported a prevalence of 76.40%, which falls between the values reported by Zhao et al. and Chavhan et al. This variation highlights differences in bacterial prevalence that could be attributed to factors such as geographical location, study design, or sample size (Table 2).

Table 2: Extended Spectrum Beta-Lactamase and AmpC Producing Enterobacteriaceae in Broiler Chicken and Goat comparison to other studies.

Study	Reference	Results	
Comparison of prevalence of Enterobacteriaceae bacteria with other studies.	Zhao et al. (2001)[11]	85.71%	
	Chavhan et al. (2004)[12]	57.10%	
	Present study (2024)	76.40%	
		Enterobacteriaceae	Other than Enterobacteriaceae
Comparison of prevalence of bacteria other than Enterobacteriaceae with	Muhammad et al. (2016)[13]	77.60%	22.30%
	Present study	76.40%	23.50%
Enterobactriaceae Isolates in Different studies	Muhammad et al. (2016)[13]	E.coli followed by Salmonella and Klebsiella	
	Present Study	E.coli followed by Klebsiella and Proteus	
		Monomicrobial Polymicrobial	Monomicrobial Polymicrobial
Monomicrobial and Polymicrobial isolation in different studies	Rasteger AR et al. (2005)[14]	75.74%	24. 52%
	Horieh et al. (2008)[15]	26.50%	73.49%
	Present Study	63.04%	26.98%
Comparision of colistin resistance E.coli with other studies	H.Hasman et al. (2017)[16]	Detected resistance by genotypic method	
	Afrah kamal Yassin (2015)[17]	Detected resistance by genotypic method	
	Present study	Detected Resistance by phenotypic method	
Comparison of ESBL detection with other studies	Sally Hassan Essawy et al. (2018)[18]	84%	
	Muhammad et al. (2016)[13]	7.76%	
	Felix reich et al. (2013)[19]	41.1%	
	Present study	17.5%	
Comparison of ESBL dection by DDST with other studies	Jakobsen et al. (2020)[20]	52%	
	Pedro A. D et al. (2004)[21]	32%	

	Present study	57%
Comparison of ESBL by E Test with other studies	Sally Hassan Essawy et al. (2018)[18]	75.7%
	Pedro A. D et al. (2004)[21]	46.9%
	Present study	71.1%
Comparison of AmpC detection with other studies	Felix et al. (2013)[19]	34.1%
	Present study	13.1%

In terms of the prevalence of bacteria other than Enterobacteriaceae, Muhammad Shoaib et al. (2016) reported that 22.30% of bacteria were nonentericobacteriaceae. In contrast, the present study reported a slightly higher prevalence of 23.50% for non-Enterobacteriaceae bacteria. These findings suggest a relatively consistent proportion of non-Enterobacteriaceae bacteria across studies, although slight variations may reflect differences in study methodologies or regional bacterial flora.

The identification of specific Enterobacteriaceae isolates varied between studies. Abdul Sajid et al. (2016) identified *E. coli* as the most common isolate, followed by Salmonella and Klebsiella. In contrast, the present study identified *E. coli* as the predominant isolate, but noted Klebsiella and Proteus as the next most common. This shift in the frequency of bacterial isolates could be due to temporal changes in bacterial resistance patterns, differences in local bacterial populations, or variations in sampling methods.

Monomicrobial versus polymicrobial isolation rates provide insight into the complexity of bacterial infections. Rasteger Lari AR et al. (2005) reported that 75.74% of isolates were monomicrobial, with 24.52% being polymicrobial. Horieh et al. (2008) reported a stark

contrast, with only 26.50% monomicrobial and 73.49% polymicrobial isolates. The present study reported 63.04% monomicrobial and 26.98% polymicrobial isolates. This intermediate finding may reflect a trend toward more complex infections or differences in the infection profiles of the populations studied.

Colistin resistance in *E. coli* was assessed via different methods across studies. H. Hasman et al. (2017) and Afrah Kamal Yassin (2015) detected resistance via genotypic methods, which provide molecular evidence of resistance. Conversely, the present study detected colistin resistance via phenotypic methods, which assess resistance on the basis of observable growth patterns in the presence of colistin. This methodological difference underscores the variety in approaches to resistance detection and the potential impact on reported resistance rates.

Extended-spectrum beta-lactamase (ESBL) detection rates vary significantly among studies. Sally Hassan Essawy et al. (2018) reported a high ESBL detection rate of 84%, whereas Asghar Ali Kamboh et al. (2016) reported a much lower rate of 7.76%. Felix Reich et al. (2013) reported an intermediate rate of 41.1%. The present study revealed an ESBL detection rate of 17.5%, indicating a lower prevalence than that reported in some studies but a higher prevalence than that reported in other studies. These variations could be due to differences in testing methodologies, local bacterial resistance profiles, or study populations.

The detection of ESBLs via the Disk Diffusion Synergy Test (DDST yielded diverse results. Abdul Sajid et al. (2016) reported a detection rate of 52%, whereas Pedro A. D et al. (2004) reported a rate of 32%. The present study revealed a detection rate of 57%, which is slightly higher than that reported in previous studies. This variation highlights the sensitivity and specificity of the DDST method in different settings and suggests that testing practices can influence reported ESBL rates.

The E-Test method for detecting ESBLs also revealed varying results. Mina Asama Raman et al. (2018) reported a high detection rate of 75.7%, whereas Carina G. Ramos et al. (2004) reported a lower rate of 46.9%. The present study's rate of 71.1% is closer to the higher end of this range, indicating that the E- test is a reliable method but that results can vary on the basis of factors such as test conditions and bacterial strains.

AmpC beta-lactamase detection rates also vary. Felix Reich et al. (2013) reported a detection rate of 34.1%, whereas the present study reported a lower rate of 13.1%. This difference may reflect variations in the prevalence of AmpC-producing bacteria in different regions or populations, as well as differences in detection sensitivity and methodology.

CONCLUSION

The study revealed the presence of Extended Spectrum Beta-Lactamase and AmpC Producing Enterobacteriaceae confirmed by phenotypic and molecular identification. The prevalence of ESBL and AmpC producing Enterobacteriaceae in this study was 76.4%. This study revealed a correlation between specific farming practices and antimicrobial resistance in *E. coli*. In particular, the results of this study revealed that broiler farms were associated with a higher prevalence of resistance, of ESBL-producing Enterobacteriaceae. Infections caused by ESBL producing organisms are associated with a limited response to many antibiotics; as a result, reliable detection of ESBL production by sensitive laboratory assays in clinical microbiology laboratories is essential to guide veterinary clinicians in providing appropriate therapy. Periodic detection of ESBL isolates, monitoring their antimicrobial

susceptibility, and rotating the use of effective antimicrobial drugs are recommended to decrease the risk of a high antibiotic resistance rate. Additionally, plasmid-mediated AmpC beta-lactamases and even carbapenemes are leading to a worsening of the situation in both human and veterinary medicine. From this study, we conclude that colistin resistance emerged around Talasari, in broiler chickens, which is alarming. The results of this study indicate that antimicrobial use for growth promotion in farms led to the growth of highly resistant bacteria, potentially posing serious health risks. The presence of plasmid-mediated colistin resistance in clinical isolates is a concern, as this potent gene may spread to other susceptible bacteria resulting in pan resistant pathogens. ESBL producers should be confirmed by disk- diffusion tests, DDSTs and E- tests and molecular methods. In phenotype method, the E-test was found to be a suitable method for detecting ESBLs.

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