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Characterization of Antimicrobial Resistance in *Escherichia coli* Isolated from Goats with Enteric Disease

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ABSTRACT

Background: Overuse and misuse of antibiotics in human and farmed animals is the key risk factor for accelerating antimicrobial resistance worldwide. The study aimed at the detection and characterization of *E. coli* associated with enteric infections in goats of Chhattisgarh, India with its antibiogram and related pathology.

Methods: The work was carried out in the Department of Veterinary Pathology, College of Veterinary Sciences and Animal Husbandry, Durg, Chhattisgarh, from December 2021 to July 2024. A total of 274 naturally died goats were screened for enteric lesions, irrespective of age and sex. Faecal samples and swabs were collected for bacteriological examination. Intestinal tissue samples were collected for molecular, histopathological and ultrastructural study. Disc diffusion technique and Congo red agar method were used for antibiogram and biofilm assay, respectively.

Result: The prevalence of *E. coli* isolates in goats, died due to enteric infection was 32.45%, based on the PCR results targeting the *ecp* gene. The 16s rRNA gene sequencing showed 99-99.4% homology with the genome sequences available in NCBI database. The phylogenetic analysis revealed the highest similarities of *E. coli* isolate of this study with the isolates of Telangana (India), Iraq and China. The study revealed 83.78% of *E. coli* isolates as biofilm producers and 64% isolates showed multidrug resistance. Various degenerative, inflammatory and necrotic lesions were seen in the intestine. In conclusion, the emergence of antibiotic resistant *E. coli* in goats poses a serious threat to public health.

Key words: *E. coli*, Goat, Multidrug resistance, Partial gene sequencing, Pathology.

INTRODUCTION

In India, goat rearing is one of the prime activities of the livestock sector after cattle. Goats are often called “poor man’s cow” because of their higher productivity, survivability in adverse environments, as well as their utility as a source of income through milk, meat, skin and manure (CIRG, 2021). Chhattisgarh’s rural areas extensively rely on goat farming, which provides supplementary income to the families dependent on agriculture.

The incidence of diseases is one of the major barriers to the development of the goat sector. Among various diseases, enteritis is a significant problem in goats, resulting in substantial economic losses to farmers (Tsegaye *et al.,* 2013). Although its aetiology is not well understood in all cases, it is thought to be caused by concurrent infection with several agents without inducing clinical illness in the host, among which *E. coli* is considered one of the most prevalent organism (Mokhbatly *et al*., 2022).

Today, antimicrobial resistance (AMR) becomes a health issue of global concern. It has been evaluated that India could witness over 2 million deaths due to AMR by the year 2050 (Prasad *et al*., 2024).

AMR in livestock directly impacts human health and environmental ecosystems. Inappropriate antibiotic use in animals causes gut bacteria to develop the resistance. These resistant bacteria can reach to environment and food chain, reducing the efficacy of common antibiotics and contributing to multidrug resistance (Al-Khalaifah *et al*., 2025).

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As long as the goats are important for human life, the bacterial infections and related antibiotic resistance pose a significant problem. Unfortunately, data on the diversity of enteric pathogens and related AMR in goats is limited

and no baseline data available regarding the *E. coli* associated molecular and pathological study of goat mortality due to enteric diseases in Chhattisgarh. Hence, the study investigates the characterization, antibiotic resistance and pathology of *E. coli* linked to enteric diseases in goats of this area.

MATERIALS AND METHODS

Study area and sample collection

The research was carried out in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, DSVCKV, Anjora, Durg, Chhattisgarh.

Commencing from December 2021 to July 2024, a total of 274 deceased goats of several organized and unorganized goat farms of Durg, Rajnandgaon, Balod and Raipur districts of Chhattisgarh were randomly screened for the presence of prominent enteric gross lesions such as enteritis, congestion and hemorrhages in intestinal mucosa, mucosal thickening, necrotic foci on wall of intestine, ballooning of intestine, presence of necrotic debris and exudate in lumen, enlarged, oedematous and haemorrhagic mesenteric lymph nodes, during post mortem examination; of which, 114 were selected for this study.

Intestinal contents and swabs from the part of intestine showing lesions were collected and immediately cultured or stored at 4°C until processing. Tissue samples of the small and large intestine were stored separately at -20°C for molecular characterization. Moreover, representative tissue samples from mesenteric lymph node, small and large intestine were preserved in 10% neutral buffer formal saline solution and 2.5% glutaraldehyde solution, for histopathology and electron microscopic studies, respectively.

Isolation of *E. coli*

Cultural isolation of *E. coli* was carried out as per the standard protocol (Quinn *et al*., 2002). Briefly, the swabs were inoculated into nutrient broth and incubated for 4-6 h at 37°C. Thereafter, the inoculum was streaked onto MacConkey agar and incubated at 37°C for 24 h. The plates were observed for characteristic pink colonies of *E. coli.* Further, a single isolated pink colony was streaked on Eosin Methylene Blue (EMB) agar, incubated for 18-24 h at 37°C and observed for the development of characteristic green metallic sheen. Pure cultures of *E. coli* isolates were taken on Brain Heart Infusion (BHI) agar slants. These slant cultures were stored at 4-8°C for further analysis.

Identification of *E. coli*

The identification of *E. coli* was done based on cultural and biochemical characteristics (Quinn *et al*., 2002). Further, *E. coli* isolates were outsourced to National Centre for Microbial Resource (NCMR), National Center for Cell Science (NCCS), Pune, Maharashtra, India for matrix-assisted laser desorption/ ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis for confirmation of its identity.

Biofilm forming assay

For determining the virulence potential of the presumptive isolates of *E. coli,* biofilm forming ability was detected using Congo Red Agar (CRA) method as described by Freeman *et al*. (1989). Black colonies with a dry crystalline consistency indicated biofilm production in *E. coli* (Karthik *et al*., 2018).

Antibiotic sensitivity profile

The antibiotic susceptibility patterns of the *E. coli* isolates were determined using Kirby-Bauer disc diffusion method on Mueller-Hilton agar following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2020). A total of 12 antibiotics were tested using disc (HiMedia Laboratories Pvt. Limited, Mumbai) of ampicillin (10 µg), amoxycillin clavulanic acid (20/10 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), cotrimoxazole (1.25/23.75 µg), gentamicin (10 µg), tetracycline (30 µg), azithromycin (15 µg), cefixime (5 µg), streptomycin (10 µg), chloramphenicol (30 µg) and norfloxacin (10 µg). After incubation, the diameter of the zone of inhibition around discs was measured with HiAntibiotic Zone Scale-C PW297 (HiMedia Laboratories Pvt. Limited, Mumbai) and compared (CLSI, 2020).

According to US and European Centers for Disease Control and Prevention (CDC and ECDC), nonsusceptibility to ≥3 antimicrobial categories is taken as Multidrug-resistance (MDR) (Magiorakos *et al.,* 2012).

Moreover, to determine the degree of bacterial resistance to antibiotics, the multiple antibiotic resistance index (MARi) was calculated (Krumperman, 1983). This index was assessed by dividing the numbers of antibiotics to which the organism were resistant to (a), by the numbers of antibiotics tested (b) and MARi greater than 0.2 is considered a health risk (Temikotan and Daniels, 2022).

Molecular characterization

The *Escherichia coli* common pilus *(ecp)* gene was targeted for molecular characterization.

The genomic DNA from *E. coli* isolates were extracted by using HiPurA® Multi sample DNA Purification kit (HiMedia Laboratories Pvt. Limited, India) as per the manufacturer's guidelines.

The PCR reaction was carried out in a total volume of 20 µL containing 3 µL of extracted DNA, 1µL of each primer ECP F (5′TGG TAA TTA CCG ACG AAA ACG GC 3′) and ECP R (5′ACG CGT GGT TAC AGTC TTC CG 3′), 10 µL 2X PCR TaqMixture (Himedia) and 5 µl nuclease free water. Amplification was carried out in a thermal cycler using an initial denaturation step at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 45 sec, annealing step at 60°C for 45 sec, elongation at 72°C for 90 sec and final extension at 72°C for 10 min. The reaction mixture was run by 1.5% agarose gel electrophoresis alongside a 100 base pair marker (Himedia). The amplified products (amplicon

size 500 bp) were viewed using the gel documentation system (Bio Rad, UK) (Avelino *et al.,* 2010).

Nucleotide sequencing

Partial nucleotide sequencing of 16s rRNA gene of one representative *E. coli* isolate was carried out by NCMR, NCCS, Pune, Maharashtra. The obtained sequences were aligned using the pairwise alignment tool BioEdit. Based on the BLAST results, sequences were further compared with other nucleotide sequences of *E. coli* in GenBank database. In addition, CLUSTAL W feature of MEGA XI software was used for phylogenetic tree analysis employing neighbor-joining algorithm with 1000 bootstrap replicates (Tamura *et al.,* 2021).

The final sequences were submitted in NCBI: https://www.ncbi.nlm.nih.gov, in order to get the accession number.

Pathomorphological study

For pathomorphological studies, formal saline fixed tissue samples were subjected to routine processing, paraffin embedding, sectioning at 4-5 µm thickness followed by staining with haematoxylin and eosin (Gridley, 1960).

Ultrastructural study

Representative tissue samples (n=10) of intestine were outsourced to Palamur Biosciences Laboratory, Telangana to explore the ultrastructural alterations using scanning electron microscope.

Statistical analysis

The data was statistically analysed using IBM SPSS statistics 24 software. P-values of correlations less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Prevalence

In the current study, the prevalence of *E. coli* in naturally dead goats with enteric lesions was recorded as 32.45% (37/114). Although *E. coli* strains are said to be harmless commensals in the intestine of animals, but this finding indicates that most of these bacterial strains were pathogenic and associated with enteric disease in goats which also corroborates with preceding reports (Singh *et al*., 2018; Sharma *et al*., 2020; Gupta *et al*., 2024). These workers isolated *E. coli* from diarrheal goats and demonstrated the bacterium as an enteric pathogen. Begum *et al*. (2016) recorded the 92% prevalence of *E. coli* in diarrhoeic goats while 84.76% prevalence of *E. coli* were recorded by Mishra *et al*. (2020), in goats suffering from diarrhoea, in their study. However, in several studies, relatively lower prevalence were reported as 18.82% and 23.5% by Kamal *et al*. (2018) and Azmat *et al*. (2024), respectively.

Identification of *E. coli*

The cultural and biochemical characteristics of *E. coli* isolates, observed in our study, are in accordance with the previous literature (Ahmed *et al*., 2010; Njoroge *et al*., 2013; Chatzopoulos *et al*., 2016), where they showed characteristic colonies on MacConkey and EMB agar (Fig 1). Biochemically, the bacteria were indole, methyl red positive and Voges-Proskauer (VP), citrate, oxidase and urease negative (Fig 2). Moreover, bacteria were able to ferment glucose, lactose, maltose, dextrose, sucrose and mannitol.

Further, in MALDI-TOF MS analysis, the bacteria showed best matching with *Escherichia coli* DH5alpha of MALDI-TOF MS biotyper database with score value of 2.457 followed by the second-best matching with *Escherichia coli* ATCC 25922 with score value 2.338 (Fig 3). Roncarati *et al*. (2021) used this technique in their study and confirmed the identity of *E. coli* isolates.

Biofilm forming assay

Biofilm formation serves as an important virulence factor for bacteria that provides protective environment for their survival and decreases the susceptibility of bacteria to the host immune response and antimicrobial agents (Zhao *et al*., 2023). The study revealed 83.78% isolates of *E. coli* were biofilm producer while 16.21% were non biofilm former.

Similar to our study, Dadawala *et al*. (2010) used Congo red (CR) assay for the assessment of biofilm production in

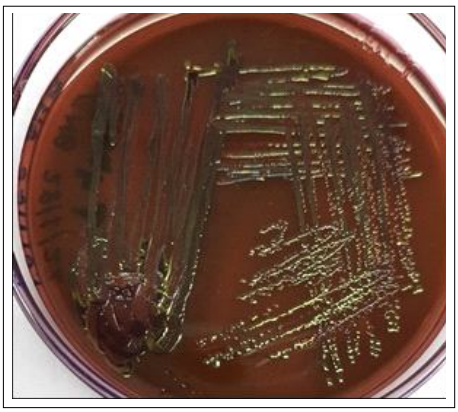


Fig 1: *Escherichia coli* isolate producing characteristic green metallic sheen on eosine methylene blue agar.

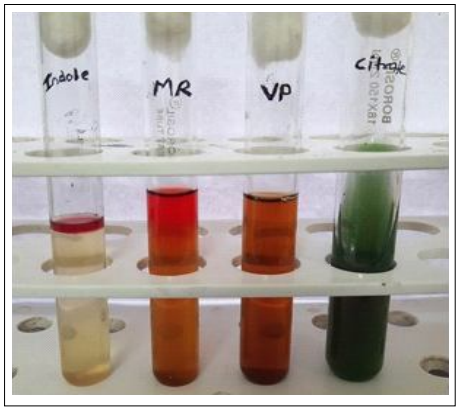


Fig 2: *Escherichia coli* isolate producing characteristic IMViC pattern + + - -.

E. coli and observed that out of 14 isolates, 12 (85.71%) were producing black colonies.

Antibiotic sensitivity profile

Antibiotic sensitivity assay revealed that none of the antimicrobials had 100% sensitivity and resistance towards *E. coli* isolates in the study area. The isolates showed the highest resistance to tetracycline (78.37%), followed by ampicillin (72.97%), amoxiclav (amoxicillin/clavulanic acid) (48.64%), co-trimoxazole (45.94%) and cefotaxime (40.54%). However, relatively lower resistance was observed against ciprofloxacin (32.43%), cefixime (24.32%), gentamicin (21.62%), norfloxacin (16.21%), azithromycin (16.21%), streptomycin (13.51%) and chloramphenicol (08.10%). In the present study, 64% of the isolates were identified as multidrug-resistant (MDR) based on a Multiple Antibiotic Resistance (MAR) index.

Moreover, it was observed that antimicrobial resistance was statistically significant ($p<0.05$) with biofilm production. Our results are in close conformity with earlier reports, where Zare *et al*. (2014), observed 65% *E. coli* isolates with multiple antibiotic resistance (MAR), in their study. Hariharan *et al.* (2004) recorded high resistance of *E. coli* to tetracycline in different animals while Adefarakan *et al.* (2014) reported higher number of resistant *E. coli* isolates to co-trimoxazole, nitrofurantoin and tetracycline in goat faeces. The present study finding agrees with the reports by Singh *et al*. (2017); Haulisah *et al*. (2021) and Kumar *et al*. (2022), who demonstrated the presence of multiple antibiotic resistance in more than 60% *E. coli* strains associated with clinical diarrhoea in goats. Also, Katongole *et al.* (2020) reported the statistically significant association between MDR and biofilm production in uropathogenic *E. coli.*

Long-term exposure of bacteria to subtherapeutic antibiotic doses is the cause of the increment in antibiotic resistance (Samreen *et al*., 2021). It may also originate in farms from contaminated water with subtherapeutic antibiotic concentrations. Antibiotic-resistant bacteria can transfer their resistance genes to DNA and plasmid of bacteria, during their multiplication, causing the number of resistant bacteria to increase (Xu *et al*., 2022). Moreover, antibiotic resistance in animals may also attributed to biofilm forming ability of bacteria. The proximity of cells within a biofilm can facilitate a plasmid exchange and hence enhance the spread of antimicrobial resistance (Watnick and Kotler, 2000).

Molecular characterization

The results of PCR amplification in the present study showed that all the *E. coli* isolates (n=37), were positive for *ecp* gene, producing an amplicon of 500 bp (Fig 4). This finding is in close agreement with the earlier reports where *E. coli* isolates were confirmed using *ecp* gene amplification (Avelino *et al*., 2010; Deshmukh *et al*., 2023; Munhoz *et al*., 2023). Munhoz *et al*. (2023) described that *E. coli* common pilus (*ecp*) fimbrae present in several diarrheagenic *E. coli* (DEC) associated pathotypes and plays an important role in host cell adherence and biofilm formation.

Nucleotide sequencing

The partial nucleotide sequencing of 16S rRNA gene of *E. coli* isolate (NE1 DSVCKV/DURG/CG/INDIA) revealed final consensus of 1008 bp sequence. The Basic Local Alignment Search Tool (BLAST) analysis discovered 99.00-99.40% homology with other nucleotide sequences of *E. coli* available in the reference database of GenBank. The accession number for the *E. coli* isolate of the present study was provided by NCBI GenBank as PQ334875.

Phylogenetic analysis showed highest similarities of *E. coli* isolate (PQ334875) of this study with the *E. coli* isolates of Telangana, India (MK716402.1), Iraq (LC796919.1) and China (MK621216.1) (Fig 5).

Gross and histopathological examination

During the necropsy procedure, the majority of the goats were found emaciated, dehydrated, with rough hair coat and pale conjunctiva. The perianal region and tail-base were soiled with diarrhoeic faeces. The gross lesions observed were enlargement, congestion and multiple necrotic foci on liver, enlarged and oedematous mesenteric

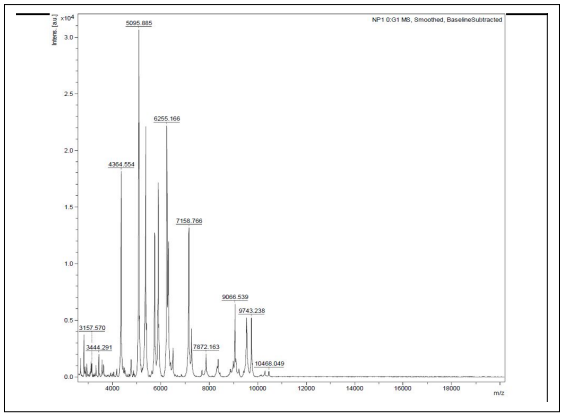
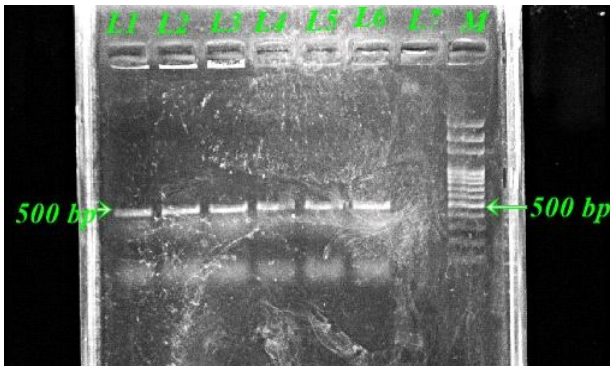


Fig 3: MALDI TOF MS spectra of *Escherichia coli* isolate.



M: 100 bp DNA ladder.
L1-L5: Positive isolates.
L6: Positive control.
L7: Negative control.

Fig 4: Gel image of amplification of *ecp* gene of *Escherichia coli* isolates producing an amplicon size of 500 bp.

lymph nodes and severe ballooning of the small intestine with hyperaemic serosa, as well as congestion and haemorrhage in mucosa (Fig 6). The microscopic examination revealed cellular swelling and vacuolation,

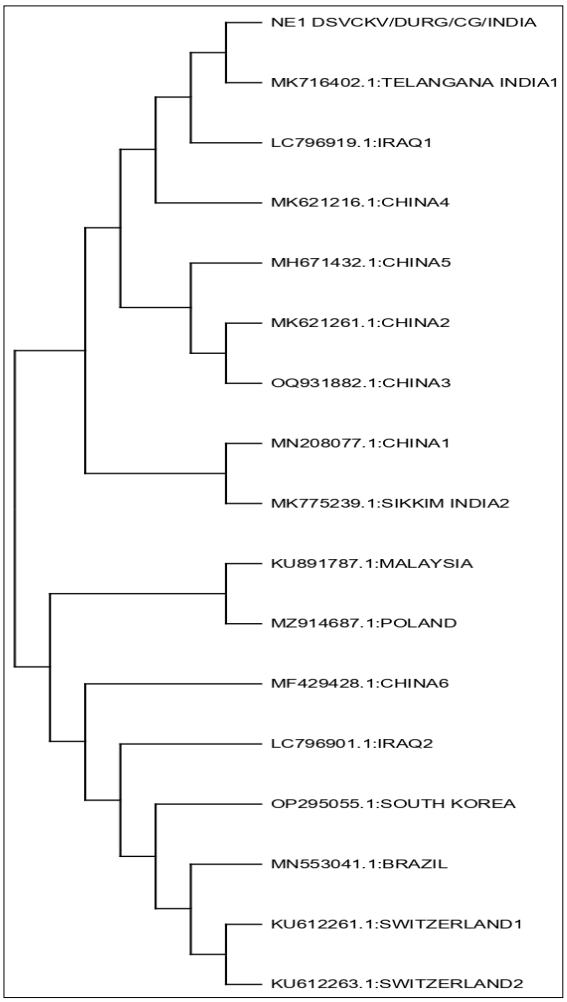


Fig 5: Cladogram of *Escherichia coli* (NE1 DSVCKV/DURG/CG/INDIA) isolate.

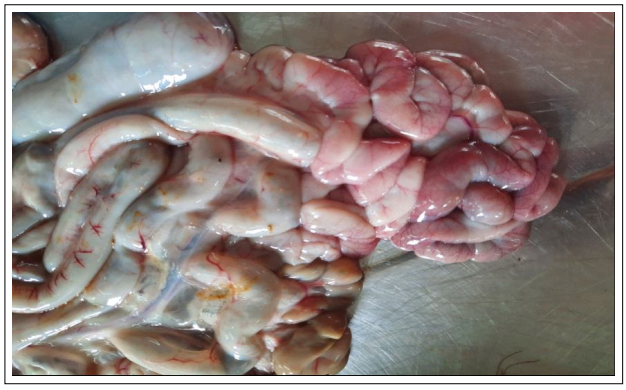


Fig 6: Jejunum of *Escherichia coli* positive goat showing congestion.

congestion and infiltration of inflammatory cells with distended sinusoids in liver (Fig 7) and infiltration of mononuclear cells over a homogenous meshwork of fibrin threads in the medulla of mesenteric lymph nodes. Moreover, desquamation of mucosal epithelium was accompanied by infiltration of neutrophils, lymphocytes in the lamina propria, villous atrophy, stunting or complete loss of villi in jejunum (Fig 8). The above lesions associated with *E. coli* infection in goats corroborate with the findings of Kumar *et al*. (2015) and Gupta *et al*. (2023).

Ultrastructural studies

Ultrastructural lesions were characterized by fusion, atrophy and loss of villi accompanied with enlarged crypt orifices in jejunal mucosa as well as covering of mucosa of small intestine with necrotic and fibrinous layer (Fig 9). The above mentioned changes corroborate with the findings of Neog *et al*. (2011), who studied the Rota virus and *E. coli* related pathology in diarrhoeic pigs.

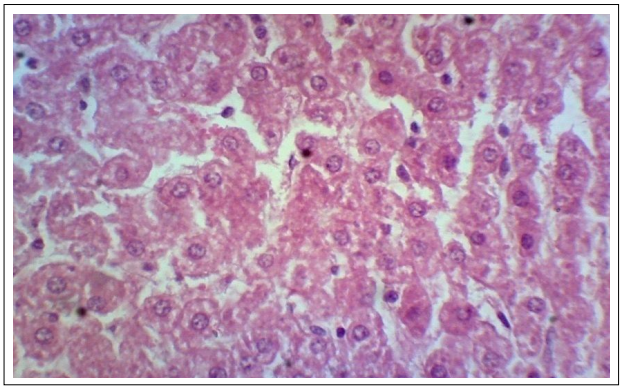


Fig 7: Photomicrograph of liver from *Escherichia coli* infected goat showing cellular swelling characterized by swollen hepatocytes with eosinophilic cytoplasm and vacuolation (H and E ×1000).

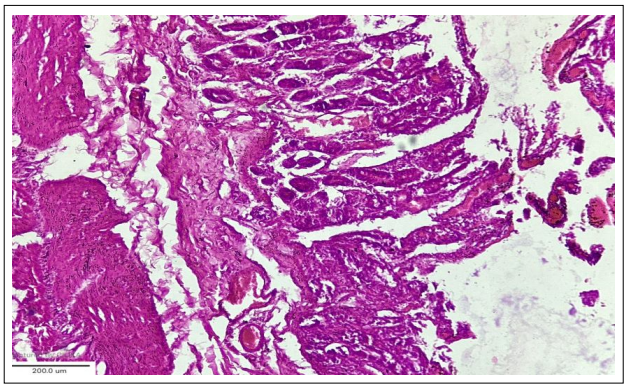


Fig 8: Photomicrograph of jejunum showing erosion of mucosal epithelium with infiltration of polymorphonuclear cells in lamina propria, haemorrhagic crypts and accumulation of sloughed off epithelial debris with inflammatory cells in lumen (H and E × 100).

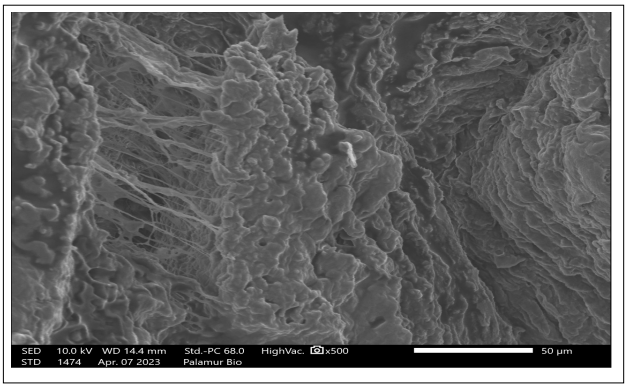


Fig 9: Ultrastructural changes in small intestine showing necrotic and fibrinous layer completely covering the mucosa (SEM ×500).

CONCLUSION

This study shows that *E. coli* is one of the major cause of enteric infection in goats, which may result in fatal infections. Moreover, goats may serve as carrier for multidrug-resistant bacteria, raising concerns about their potential introduction into environment and eventually to the food chain posing threat to other animals and human food safety; hence, the irrational use of antibiotics in animals should be discouraged.

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Disclaimers

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Conflict of interest

The authors declare no conflicts of interest regarding the publication of this article.

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