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Original Article

Antimicrobial resistance patterns of multidrug resistant ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from sheep and goats in Punjab, India

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Abstract

Background: A serious community concern worldwide is the alarming spread of enterobacteria producing extended-spectrum beta-lactamase (ESBL) enzymes. The food-producing animals harbor ESBL-producing enterobacterales and disseminate these resistant strains of pathogens through faeces. ESBL producers are multidrug-resistant phenotype posing a challenge to the disease management in clinical settings. **Aims:** The objective of the present study was to investigate the prevalence of ESBL-producing *Escherichia coli*, and *Klebsiella pneumoniae* from fecal samples (n=340) of healthy (n=300) and diarrhoeic (n=40) sheep and goats and hand swabs (n=70) of animal handlers. **Methods:** Presumptive ESBL producing bacteria were identified using ESBL agar, which were then confirmed by combined disc method (CDM). Genotypic detection of beta-lactamase, quinolones and tetracycline resistance genes was done using PCR. **Results:** A total of 156 enteropathogenic *E. coli* were recovered from sheep (79/170) and goats (77/170). Phenotypic antimicrobial resistance data revealed high resistance against cefazolin with 91.13% and 84.41% in sheep and goats, respectively. Low resistance was recorded against carbapenems. All the ESBL producing isolates revealed a multiple antibiotic resistance (MAR) index >0.2. The predominant β-lactamase gene found was the *blaTEM* detected in 110 (70.51%) isolates followed by *blaCTXM* in 80 (51.28%) isolates. **Conclusion:** The findings of the present study revealed a high prevalence of multidrug resistance *E. coli* and *K. pneumoniae* in food-producing animals. It represents an issue of concern and requires serious need for implementing effective approaches to reduce multidrug resistance in commensal organisms.

Key words: Antimicrobial resistance, Combined disc method, Extended-spectrum beta-lactamase, Multiple antibiotic resistance

Introduction

Antimicrobial resistance (AMR) is currently on the rise and is seen as a potential threat posing health hazard to both humans and animals (Wall *et al*., 2016; WHO, 2019). Each year, AMR causes thousands of mortalities among humans and results in significant economic losses that, by 2050, might cause the global GDP to fall by 1-4% (Wall *et al*., 2016; Bank, 2017). The primary factor causing the onset and rapid spread of AMR is the extensive use of antibiotics in both human and livestock sector (WHO, 2014; Wall *et al*., 2016). Broad spectrum antibiotics are indiscriminately added to animal feed for prophylaxis and treatment purposes which can lead to the distribution and propagation of resistant strains of microbes in the environment (Brower *et al*., 2017). The possibility of transmission to humans through the food chain and/or the environment is raised when such resistance manifests in food producing animals (Laxminarayan *et al*., 2013; Woodford *et al*., 2014).

A serious community concern worldwide is the emergence and transmission of members of *Enterobacteriaceae* producing enzymes known as extended-spectrum beta-lactamases (ESBLs) (Liu *et al*., 2016). Food-producing animals harbor ESBL-producing bacteria and are capable of spreading these resistant bacteria through feces (Doosti *et al*., 2015). The plasmid-encoded ESBL enzymes confer resistance to major beta-lactams like penicillins, cephalosporins as well as aztreonam but not cephamycins or carbapenems (Alegria *et al*., 2020). ESBL producers commonly have a multidrug-resistant phenotype posing a challenge to disease management in clinical settings (Bradford, 2001). The ESBL genes in *Enterobacteriaceae* which are most frequently found in *E. coli* and *K. pneumoniae* are acquired either through mutation or horizontal transfer of plasmids (Abrar *et al*., 2019), most common genes include *bla*CTX-M, *bla*TEM and *bla*SHV (Bush and Jacoby, 2010).

Various studies have highlighted the prevalence of beta-lactam antibiotic resistance genes in *E. coli* isolated from animal fecal matter. In Spain, a high prevalence of ESBL-producing *E. coli* strains with various beta-lactamase genes was detected in faeces of different animal species, representing a potential zoonotic transmission vector (Suay-Garcia *et al*., 2019). ESBL-producing *E. coli* strains isolated from livestock in Greece exhibited diverse antimicrobial resistance genes with *bla*CTXM-1 and *bla*CTXM-15 genes harbored by all the isolates (Athanasakopoulou *et al*., 2021). A study conducted in dairy cattle farms in Tulungagung, Indonesia, identified *bla*TEM and *bla*CTXM genes encoding multidrug-resistant ESBL-producing *E. coli* isolates in milk samples (Widodo *et al*., 2023). A similar study conducted in Bogor, Indonesia, revealed that 60% of *E. coli* isolates recovered from cattle manure samples harbored at least one beta-lactam resistance gene, with *bla*TEM being the predominant gene (Elsharkawy *et al*., 2024).

Animal fecal matter can potentially be considered as a source of zoonotic pathogens like *Campylobacter*, *E. coli* O157:H7 and *Salmonella* (Leifert *et al*., 2008). Food-producing animals harbor these enteric pathogens and represent the major reservoirs responsible for causing outbreaks and fatalities worldwide (Heredia and García, 2018). The overall prevalence of *Campylobacter coli* isolated in Tanzania was 7.71% in poultry (Nshama *et al*., 2022), 32.5% in pigs, 35.4% in dairy cattle and 19.6% in beef cattle samples (Kashoma *et al*., 2015).

The earlier reports published from various parts of India demonstrate multidrug resistant enteric microbes of animal origin, however, there is limited information available about multidrug resistant bacteria in sheep, goats and their handlers. It is, therefore, imperative to investigate the prevalence of multidrug resistance (MDR) in bacteria from these animal species along with the phenotypic and genotypic characterization. This study intended to investigate the occurrence and AMR profile of ESBL-producing *E. coli*, and *K. pneumoniae* isolated from sheep and goats and animal handlers from various districts in Punjab, India.

Materials and Methods

Ethical permission

The experiment protocols for animals were permitted by the Institutional Biosafety Committee (IBSC/2021/1765-66, Dated: 13/10/2021), and Institutional Animal Ethics Committee (GADVASU/2021/IAEC/61/23, Dated: 19/10/2021) GADVASU, Ludhiana, India. The permission for collecting hand swabs from animal handlers was authorized by Institutional Ethics Committee (DMCH/R&D/2021/104, Dated: 10/09/2021) of Dayanand Medical College & Hospital, Ludhiana, India.

Sample collection

Overall, 340 fecal samples (300 from apparently healthy animals; 150 sheep, 150 goats and 40 from diseased (diarrheic) animals; 20 sheep and 20 goats) and 70 hand swabs were taken from animal handlers. The pre-moistened cotton swabs were used for sample collection. The swabs were inserted inside the rectum to collect the fecal sample or rubbed gently against the palm, on and between the fingers, and around the nail beds of hands from handlers. All samples were labelled and kept in containers with ice packs until transported to the laboratory. All the samples in this study were collected randomly from 6 sheep farms and 9 goat farms across various districts of Punjab.

Isolation of bacteria

The collected samples were further processed for bacterial isolation by inoculating and streaking them on Brain Heart Infusion agar (BHI) (HiMedia, India). The inoculated culture plates were incubated overnight at temperature of 37°C for 24 h. The next day plates containing discrete and pure colonies were spotted on the target plate of matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) for identification. The results with score values over 2.00 were accepted for bacterial species identification (Klotz *et al*., 2019). The score value above 2.00 was considered for species identification.

ESBL phenotype testing

The ESBL production by bacteria was tested using HiCrome ESBL agar (HiMedia, India). A liquid suspension of 0.5 McFarland turbidity was made from an isolated colony and a loopful of it was streaked on HiCrome ESBL agar. After incubating these plates at 37°C for 24 h, the change in color to pink/purple (*E. coli*) and blue (*Klebsiella*) as per the manufacturer's color guidelines was noted. However, in case the plate remained colorless other Gram-negative bacilli were suspected.

Combined Disc Method (CDM)

The CDM was used to retest the presumed ESBL-positive isolates for phenotypic confirmation. In this method antibiotic discs containing ceftazidime and cefotaxime alone and combined with clavulanic acid were applied. Positive results were assessed by examining the inhibition zones surrounding the discs, with the zones around the combination discs demonstrating an increase of 5 mm or greater compared to the individual antibiotic discs (Brossier *et al*., 2008).

Antibiotic susceptibility testing (AST)

All the ESBL-producing bacterial isolates were examined for AST on Mueller Hinton Agar employing the Kirby-Bauer disk diffusion method as per CLSI guidelines 2020. A total of 20 antibiotic discs (Himedia, Mumbai, India) namely amikacin (AK) (30 µg), gentamicin (GEN) (30 μg), amoxyclav (AMC) (25 μg), ampicillin (AMP) (10 μg), ampicillin/sulbactam (A/S) (10 μg), cefazolin (CZ) (30 μg), cefepime (CPM) (30 μg), cefotaxime (CTX) (30 μg), cefoxitin (CX) (30 μg), ceftazidime (CAZ) (30 μg), ceftriaxone (CTR) (30 μg), cefuroxime (CXM) (30 μg), piperacillin (PI) (30 μg), aztreonam (AT) (30 μg), ertapenem (ETP) (30 μg), imipenem (IMP) (10 μg), ciprofloxacin (CIP) (30 μg), nitrofurantoin (NIT) (200 μg), cotrimoxazole (COT) (25 μg) and tetracycline (TE) (30 μg) were used. The clear zones formed around the antibiotic discs known as the zones of inhibition were measured and the isolates were classified as sensitive, intermediate, or resistant as per CLSI guidelines (CLSI, 2020).

An isolate was categorized as multidrug-resistant strain, when it exhibited resistance to three or more classes of antibiotics. The MAR index of the isolates was calculated using the standard formula suggested by Krumperman (1983). The formula for MAR is denoted by MAR = a/b, where "a" represents the number of antibiotics to which an organism shows resistance and "b" represents the total number of antibiotics.

DNA extraction and molecular analysis of AMR genes

DNA extraction from ESBL-producing isolates was done using the Hot-Cold Lysis method. For this, 150 μL of nuclease free water was taken in a 1.5 ml microcentrifuge tube (MCT) and 4-6 bacterial colonies from the pure growth were suspended in it and were vortexed for a few seconds to form a homogenous suspension. The dry bath was preheated at 100°C and the suspension was kept in it for 10 min. The MCTs were removed from the dry bath and immediately placed in ice for 10 min. The suspension in the MCTs was then centrifuged at 12000 rpm for 3 min. The supernatant was taken out carefully in a fresh MCT without disturbing the pellet. The supernatant obtained was then used as a source of DNA and stored at -20°C until further use. The extracted template was amplified to detect various AMR genes using PCR. The details of primers used for amplification of beta-lactams, quinolones, and tetracycline groups are listed in Table 1 along with their expected amplicon sizes. After loading 10 μL of PCR product and a DNA ladder, the agarose gel electrophoresis was carried out at 80 V for 45 min and results were analyzed.

Results

ESBL-producing *E. coli* and *K. pneumoniae* in sheep and goats

Overall, 267 *E. coli* isolates from apparently healthy animals (130 (86.6%) from sheep and 137 (91.3%) from goats) were obtained in our study. In total 9 *K. pneumoniae* isolates from apparently healthy animals (4.3% from sheep and 5.3.3% from goats) were acquired. The prevalence of ESBL-producing *E. coli* observed was

Table 1: Primers for the detection of AMR genes

Class of antibiotics	Gene	Sequence	Amplicon size	Reference
Beta-lactams	*bla*CMY-2	F: ATGATGAAAAAATCGTTATGC R: TTGCAGCTTTTCAAGAATGCGC	1143	Navarro *et al.* (2001)
	*bla*CTXM	F: TTTGCGATGTGCAGTACCAGTAA R: CGATATCGTTGGTGGTGCCATA	544	Das *et al.* (2020)
	*bla*CTXM-1	F: CCCATGGTTAAAAAATCACTGC R: CAGCGCTTTTGCCGTCTAAG	942	Huang *et al.* (2020)
	*bla*CTXM-3	F: AATCACTGCGTCAGTTCAC R: TTTATCCCCCACAACCCAG	701	Maynard *et al.* (2003)
	*bla*CTXM-15	F: CAATGTGCAGCACCAAGTAA R: CGCGATATCGTTGGTGGTG	540	Dutta *et al.* (2013)
	*bla*CTXM-25	F: GCACGATGACATTCGGG R: AACCCACGATGTGGGTAGC	327	Huang *et al.* (2020)
	*bla*SHV	F: TCGCCTGTGTATTATCTCCC R: CGCAGATAAATCACCACAATG	768	Maynard *et al.* (2003)
	*bla*TEM	F: CAGCGGTAAGATCCTTGAGA R: ACTCCCCGTCGTGTAGATAA	643	Chen *et al.* (2004)
Quinolone group	*qnr*A	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	516	Robicsek *et al.* (2006)
	*qnr*B	F: GGMATHGAAATTCGCCACTG R: TTTGCYGYYCGCCAGTCGAA	264	Schmiedel *et al.* (2014)
	*qnr*S	F: GCAAGTTCATTGAACAGGCT R: TCTAAACCGTCGAGTTCGGCG	428	
Tetracycline group	*tet*A	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	210	Ng *et al.* (2001)
	*tet*B	F: TTGGTTAGGGGCAAGTTTTG R: GTAATGGGCCAATAACACCG	659	
	*tet*C	F: CTTGAGAGCCTTCAACCCAG R: ATGGTCGTCATCTACCTGCC	418	

51.5% from sheep (67/130) and 45.2% from goats (62/137). None of the *K. pneumoniae* isolates were found positive for ESBL production.

Overall, 40 *E. coli* isolates were aquired from diarrheic animals (20 from sheep and 20 from goats). The prevalence of ESBL-producing *E. coli* isolates from these animals was 60% from sheep (12/20), and 75% from goats (15/20).

Hand swabs of animal handlers

Only 5 (7.14%) *E. coli* isolates were identified in hand swabs from handlers, but ESBL production was not detected in any of these isolates.

Antibiotic sensitivity test (AST)

The majority of ESBL-producing *E. coli* isolates from sheep (91.13%) and goats (84.41%) were resistant to cefazolin. More than 60% of the isolates from sheep and goats were resistant to cefoxitin, ceftazidime, aztreonam, cotrimoxazole, and nitrofurantoin. More than 50% of the isolates from sheep were sensitive to ampicillin/sulbactum, amoxiclav, and amikacin while 50% of the isolates from goats were sensitive to cefepime only. Most of the isolates from sheep and goats were sensitive to imipenem and ertapenem. AST pattern of ESBL-producing *E. coli* isolates from sheep and goats are shown in Figs. 1 and 2, respectively.

Multidrug resistance (MDR)

If a bacterial isolate was found resistant to three or more than three classes of antimicrobial drugs, it was assigned to MDR category (Magiorakos *et al*., 2012). According to this criterion, all the ESBL-producing *E. coli* isolates from sheep and goats were seen to be MDR.

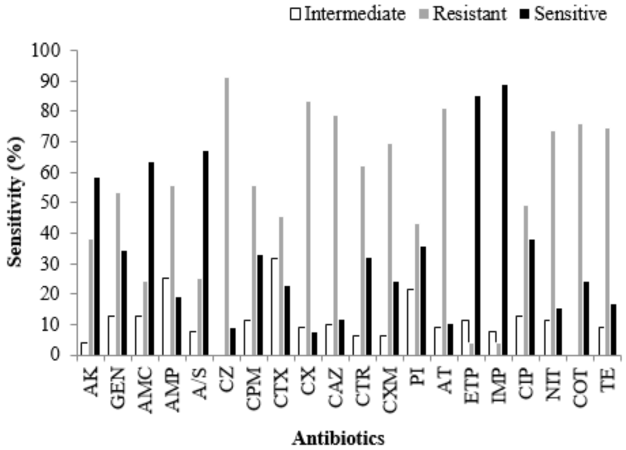

Fig. 1: AST pattern of ESBL producing *E. coli* isolates from sheep (n=79). AK: Amikacin, GEN: Gentamicin, AMC: Amoxyclav AMP: Ampicillin, A/S: Ampicillin/sulbactum, CZ: Cefazolin, CPM: Cefepime, CTX: Cefotaxime, CX: Cefoxitin, CAZ: Ceftazidime, CTR: Ceftriaoxone, CXM: Cefuroxime, PI: Piperacillin, AT: Aztreonam, ETP: Ertapenem, IMP: Imipenem, CIP: Ciprofloxacin, NIT: Nitrofurantoin, COT: Cotrimoxazole, and TE: Tetracycline

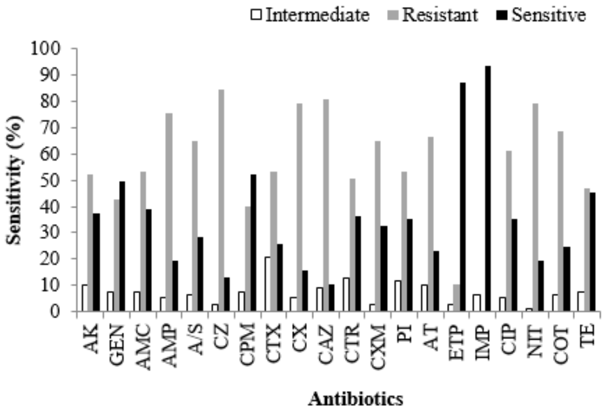

Fig. 2: AST pattern of ESBL producing *E. coli* isolates from goats (n=77). AK: Amikacin, GEN: Gentamicin, AMC: Amoxyclav AMP: Ampicillin, A/S: Ampicillin/sulbactum, CZ: Cefazolin, CPM: Cefepime, CTX: Cefotaxime, CX: Cefoxitin, CAZ: Ceftazidime, CTR: Ceftriaoxone, CXM: Cefuroxime, PI: Piperacillin, AT: Aztreonam, ETP: Ertapenem, IMP: Imipenem, CIP: Ciprofloxacin, NIT: Nitrofurantoin, COT: Cotrimoxazole, and TE: Tetracycline

Table 2: MAR indices of ESBL producing *E. coli* isolates from sheep (n=79)

MAR index	No. of isolates (%)
00-0.20	00 (00.00)
0.21-0.40	03 (03.79)
0.41-0.60	47 (59.49)
0.61-0.80	28 (35.44)
0.81-1.00	01 (01.26)
Total	79

Table 3: MAR indices of ESBL producing *E. coli* isolates from goats (n=77)

MAR index	No. of isolates (%)
00-0.20	00 (00.00)
0.21-0.40	12 (15.58)
0.41-0.60	43 (55.84)
0.61-0.80	21 (27.27)
0.81-1.00	01 (01.29)
Total	77

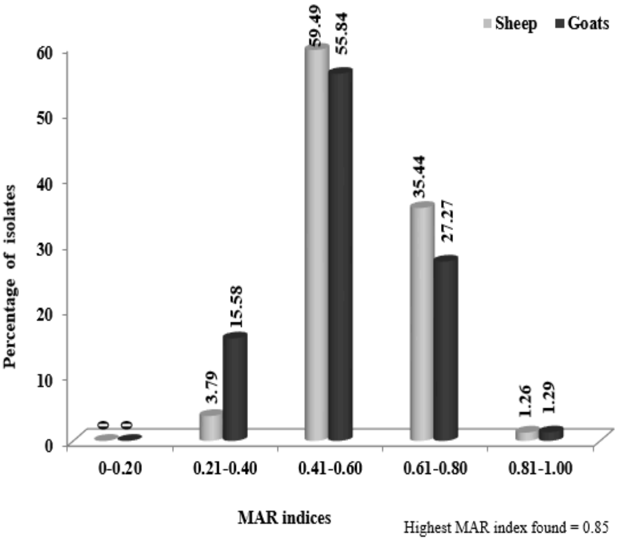

Fig. 3: MAR indices in sheep and goats

Multiple antibiotic resistance (MAR) index

All the ESBL-producing *E. coli* isolates from sheep and goats had a MAR index higher than 0.2. The MAR indices calculated for ESBL-producing *E. coli* isolates from sheep and goats are given in Tables 2 and 3, respectively and in Fig. 3.

Molecular detection of AMR genes in ESBL-producing isolates

The PCR results revealed the occurrence of *bla*CTX-M gene in 80 (51.28%) ESBL-producing *E. coli* isolates. The occurrence of the *bla*CTXM-1 gene was detected in 10 (6.41%), *bla*CTXM-3 in 30 (19.23%), and *bla*CTXM-15 in only 6 (3.84%) ESBL-producing *E. coli* isolates. None of the ESBL-producing isolates harbored *bla*CMY-2, *bla*CTXM-25, and *bla*SHV gene as shown by PCR results. Out of 156 EPEC isolates, most of the isolates (110, 70.51%) were found to be positive for the *bla*TEM gene.

In the present study, *qnr*S (22.43%) was the predominant PMQR gene, whereas a low prevalence of *qnr*A (3.20%) and *qnr*B (3.84%) was detected among ESBL-producing *E. coli* isolates. The occurrence of *tet*A, *tet*B and *tet*C genes was also investigated, and *tet*A (76.92%) was the predominant gene detected among ESBL-producing *E. coli* isolates, followed by *tet*C gene (19.23%) and *tet*B gene (6.41%).

Discussion

Ever since the discovery of ESBL-producing enterobacterial isolates, practitioners are facing hurdles in the treatment of clinical infections presented in the hospitals. The failure in the therapeutic strategies against most common bacteria like *E. coli*, *K. pneumoniae* and *Salmonella* spp., has immensely increased over the past years leading to poor patient outcome (Lalruatdiki *et al.*, 2018). ESBL-producing *E. coli* and *K. pneumoniae* are recognized as significant “One Health” pathogens prevalent in humans, animals and the environment (Ramatla *et al.*, 2023). The spread of ESBLs through animal waste to humans and the environment is the leading cause of multidrug resistance due to their persistence in various settings (Widodo *et al.*, 2020; Ramatla *et al.*, 2023).

K. pneumoniae, an important member of ESKAPE pathogens contributes to antimicrobial-resistant infections and causes increased mortality and healthcare risks, particularly in developing countries (Founou *et al.*, 2017). They are the leading causes of nosocomial infections and present a major challenge to clinicians by exhibiting MDR necessitating the development of new treatment strategies (Mulani *et al.*, 2019). In developed countries like Germany, multidrug resistant and ESBL-producing *K. pneumoniae* isolates have been identified in animals, food and environment (Wareth and Neubauer, 2021). In South Korea, a study on companion animals revealed high levels of resistance to various antibiotics (Lee *et al.*, 2021).

Antimicrobial agents are extensively used in food-producing animals and the transfer of resistant strains to human consumers can occur in a variety of ways (Torres *et al.*, 2021). In India, food-producing animals are raised for meat and milk production and are generally in close association with the handlers and their families. Currently, limited information is available on ESBL-producing *E. coli*, and *K. pneumoniae* isolates from sheep and goats, and their potential spread to humans. Therefore, the current study provides further information regarding ESBL production in bacteria from food-producing animals (healthy and diseased) and their handlers.

It was surprising to find a high occurrence of ESBL-producing isolates among apparently healthy animals investigated in this study. ESBL-producing *E. coli* isolates are widespread in healthy food animals throughout Asia. A previous study conducted in Northeast India, reported a high prevalence of ESBL-producing *E. coli* (75%) in livestock and poultry (Tewari *et al.*, 2019). High prevalence rates were also observed in pigs (76.7%) and broilers (40%) in Thailand (Boonyasiri *et al.*, 2014), much higher rates were reported in chickens (94.1%) and pigs (69.5%) in South Korea (Song *et al.*, 2020). The major cause responsible for the advent of AMR in animal farms is the non-therapeutic use of antibiotics over a long time (Durso and Cook, 2014). This emphasizes the fact that healthy animals can be a crucial reservoir for ESBL-producing isolates (Yang *et al.*, 2022) which might increase the risk of the spread of resistant pathogens or their genes through various means (food-borne or environmental transmission) (Da Costa *et al.*, 2013).

In the current study, results of ESBL-producing *E. coli* isolates obtained from diarrheic animals were also high with a percentage of 60% from sheep (12/20) and 75% from goats (15/20). The results of a previous study report high isolation rates (97.5%) of ESBL-producing *E. coli* recovered from dairy cattle having a uterine infection (Agrawal *et al.*, 2021). High occurrence rates (55%) of ESBL-producing *E. coli* are reported from diarrheic animals in China (Wang *et al.*, 2020), from food producing animals (53.6%) in Hong Kong (Ho *et al.*, 2011) and from bovine fecal samples (42.77%) in India (Shikha *et al.*, 2022). The main reasons could be the availability of antibiotics without the need of prescriptions (Ayukekbong *et al.*, 2017), treatment advice by fellow farmers and counterfeit medicines (Moudgil *et al.*, 2018; Tacconelli *et al.*, 2018). Other reasons include the use of β-lactam antibiotics and even 4th generation cephalosporins in Veterinary practices contributing to a high occurrence of ESBL isolates in diseased animals (Medeiros, 1997; Cavaco *et al.*, 2008).

None of the *E. coli* isolates identified from hand swabs of handlers showed ESBL production in the present study. Although hands are considered as one of the prime routes for the spread of AMR (Espadale *et al.*, 2018), negative results may be because of good sanitation and hygiene followed by the handlers. Besides this, microbiological tests performed on the hand swabs reflect only a preview of the entire transmission course. As the hand-animal encounter is usually high during animal handling, the tests cannot completely reveal the entire events of transmission occurring in these settings (Schmitt *et al*., 2021).

It is well established that the persistent or frequent administration of antimicrobials for therapeutic, prophylactic or growth promotion purposes causes extreme resistance to antimicrobials in various microbes including *E. coli* in farm animals (Yang *et al*., 2004; Liu *et al*., 2007). In the present study, the majority of the ESBL-producing *E. coli* isolates (>80%) recovered from sheep and goats were resistant to cefazolin. Resistance to cefoxitin and ceftazidime were quite high (>70%). The resistance rates of ESBL-producing *E. coli* isolates in the present study were quite similar to reports by Liu *et al.* (2007) for sheep isolates and Singh *et al.* (2017) for goat isolates. There has been a tremendous increase in resistance rates against two cephalosporin antibiotics, namely cefazolin and cephalothin in the past years (from 2001 to 2006) (Dai *et al*., 2008). A report analyzing the antimicrobial resistance of microbes isolated from animals in developing countries identified China and India as the largest hotspots of resistance (Van Boeckel *et al*., 2019). Global research on antimicrobial resistance in food-producing animals from 2015 to 2019 suggests a major increase (48%) in recent years. India ranks first when publications are standardized by income and population size (Sweileh, 2021). Moreover, studies highlighting recent trends in cephalosporin resistance in animals show a global increase in MDR in *E. coli* from food animals by 1.6 times between 1980 and 2018 (Pires *et al*., 2022).

Carbapenems are crucial antibiotics administered for treating severe Gram-negative infections but are unavailable in developing countries due to high costs (Njeru, 2020). Limited access to these antibiotics may be the reason for relatively low levels of resistance in developing countries. Most of the isolates in the present study were sensitive to imipenem and ertapenem representing low carbapenem resistance rates. The results of a cross-sectional study in India found a relatively low level (9.78%) of carbapenem-resistant Gram-negative bacteria in animals (Arun *et al*., 2022).

Exposure to different antimicrobials leads to higher chances for resistant strains of organisms getting selected (Cantòn *et al*., 2003). In co-selection processes, a single antimicrobial agent might select different multidrug-resistant isolates, and different antimicrobials might select a multidrug-resistant isolate (Sundqvist *et al*., 2010). As the majority of the ESBL-producing strains of bacteria determined in this study exhibited MDR, the co-selection processes might be involved (Sundqvist *et al*., 2010). The increasing prevalence of MDR reveals an urgent requirement for a serious antibiotic surveillance program (Osundiya *et al*., 2013).

Plasmids bearing resistance genes, each encoding a distinct antibiotic resistance phenotype are mainly responsible for the emergence of MAR in resistant bacteria. Other microbes that belong to the same or different species may acquire these antibiotic-resistance genes (Osundiya *et al*., 2013). The method of calculating the MAR index is useful in tracing the source of antibiotic-resistant organisms (Sandhu *et al*., 2016). Reports of numerous studies have revealed that MAR indexing can help to differentiate *E. coli* isolates from various sources involving humans and animals (Vantarakis *et al*., 2006; Mthembu *et al*., 2010). In a study, the MAR index was used to trace the source of water contamination by *E. coli* to distinguish between human and non-human sources (Mthembu *et al*., 2010). It is a valid, reliable, and cost-effective method (Sandhu *et al*., 2016). If the value of the MAR index calculated is greater than 0.2, it indicates that antibiotics are frequently used and there is a substantial danger of contamination (Davis and Brown, 2016). On analyzing the MAR index of all the ESBL-producing *E. coli* isolates from sheep and goats the figures were greater than 0.2. This indicates frequent use of antibiotics in that area and a significant rise in the MDR strains. The present observations are consistent with studies conducted by Osundiya *et al.* (2013), Mthembu *et al.* (2019) and Vinodhkumar *et al.* (2019).

Many types of ESBL-encoding genes are found but studies conducted on animals mainly focus on *bla*TEM, *bla*CTX-M, and *bla*SHV genes (Paterson and Bonomo, 2005; Castanheira *et al*., 2021). In this study, various antibiotic resistance genes were investigated and among them, *bla*TEM and *bla*CTX-M genes were the most frequent ESBL variants observed. The results of this study were consistent with the study reporting *bla*TEM as the most frequent ESBL variant carried by *E. coli* isolates (41/50) in farm animals from China (Liu *et al*., 2007). Similar results from a previous study report predominant presence of the *bla*TEM gene in food animals from India (Kumar *et al*., 2022). The majority of the ESBL-producing *E. coli* isolates carried the *tet*A gene and it was the most commonly found gene among all the tetracycline resistance genes studied. Many other authors have also reported a high rate of occurrence of *tet*A gene in sheep and goats in India (Banerjee *et al*., 2022; Kumar *et al*., 2022). The high rate of resistance occurring against this antibiotic may be due to the overuse of tetracycline compounds in the treatment of animal diseases and as growth promoters as well as their use in prophylaxis.

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Conflict of interest

The author(s) declare that there is no conflict of interest.

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