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Antimicrobial resistance gene carriage in *Escherichia coli* isolated from healthy sheep and goats

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Abstract

Faecal samples from small ruminants represent an important yet underexplored reservoir of antimicrobial-resistant bacteria with potential public health implications. In the present study, a total of 50 faecal samples, comprising 25 from sheep and 25 from goats, were collected for the isolation, phenotypic characterization, and molecular detection of antimicrobial resistance in *Escherichia coli*. Primary isolation was performed on Brain Heart Infusion (BHI) agar, followed by preliminary identification using Gram staining, catalase, oxidase, and potassium hydroxide (KOH) tests. Presumptive isolates were further processed on MacConkey agar and Eosin Methylene Blue (EMB) agar for selective and differential identification. Out of the 25 goat samples, 18 were positive for *E. coli*, while in sheep, 13 out of 25 samples were confirmed positive. Confirmed *E. coli* isolates were subjected to antimicrobial susceptibility testing (ABST) to assess resistance patterns. Molecular analysis was carried out using polymerase chain reaction (PCR) for the detection of key antimicrobial resistance-associated genes, including *bla*CMY, *sul1*, and the class 1 integron gene *int1*. The detection of β -lactamase genes, sulphonamide resistance determinants, and integron-associated elements highlights the circulation of genetically equipped *E. coli* strains in sheep and goats. The findings indicate that small ruminants may harbor antimicrobial-resistant *E. coli*, including strains carrying clinically relevant resistance genes, highlighting their potential role in the dissemination of resistance determinants at the animal-environment interface within a One Health context.

Keywords: *Escherichia coli*, phenotypic characterization, antimicrobial resistance, β -lactamase genes, integrons, polymerase chain reaction, one health

Introduction

Escherichia coli is a common commensal inhabitant of the gastrointestinal tract of small ruminants; however, fecal shedding from apparently healthy sheep and goats represents an important reservoir of pathogenic and antimicrobial-resistant strains with potential zoonotic significance (Moriarty *et al.*, 2011; Orden *et al.*, 2003) [5, 7]. Several studies have demonstrated that fecal *E. coli* populations in small ruminants frequently harbor virulence determinants, including Shiga toxin-producing genes, even in the absence of clinical disease, highlighting their role in silent transmission within animal populations and the environment (Ramatlal *et al.*, 2024) [8]. In addition to pathogenicity, the emergence and dissemination of antimicrobial resistance (AMR) among fecal *E. coli* isolates have become a major concern, with reports indicating high levels of resistance to commonly used antibiotics and the presence of mobile genetic elements facilitating horizontal gene transfer. The persistence and environmental survival of *E. coli* in fecal matter further enhance the risk of transmission across the animal-human-environment interface, emphasizing its importance within the One Health framework (Moriarty *et al.*, 2011) [5]. Therefore, systematic detection and characterization of *E. coli* from fecal samples of small ruminants, along with assessment of their antimicrobial resistance profiles, are essential for understanding their epidemiological role and mitigating potential public health risks.

Materials and Methods

Sample Collection

A total of 50 faecal samples were collected aseptically from apparently healthy small ruminants, comprising 25 sheep and 25 goats.

Samples were collected in sterile containers and transported to the laboratory under refrigerated conditions (4 °C) for processing within 24 h.

Isolation and Identification of *Escherichia coli*

Faecal samples were enriched in Brain Heart Infusion (BHI) broth and incubated at 37 °C for 18-24 h. A loopful of enriched culture was subcultured onto MacConkey agar and incubated at 37 °C for 24 h. Presumptive lactose-fermenting colonies were further streaked onto Eosin Methylene Blue (EMB) agar for selective and differential identification. Colonies showing characteristic metallic green sheen on EMB agar were considered presumptive *Escherichia coli*. Preliminary confirmation was performed using Gram staining, catalase test, oxidase test, and standard biochemical tests (Cowan and Steel, 1974) [2].

Molecular Confirmation of *Escherichia coli*

Genomic DNA was extracted from presumptive isolates using a standard boiling method. Molecular confirmation of *E. coli* was performed by polymerase chain reaction (PCR) using species-specific primers:

Table 1: *E. coli* Primer

Primer Type	Primer Sequence (5'-3')	Amplicon Size (bp)
Forward	ATC AAC CGA GAT TCC CCC AGT	232
Reverse	TCA CTA TCG GTC AGT CAG GAG	232

PCR was carried out in a total reaction volume of 20 µL containing 10 µL master mix, 1 µL forward primer, 1 µL reverse primer, 3 µL template DNA, and 5 µL nuclease-free water. Thermal cycling conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 64 °C for 45 s, and extension at 72 °C for 90 s, with a final extension at 72 °C for 10 min.

Amplified products were resolved by agarose gel electrophoresis and visualized under ultraviolet illumination to confirm the presence of *E. coli*-specific amplicons (Riffon *et al.*, 2001) [9].

Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing of confirmed *E. coli* isolates was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. The antibiotics tested included enrofloxacin, gentamicin, amikacin, ciprofloxacin, and other commonly used antimicrobials in small ruminant practice. Zone diameters were measured and interpreted as susceptible, intermediate, or resistant according to Clinical and Laboratory Standards Institute guidelines (CLSI M100, 2023).

Molecular Detection of Antimicrobial Resistance Genes

Genomic DNA extracted from confirmed *E. coli* isolates was used for detection of antimicrobial resistance-associated genes, including *blaCMY*, *sul1*, and *intI1*.

PCR amplification for the *blaCMY* gene was carried out with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 60 s, annealing at 47 °C for 90 s, and extension at 72 °C for 60 s, with a final extension at 72 °C for 10 min.

For the *sul1* gene (822 bp), thermal cycling conditions included initial denaturation at 94 °C for 5 min, followed by denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min, with a hold at 4 °C.

Detection of the class 1 integron integrase gene (*intI1*, 565 bp) was performed with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 60 s, annealing at 54 °C for 60 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 10 min.

PCR products were resolved by agarose gel electrophoresis and visualized under ultraviolet illumination.

Table 2: Antimicrobial Resistance Genes Primer Sequences

Target Gene	Primer Type	Primer Sequence (5'-3')	Amplicon Size (bp)	Reference
<i>Sul1</i>	Forward	TTCGGCATTCTGAATCTCAC	822	Shehata <i>et al.</i> , (2016)
	Reverse	ATGATCTAACCCTCGGTCTC		
<i>IntI1</i>	Forward	ACGAGCGCAAGGTTTCGGT	565	Goudarzi and Eftekhari (2014)
	Reverse	GAAAGGTCTGGTCATACATG		
<i>blaCMY</i>	Forward	TGGCCAGAACTGACAGGCAAA	462	Van <i>et al.</i> , (2008)
	Reverse	TTTCTCCTGAACGTGGCTGGC		

Results

A total of 50 small ruminant faecal samples, including 25 goats and 25 sheep, were analyzed. Out of the 25 goat samples, 18 were positive for *Escherichia coli*, while in sheep, 13 out of 25 samples were confirmed positive. The isolates were further screened for selected antimicrobial resistance genes. Among goat isolates, the *blaCMY* and *sul1* genes were each detected in 13 out of 25 samples (52%), while the *intI1* gene was identified in 9 out of 25 samples (36%). In sheep isolates, *blaCMY* and *sul1* were each detected in 11 out of 25 samples (44%), whereas *intI1* was observed in 7 out of 25 samples (28%). Overall, across all samples analyzed, *blaCMY* and *sul1* were the most prevalent resistance genes, each identified in 24 out of 50 samples (48%), followed by *intI1* in 16 out of 50 samples (32%).

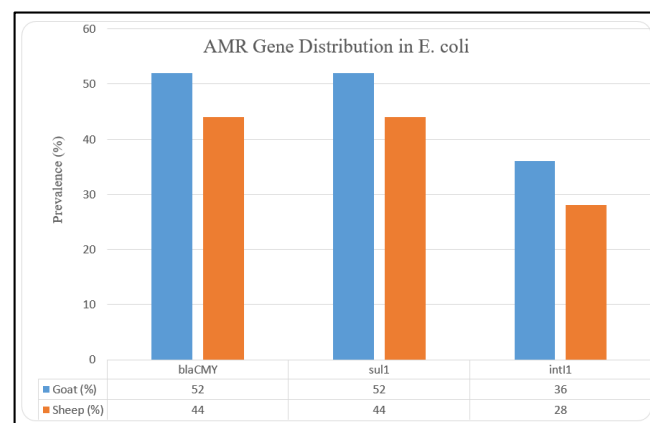


Fig 1: AMR gene distribution in *E. coli*

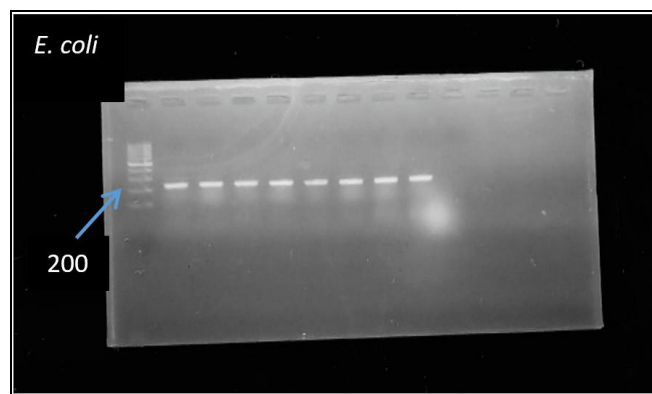


Fig 2: Agarose gel showing amplified product For *E. coli* isolates (Approx 232bp)

Table 3: Comparative Statistical Analysis of Antibiotic Resistance Between Sheep and Goats (Chi-square Test)

Antibiotic	Sheep (S)	Sheep (R)	Goat (S)	Goat (R)	χ^2 Value	p-value	Significance
Amoxicillin/Clav (Amc)	15	35	33	15	13.89	<0.001	Significant
Amikacin (Ak)	42	7	45	5	0.38	0.53	NS
Ciprofloxacin (CIP)	12	30	39	7	26.14	<0.001	Significant
Levofloxacin (LE)	11	37	44	5	41.02	<0.001	Significant
Co-trimoxazole (COT)	16	31	35	12	13.52	<0.001	Significant
Ceftriaxone (CTR)	45	4	47	3	0.14	0.70	NS
Colistin (CS)	38	12	41	9	0.54	0.46	NS
Meropenem (MRP)	40	8	42	7	0.09	0.76	NS
Erythromycin (E)	3	47	5	45	0.54	0.46	NS
Cefotaxime (CTX)	14	35	26	21	7.68	0.006	Significant
Polymyxin B (PB)	39	11	36	14	0.49	0.48	NS
Tetracycline (TE)	35	12	40	8	1.02	0.31	NS
Gentamicin (GEN)	41	7	43	6	0.07	0.79	NS
Cefoperazone (CPZ)	36	10	40	8	0.47	0.49	NS

NS = Not Significant ($p > 0.05$)

Discussion

The present study demonstrates a high prevalence of *Escherichia coli* in small ruminants, with higher occurrence in goats (72%) than sheep (52%), suggesting species-related differences in exposure or colonization. These findings support earlier reports identifying small ruminants as important reservoirs under grazing conditions (Moriarty *et al.*, 2011)^[5].

Significant interspecies differences in antimicrobial resistance were observed, with sheep isolates showing higher resistance to key antibiotics, including ceftiofur, amoxicillin/clavulanic acid, fluoroquinolones, and co-trimoxazole ($p < 0.001$). This indicates differential selective pressure, possibly due to variations in antimicrobial usage or management practices, consistent with previous studies (Ndegwa *et al.*, 2019; Basnet & Kilonzo-Nthenge, 2024)^[6]. In contrast, no significant differences were found for several critically important antibiotics, suggesting retained efficacy in the study population.

The high prevalence of blaCMY and sul1 (48%) and the detection of int11 (32%) indicate widespread distribution of resistance genes associated with mobile genetic elements, facilitating horizontal gene transfer (Shabana & Al-Enazi, 2020)^[10]. Although virulence factors were not assessed, prior evidence of pathogenic *E. coli* in small ruminants raises potential zoonotic concerns (Orden *et al.*, 2003; Ramatla *et al.*, 2024)^[7, 8].

However, the limited sample size and absence of virulence gene characterization constrain comprehensive risk assessment. Overall, the findings highlight small ruminants as reservoirs of antimicrobial-resistant *E. coli*, emphasizing

the need for targeted surveillance and antimicrobial stewardship.

Conclusion

In conclusion, the study highlights a notable prevalence of *Escherichia coli* in small ruminants, with goats showing higher occurrence than sheep. The detection of blaCMY, sul1, and int11 genes indicates the presence of important antimicrobial resistance determinants and the potential for their dissemination. These findings suggest that small ruminants may serve as reservoirs of antimicrobial resistance, emphasizing the need for continuous monitoring and prudent antimicrobial use in livestock systems.

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