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Molecular identification and antimicrobial resistance profiling of pathogenic *E. coli* isolates from smallholder livestock households in Central Ethiopia

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ABSTRACT

Escherichia coli of different pathotypes are frequently involved in morbidity and mortality in animals and humans. The study aimed to identify *E. coli* pathotypes and determine antimicrobial resistance (AMR) profiles in Ethiopian smallholder livestock households. The pathotyping included 198 *E. coli* isolates identified from human and environmental samples collected from 98 households. AMR profiling was conducted on selected *E. coli* pathotypes from 89 households, along with known isolates from calf samples obtained from the same households. Morphological and biochemical tests were used to identify presumptive *E. coli* isolates. DNA was extracted and then singleplex PCR was used to amplify virulence genes. A disc diffusion test was applied for AMR profilings in *E. coli* pathotypes. Data were evaluated using chi-square tests and logistic regression. Calf (79.8 %) and human (73.7 %) samples were more likely to contain pathotypes (OR 3.2; 95 % CI: 1.7, 5.9; $p = 0.001$ and OR 2.3; 95 % CI: 1.2, 4.1; $p = 0.008$, respectively) than the environmental samples (55.6 %). ETEC (32.3 %) and STEC (15.2 %) were the most common pathotypes detected in the study samples. Out of the 176 isolates selected for AMR profiling, 85 % were resistant to at least one drug and 36 % were multi-drug resistant (MDR). The MDR isolates were found in 44 households, with 11 sharing identical pathotypes and resistance profiles among the different samples. Thus, *E. coli* strains were likely circulated among humans, animals, and the environment. This in turn calls for a One-health approach to improve antimicrobial usage standards and promote proper waste disposal practices.

1. Introduction

It has been estimated that 60 % emerging infectious diseases in humans originate from animals and over 36 % of these emerging zoonotic diseases are associated with food producing animals [1]. *Escherichia coli* is known to cause mild to severe gastrointestinal tract infections (GITIs) in animals and humans. Most GITIs are foodborne caused by contaminated food or water. Health problems associated with a lack of access to safe food and water are more common in low-income countries. According to World Health Organization (WHO) one-third of the populations in low-income countries suffered from foodborne diseases [2]. Enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) accounted for 37,000 and 26,000 deaths annually [2] . In addition, newborn calves are known to be affected by diarrheal diseases caused by bacteria. Although they are not always the only causative agents, *E. coli* pathotypes are commonly found in diarrheal calves [3–5].

The *E. coli* strains known to affect humans and animals are classified into distinct pathotype groups according to their specific virulence gene profiles [6]. For instance, enterotoxigenic *E. coli* (ETEC) produces heat-labile (*lt*) and heat-stable (*st*) toxins, while enteropathogenic *E. coli* (EPEC) is characterized by bundle-forming pilus (*bfp*) and locus of enterocyte effacement (LEE). Enterohemorrhagic *E. coli* (EHEC) produces shiga toxin 1 (*stx1*), shiga toxin 2

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(*stx2*), and hemolysin (*hly*), whereas some shiga toxin-producing *E. coli* (STEC) strains also fall within this category but typically lack the *hly* virulence gene. Enteroaggregative *E. coli* (EAEC) possesses aggregative adherence fimbriae (aatA) and dispersin, and diffusely adherent *E. coli* (DAEC) is characterized by Afa/Dr virulence genes. The type and severity of diseases associated with these *E. coli* pathotypes depend on the specific virulence genes they possess [6].

Antibiotics are extensively used in treating and preventing bacterial infections in animals and humans [7–9]. In addition, in some settings farmers use antimicrobials as an additive in animal feed to improve the growth of their animals [10,11]. The worldwide consumption of antimicrobials in the livestock sector in 2010 was estimated to 63,151 tonnes and by 2030, it has been predicted to rise by 67 percent [11]. Bacteria that are resistant to all available antibiotics have increased because of the extensive use and misuse of antimicrobials in humans and animals [12].

Antibiotic-resistant *E. coli* strains have been found in calf [13], human [14] and environmental [15] samples. In Ethiopia, one study found that fecal *E. coli* isolated from calves were resistant to tetracycline (58 %) and polymixin B (21 %) [16], and in another study 100 % of the isolates from meat samples were resistant to ampicillin, penicillin, and erythromycin [17]. In a study from Switzerland, fecal *E. coli* isolates from veal calves demonstrated tetracycline (56 %), and sulfonamide (56 %) resistance [10]. About 41 % of *E.coli* isolates from soil samples collected from 14 dairy farms in the USA were multi-drug resistant [18].

Resistant bacterial strains in animals and the environment may pose significant health threats [18]. In Africa, many of the antimicrobials currently in use are not effective against bacterial infections that threaten animal and human health [19,20]. Globally, it has been predicted that by 2050, up to 10 million people will die annually due to infections caused by drug-resistant bacteria [21,22].

Although *E. coli* of various pathotypes are frequently associated with diarrheal illness in humans and calves, comprehensive pathotyping and antibiotic resistance profiling data are rarely available from the very same household. Data from humans and livestock from the same household are of great interest to assess the risk of bacterial transmission between species. Applying a One-health approach, this study aimed to describe the occurrence of *E. coli* of different pathotypes and determine their AMR profiles, in households involving diarrheal calves, humans and environmental samples in Central Ethiopia. The study also aimed to assess risk factors for human exposure to *E. coli* infections in households.

2. Materials and methods

2.1. Study area

The study was conducted in ten subdistricts including nine from Basona Werana District and one from Angolela Tera District, Amhara Region, in Central Ethiopia (Fig. 1). Basona Werana, the main sampling district, has a population size of 120,930 people in 27,686 households [23]. The district contains 22 subdistricts and practices mixed crop-livestock production, with cattle being the most common livestock species.

2.2. Sample collection, E. coli isolation and pathotype identification

Sample collection, isolation, pathotype identification, and selection for AMR profiling are summarized in Table 1 and in the (Supplementary material, Figs. 1, 2, and 3). Study samples were obtained from smallholder households with calf diarrheal cases. The study samples comprised; fecal from diarrheal calves, soil from areas close to where the calves were housed, and stool from humans of diverse age and gender who had direct contact with the diarrheal calves. Samples were collected concurrently during a single visit to each household.The calf samples have been described previously, and pathotyping in the human and environmental samples were conducted as described in the previous study [24]. In brief, about 5 gs of human stool and 5 gs of environmental soil samples were collected from each household with diarrheal calves. Within 24 h of collection, samples were placed in a phosphate-buffered saline solution and transported to the laboratory in an icebox. Presumptive *E. coli* isolates were obtained by enriching the collected samples in tryptic soy broth and subsequently culturing them on eosin-methylene blue agar (EMB) medium. These isolates then underwent subsequent morphological and biochemical characterizations. Confirmed *E. coli* isolates were then subjected to DNA extraction. Finally, single-plex PCR was run to amplify ten virulence genes corresponding to different *E. coli* pathotypes. After the virulence genes (shown in *italics*) were detected, *E. coli* isolates were identified as EPEC (*bfp, eae*), STEC (*stx1, stx1-stx2, stx2,*), ETEC (*lt, lt-st, st*), EAEC (*aatA*), DAEC (*daaE*), and EHEC (*stx1-stx2-hly*) pathotype.

2.3. Antimicrobial resistance (AMR) profiling

Due to resource constraints, only selected isolates was subjected to AMR profiling. In total, 176 *E. coli* isolates from 89 households were selected. These included 104 isolates from humans ($n = 63$) and the environment ($n = 41$) and additionally 72 isolates from calves, as described in our previous study [24]. For AMR profiling, three to four *E. coli* colonies of each isolate grown on an EMB agar medium were suspended in a standard sodium saline solution. The inoculum turbidity was adjusted to 0.5 McFarland standards, which was equivalent to 1.5×10^8 CFU/ml [25]. Six antibiotics commonly used in the study districts were included in the disc diffusion assay, following the protocols of the Clinical Laboratory Standards Institute (CLSI) [26]. The included antibiotic discs contained gentamicin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), ampicillin (10 µg), ciprofloxacin (5 µg), and trimethoprim (5 µg). The discs (Oxoid, UK) were placed on Mueller-Hinton agar plates (Mumbai, India) that had been uniformly seeded with pre-made inoculums. The inoculated plates were then incubated for 24 h at 37 °C. Bacterial growth inhibition zones were measured and recorded. The reference *E. coli* strain ATCC 25,922 was used as a control [27]. Data from AMR profiles were utilized to categorize isolates as susceptible (S), intermediately resistant (IR), and resistant (R) based on the comparisons between inhibition zones and the CLSI cut-off points (Table 2). Additionally, isolates that exhibited resistance to at least one substance from three or more antimicrobial classes were classified as multi-drug resistant, MDR [28].

2.4. Household data collection

Concurrently with the collection of stool samples from participants, data on risk factors for exposure to pathogenic *E. coli* were also collected. Participants who had direct contact with diarrheal calves were interviewed about potential risks using a structured and pre-tested questionnaire (Supplementary material; Questionnaire). The assessed risk factors included age, gender, educational level, awareness of disease transmission between animals and humans, and observational assessment of personal and environmental hygiene standards. For hygiene-related data, a four-point scale, ranging from very poor to very good was employed. The questionnaire, initially prepared in English, was subsequently translated into the local language, "Amharic," and utilized in a face-to-face interview format.

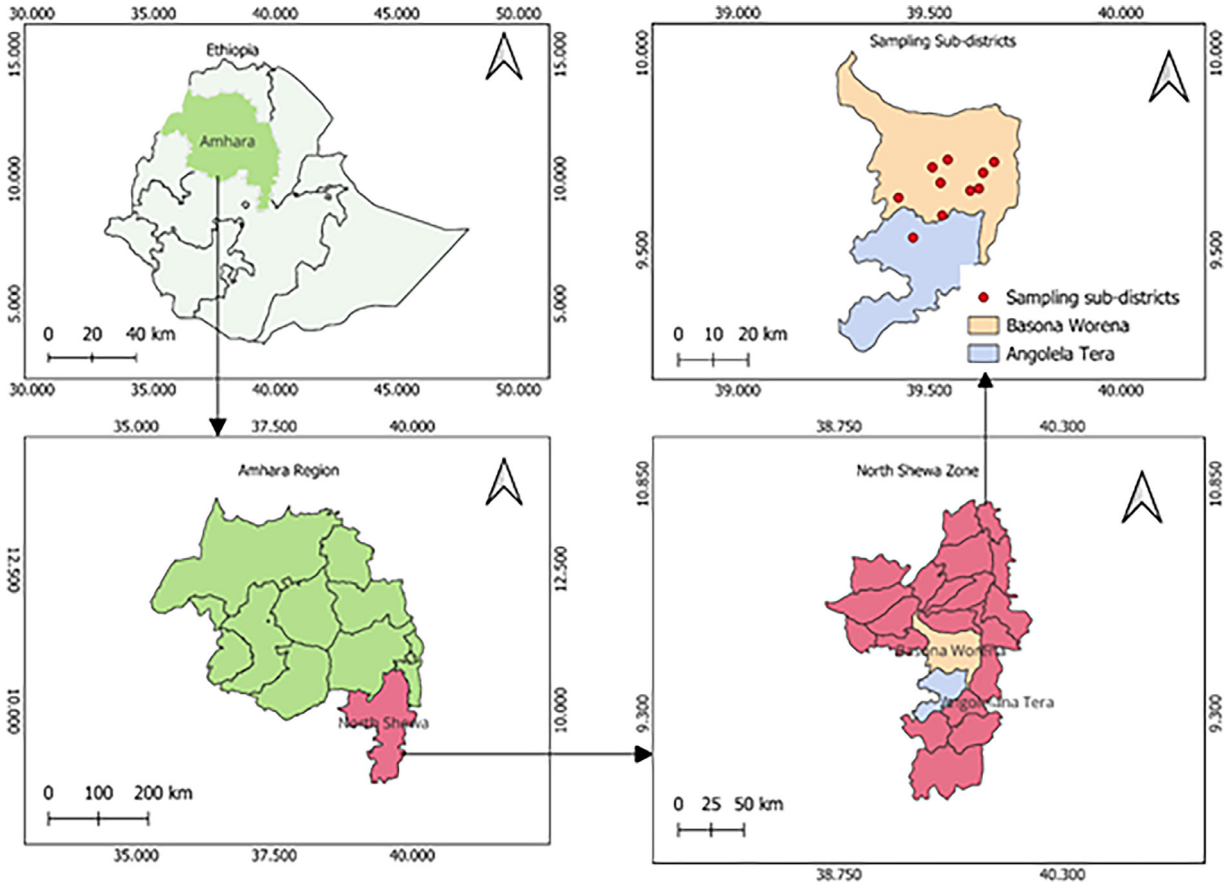

Fig. 1. The study area, located in the top right map, includes ten sampling subdistricts in the two districts. *Escherichia coli* isolates were obtained from calf, environmental and human samples collected in the HHs within these subdistricts.

Table 1
The number of studied households (HHs), calf fecal samples (CS), environmental soil samples (ES) and human stool samples (HS) from farms in central Ethiopia, including count of detected pathotypes and selected pathotypes for antimicrobial resistance testing are shown. Data from calf samples were sourced from a previous study [24].

Procedures	No. of HHs	No. of samples			No. of *E. coli* isolates			Remark
		CS	ES	HS	CS	ES	HS	
Sample collection	98	100	100	100	–	–	–	≥ 3 samples/HH
E. coli enrichment	98	100	100	100	300	300	300	3 isolates/sample
Biochemical characterizations	98	99	99	99	281	281	281	Presumptive *E. coli* obtained
E. coli pathotyping (PCR)	98	79	55	73	160	73	109	*E. coli* pathotypes found
AMR profile test	89	72	41	63	72	41	63	Isolates selected for AMR testing

Table 2
List of antimicrobial discs with respective concentrations and cut-off points, as recommended by the Clinical Laboratory Standard Institute [26].

Substance	Class	Code	Concentration	CLSI inhibition zone (mm)		
				R ≤	IR	*S* ≥
Ampicillin	Penicillins	AMP	10 µg	13	14–16	17
Chloramphenicol	Phenicols	CPH	30 µg	12	13–17	18
Ciprofloxacin	Fluoroquinolones	CIP	5 µg	15	16–20	21
Gentamicin	Aminoglycosides	GEN	10 µg	12	13–14	15
Tetracycline	Tetracyclines	TET	30 µg	11	12–14	15
Trimethoprim	FPI	TMP	25 µg	10	11–15	16

R, resistant; IR, intermediately resistant; *S*, susceptible; FPI, folate pathway inhibitors.

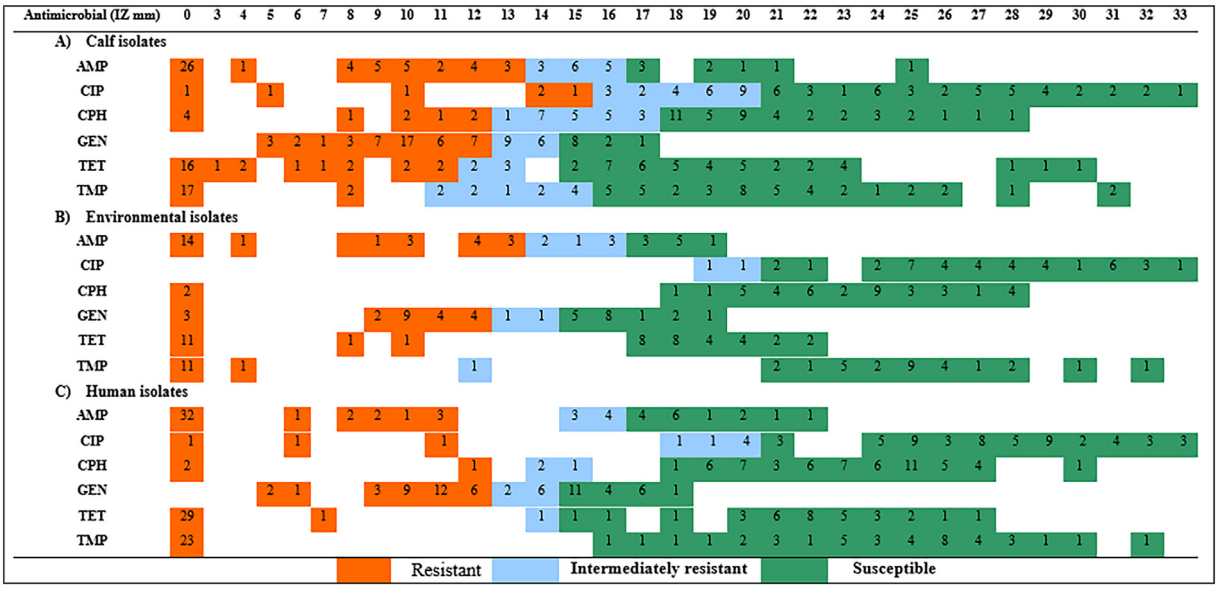

Fig. 2. Antimicrobial resistance profiles of *E. coli* isolates against six antimicrobials corresponding to inhibition zones (IZs) in calves, environment, and humans in central Ethiopia ($n = 176$). CS, Calf Sample; ES, Environmental Sample and HS, Human Sample; AMP, Ampicillin; CIP, Ciprofloxacin; CPH, Chloramphenicol; GEN, Gentamicin; TET, Tetracycline and TMP, Trimethoprim.

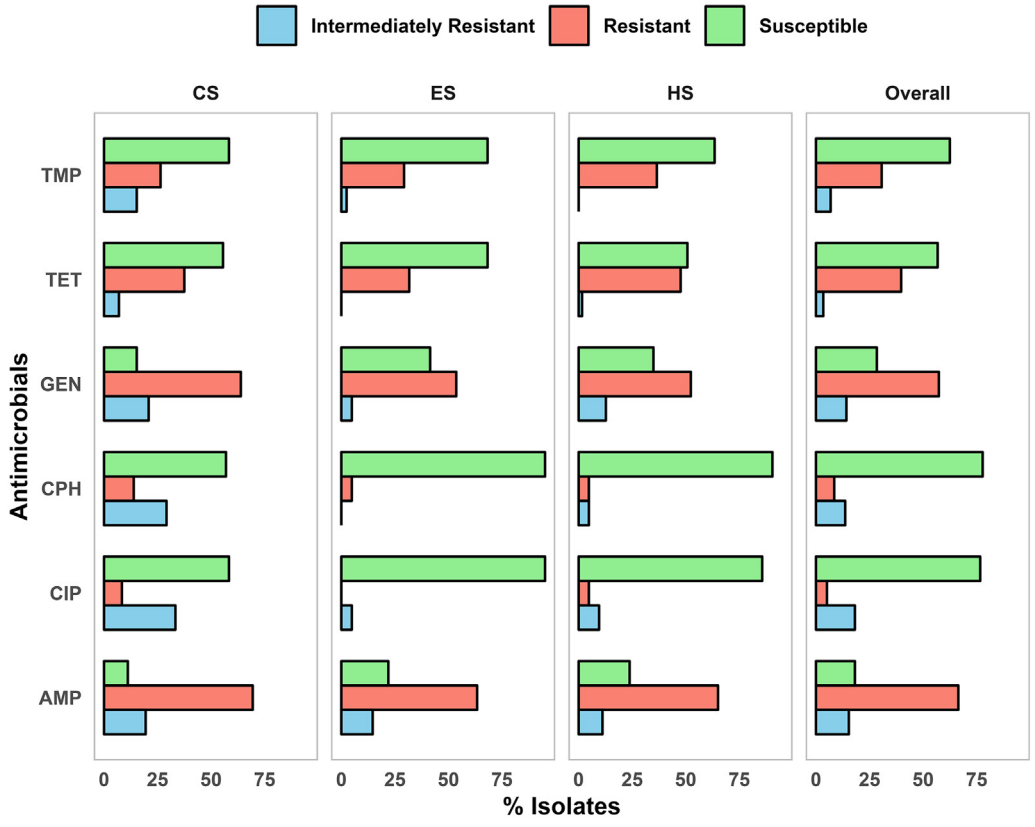

Fig. 3. AMR profiles of the 176 *E. coli* isolates obtained from calf, environmental, and human samples in central Ethiopia. The bar graphs show how resistance, intermediately resistance, and susceptibility to different antimicrobial substances vary across sample sources. The overall category provides a complete summary of AMR profiles from all sample sources. AMP, Ampicillin; CIP, Ciprofloxacin; CPH, Chloramphenicol; GEN, Gentamicin; TET, Tetracycline and TMP, Trimethoprim.

2.5. Data analysis

Microsoft Excel was used to record, code, and compile generated data from the identification of *E. coli* pathotypes and AMR profiles. A "Yes" or "No" response was used to indicate the presence and absence of virulence genes and pathotypes. AMR susceptibility was identified as "resistant", "intermediately resistant" and "susceptible". Several R scripts, including crosstable [29] gmodels [30] and ggplot2 [31] were used for descriptive statistics, logistic regression and plotting graphs, respectively. The prevalence of pathotypes and AMR profiles were reported using descriptive statistics and logistic regression. In the logistic regression analy-

Table 3
The proportion of *E. coli* pathotypes and associated virulence genes in the environmental and human samples obtained in a study in central Ethiopia (n = 198).

Pathotype	Virulence gene(s)	No. of samples				Overall N = 198	
		ES N = 99		HS N = 99			
		n	%	n	%	N	%
EAEC	*aatA*	2	2	4	4	6	3
EHEC	*stx1-stx2-hly*	1	1	–	–	1	0.5
EPEC	*eae*	1	1	3	3	4	2
ETEC	*lt-st*	–	–	1	1	1	0.5
	st	26	26.3	37	37.4	63	31.8
STEC	*stx1*	4	4	5	5.1	9	4.6
	stx1-stx2	1	1	6	6.1	7	3.5
	stx2	10	10.1	4	4	14	7.1
EAEC/ETEC	*aatA - st*	2	2	3	3	5	2.5
EAEC/STEC	*aatA-stx2*	1	1	3	3	4	2
EPEC/STEC	*eae-stx2*	1	1	–	–	1	0.5
STEC/ETEC	*stx1-st*	1	1	1	1	2	1
	stx1-stx2-st	–	–	1	1	1	0.5
	stx2-st	5	5.1	5	5.1	10	5.1
Total		55	55.6	73	73.7	128	64.6

N, number of samples; n (%), number and percentages of samples with pathotypes; ES, environmental sample; HS, human sample; EAEC, enteroaggregative *E. coli*; EPEC, enteropathogenic *E. col*; ETEC, enterotoxigenic *E. coli*; STEC, shigatoxigenic *E. coli; aatA*, aggregative adherence; *eae*, intimin; *lt*, heat liable toxin; heat *st*, stable toxin; *stx1/2*, shigatoxin 1/2.

sis, data on pathotyping of isolates from calves reported elsewhere were used for comparison [24]. The chi-square ($\chi 2$) test and logistic regression analysis were used to examine AMR profile variations among the different sample types. A statistically significant difference between the study groups was indicated by a *p-value* < 0.05.

2.6. Ethical clearance and consent for human participants

This study obtained ethical clearance (Approval Reference: CNCSDO/423/14/2022) from the Institutional Review Board Committee at the College of Natural and Computational Sciences, Addis Ababa University, Ethiopia. Participants were fully informed about the study's objectives before data collection. Their data were used exclusively for the study's intended purposes, with their explicit consent, ensuring well-informed and voluntary participation.

3. Results

3.1. Isolation and detection of E. coli pathotypes

Presumptive *E. coli* isolates were identified in 198 of the 200 samples collected from the environment and humans on the 98 study farms (Table 1). PCR results indicated that 128 (64.6 %) of the presumptive isolates harbored at least one virulence gene (Table 3). The prevalence of virulence genes in human isolates was higher (73.7 %) than in isolates from environmental samples (55.6 %). The ETEC pathotype was the most prevalent, followed by the STEC pathotype while the EAEC and EPEC pathotypes were less common. In total, 23 samples (11.6 %) contained mixed pathotypes, defined as having virulence genes from two or more distinct pathotypes.

The occurrence of *E. coli* pathotypes in samples from calves, environment, and humans is provided in Table 4. *E. coli* of the different pathotypes were found more frequently in samples from calves and humans than in the environment (p < 0.01). Samples from calves (79.8 %) and humans (73.7 %) had a higher likelihood of harboring pathotypes than environmental samples (55.6 %). The odds ratios (OR) for calves and humans were 3.2 (95 % CI: 1.7, 5.9; p = 0.001), and 2.3 (95 % CI: 1.2, 4.1; p = 0.008), respectively.

3.2. Antimicrobial resistance profile

Of the 176 tested *E. coli* isolates, 41 % were from diarrheal calves, 23 % were environmental soil samples, and 36 % were from humans who had direct contact with the diarrheal calves (Table 1). Figs. 2 and 3 show the detailed antimicrobial resistance profiles of isolates across sample sources and tested antimicrobials. Ampicillin (69 %) and gentamicin (64 %) resistance levels of calf isolates were higher than those of human isolates (65 % and 52 %, respectively) and environmental (63 % and 54 %, respectively) isolates for the same antibiotics. In contrast, human isolates exhibited more resistance to tetracycline (47.6 %) and trimethoprim (36.5 %) than calf isolates (37.5 % and 26.4 %, respectively) and environmental isolates (31.7 % and 29.3 %, respectively). Overall, the tested *E. coli* isolates demonstrated high susceptibility to ciprofloxacin and chloramphenicol.

The overall antibiotic susceptibility profiles of *E. coli* isolates were divided into two general categories: (i) resistant to at least one drug (ODR; ≥ 1 antibiotic) and (ii) resistant to antibiotics from at least three different classes of antibiotics (MDR; ≥ 3 antibiotics) (Table 5). Most tested isolates (85 %) were found to be ODR and about 37 % were MDR. There was no significant association between the sample origin and ODR or MDR. There was a significant association between pathotypes and MDR (p = 0.009), with 80 % of EAEC, 39 % of ETEC 37 (39 %) and 29 % of STEC being MDR while no EPEC isolates were found to be MDR. Within the resistant isolates, 48 % exhibited resistance to only one or two antimicrobials. Among the MDR isolates, 19.3 %, 13.7 %, and 4.5 % displayed resistance to three, four, and five antimicrobial types, respectively. The remaining 15.3 % isolates were either intermediately resistant or susceptible to all classes of antimicrobials.

AMR occurrence among isolates with different virulence genes (VGs) are shown in Fig. 4. All VGs were associated with ODR levels of 50 % or more, whereas isolates with the *aatA, st*, and *stx2* VGs had higher MDR proportion (80, 38.5, and 39 %, respectively).

The AMR patterns of tested isolates are shown in Table 6. Co-resistance occurrences were high among *E. coli* isolates with AMP/GEN (17.6 %) being the most common. Only 15.3 % of isolates showed no resistance to any antimicrobials tested and 19.9 % exhibited resistance to only one drug. About 65 % of the isolates were resistant to ≥ 2 antimicrobials.

Table 4
Occurrence of *E. coli* pathotype and associated odds ratios from calf, environmental and human sample sources in central Ethiopia (n = 297).

Sample source	No. of tested samples	Pathotype (%)		p –value[a]	Odds ratio [95 % CI[b]]	p-value[c]
		Yes	No			
Environment	99	55.6	44.4		1.0 [Ref]	
Calf	99	79.8	20.2	<0.001	3.2 [1.7, 5.9]	0.001
Human	99	73.7	26.3		2.3 [1.2, 4.1]	0.008
Overall	297	69.7	30	–	–	–

p-value[a] (Chi-squared test).
CI[b] (Confidence Interval).
p- value[c] for the logistic regression, and data from diarrheal calves were taken from a previous study [24].

Table 5
Proportions of *E. coli* isolates of different pathotypes resistant to at least one drug (ODR, ≥ 1 antibiotic) and multidrug-resistant (MDR, ≥ 3 antibiotics) in calf faeces samples (CS), environmental soil samples (ES) and human stool samples (HS) in central Ethiopia (n = 176).

Category	Number of isolates	Resistant isolates	
		ODR (≥ 1 antibiotic)	MDR (≥3 antibiotics)
Overall	176	84.7 %	65 (36.9 %)
Sample			
CS	72	89 %	40.3 %
ES	41	83 %	29 %
HS	63	81 %	38 %
p-value[a]		0.416	0.492
Pathotype			
EAEC	10	80 %	80 %
EPEC	3	67 %	0 %
ETEC	98	88 %	38.8 %
STEC	65	82 %	29 %
p-value[b]		0.546	0.009

[a] Pearson's Chi-squared test;
[b] Fisher's exact test; CS, calf sample; ES, environmental sample and HS, human sample; EAEC, enteroaggregative *E. coli*; EPEC, enteropathogenic *E. col*; ETEC, enterotoxigenic *E. coli*; STEC, shigatoxigenic *E. coli*; ODR, at least one drug resistant and MDR, multiple-drug resistance.

Fig. 4. The proportion of resistant *E. coli* isolates obtained from calves, their environment and in-contact humans in central Ethiopia containing different virulence genes (VGs). AMP, Ampicillin; CIP, Ciprofloxacin; CPH, Chloramphenicol; GEN, Gentamicin; TET, Tetracycline and TMP, Trimethoprim; ODR, at least one drug resistant and MDR, multiple-drug resistance; *aatA,* aggregative adherence; *eae,* intimin; *lt,* heat liable toxin; **st,** heat stable toxin and *stx1/2,* shigatoxin 1/2.

Table 6
Phenotypic AMR patterns of *E. coli* isolates obtained from calves, environment and humans in central Ethiopia (n = 176).

AMR pattern	Antibiotic classes	Isolates % (n)
AMP	Single class	5.7(10)
CIP		1.1(2)
CPH		0.6(1)
GEN		11.9(21)
TET		0.6(1)
Total		19.9(35)
AMP/CIP		0.6(1)
AMP/CPH	Two classes	0.6 (1)
AMP/GEN		17.6(31)
AMP/TET		6.3(11)
AMP/TMP		0.6(1)
GEN/TET		1.7(3)
TET/TMP		0.6(1)
Total		27.8(49)
AMP/CPH/GEN	Three Classes	1.1(2)
AMP/CPH/TET		1.1(2)
AMP/GEN/TET		4.5(8)
AMP/GEN/TMP		3.4(6)
AMP/TET/TMP		8(14)
GEN/TET/TMP		1.1(2)
Total		19.3(34)
AMP/CPH/TET/TMP		1.1(2)
AM/CIP/GEN/TET	Four classes	0.6(1)
AMP/CIP/TET/TMP		0.6(1)
AMP/CIP/CPH/GEN		0.6(1)
AMP/GEN/TET/TMP		9.7(17)
CPH/GEN/TET/TMP		0.6(1)
Total		13.1(23)
AMP/CPH/GEN/TET/TMP		2.8(5)
AMP/CIP/GEN/TET/TMP	Five classes	1.7(3)
Total		4.5(8)
Susceptible and Intermediately resistant	Six classes	15.3(27)
Overall		100(176)

AMP, Ampicillin; CIP, Ciprofloxacin; CPH, Chloramphenicol; GEN, Gentamicin; TET, Tetracycline and TMP, Trimethoprim.

3.3. Pathotypes and their AMR profile on household level

Isolates of different pathotypes were found in 93 of the 98 households, and in 59 of these households, the same pathotypes were detected in at least two different sample sources (Table 7). Of the 89 households selected for AMR assessment, 83 had *E. coli* pathotypes in their samples that showed resistance to at least one of the tested drugs (ODR), and 44 of these households had samples containing pathotypes that were resistant to at least three drugs (MDR). In 18 households, the same resistance profiles and pathotypes were detected in at least two of the sample sources.

The study found no significant association between the identification of *E. coli* pathotypes and any of the studied risk factors ($p > 0.05$) (Supplementary material; Table 1). Humans with lower educational levels were more likely to carry resistant pathotypes (91 % of illiterates and 76 % of those who read and write) compared to 40 % in high school ($p = 0.019$). AMR was found

Table 7
Households with *E. coli* of the same pathotype and resistance profiles obtained from calves, environment and human sample sources in central Ethiopia.

Characteristics	Category	Number of households	
		Detected (≥ 1 sample sources)	Shared[a] (≥ 2 sample sources)
Pathotype	All types	93	59
	Mixed	50	6
Resistance	ODR	83	18
	MDR	44	11

"[a]"indicates that same pathotype (s) and resistance profile (s) were found in at least 2 or 3 of the sample sources within households; ODR, at least One Drug-Resistant (≥1 drug) and MDR, Multiple-Drug Resistance (≥3 drugs).

in 100 % of pathotypes isolated from young children aged 1–14, people who handled manure and people with no awareness about possible animal-human disease transmission.

4. Discussion

This study chracterised *E. coli* isolates in samples from diarrheal calves, in-contact humans, and the environment in the same households by pathotyping and AMR profiling. Such studies are critical for developing hands-on strategies to prevent infections brought by drug-resistant bacteria in settings where livestock are kept close to humans.

Overall, different pathotypes were identified in 64.6 % of the 198 samples, including samples from the environment (55.6 %), and humans (73.7 %). Other studies with sampling from different sources within the same household are rare, therefore, comparisons become difficult. Still the findings here are in agreement with pathotype detection studies from diarrheal children in Ethiopia, with 77 % positive samples [32], and in south Africa, with 81 % positive samples [33]. The figures are, however, substantially higher than the 22 % of samples with pathotypes reported from drinking water in Jordan [34] and 27 % from various source samples in India [35]. Yet other studies report intermediate levels, where pathotypes were found at a rate of 35.4 % in Iranian riding horses [36] and 36.4 % in Ethiopian diarrheal children [37]. These differences in prevalence of pathotypes likely reflect the differences in the conditions at the study sites and in the health status of the sampled humans and animals.

The likelihood of obtaining one of the *E. coli* pathotypes in the samples from calves and humans compared to their soil environment was 3.2 and 2.3 times greater, respectively. This may be due to several factors: (1) *E. coli* pathotypes are most likely adapted to the intestines of warm-blooded animals where suitable nutrients are available, (2) samples from calves were obtained from active diarrheal cases, and (3) personnel involved in dairy herd management may be repeatedly exposed to *E. coli* shed from their livestock. Multiple studies have shown that there is a higher probability of detecting pathogenic *E. coli* isolates from diarrheal calves than from healthy calves [38,39]. Similarly, in a study in India, children with acute diarrhea had a higher prevalence of EAEC (16 %) compared to controls without diarrhea (2.7 %) [40]. Pathogenic *E. coli* isolates are frequently reported from intestinal samples of warm blooded animals, including humans [41] and cattle [42].

In this study, ETEC and STEC pathotypes were the most abundant, at 32.3 % and 15.1 %, respectively. Some studies suggest that different *E. coli* pathotypes have little host preference [42,43], and in the present study ETEC and STEC predominated in all of the samples. This may indicate that these pathotypes could be transmitted and shared within the households, while EAEC and EPEC pathotypes were less frequently detected. Nevertheless, a previous study in Ethiopia revealed that EPEC was present in 6 % and 7 % of samples from calves and diarrheal children, respectively [32].

It has been suggested that differences in detection rates might be due to the pathotypes' overall competency, virulence, and their interaction with their hosts and their environment. For instance, EPEC is more common in children than in older people [44,45]. The overall low occurrence of EPEC and EAEC pathotypes in the human samples may be attributed to the fact that the majority of samples were obtained from humans well above 15 years old.

In this study, ampicillin resistance was the most prevalent(66.5 %), followed by gentamicin and tetracycline. This finding is consistent with the high rates of ampicillin resistance reported among *E. coli* isolates from swab and meat samples in Ethiopia (88.9 %) [46], from neonatal calf diarrhea in Egypt(100 %) [47] and from calves and pigs in Brazil (75 %) [48]. In a totally different setting, a study conducted in sixty dairy farms in Sweden found that ampicillin, streptomycin, sulfamethoxazole, and tetracycline resistances were the most prevalent among *E. coli* isolates [49].

Most isolates investigated in the current study were susceptible to ciprofloxacin (77.8 %) and chloramphenicol (76.7 %). Other studies in Ethiopoia have indicated that *E. coli* isolates from different sample sources had comparable high ciprofloxacin [17,32,50] and chloramphenicol [51] susceptibility. As ciprofloxacin has been used for decades and ranked as the fourth most consumed antimicrobial by humans in Ethiopia [52], these low resistance rates are surprising. The high susceptibility to chloramphenicol could be due to its infrequent usage in Ethiopia. According to a three-year (2017–2019) antimicrobial consumption surveillance, chloramphenicol did not rank among the top 20 antimicrobial substances consumed by humans in Ethiopia [52]. Instead, the most consumed antimicrobials include doxycycline, norfloxacin, azithromycin, and ciprofloxacin, accounting for about 70 % of the total antimicrobial consumption [52].

About 85 % of the pathogenic *E. coli* isolates in this study were found to be resistant to at least one antimicrobial and 36 % were MDR. This indicates extensive distribution of AMR among the *E. coli* isolates in the region. A high proportion of MDR was found in isolates of the EAEC (80 %), ETEC (38 %), and STEC (29 %) pathotypes while no MDR was detected in the EPEC isolates. Similarly, all STEC isolates from cattle and swine in a study from Chile were resistant to at least one antimicrobial [42]. Contrary to the findings of the current study, all EPEC isolates from diarrheal calf, milk, and dairy workers in an Egyptian study were resistant to at least one antimicrobial [53].

In this study, 50 of the 98 households had samples with mixed *E. coli* pathotypes. Different sample sources shared the same mixed pathotypes in 6 of these households. MDR isolates were found in samples from 44 households, and isolates with the same pathotypes and the same AMR profiles were found in samples from at least two sources in 11 of those households. This could be due to transmission within households and such transmission could be associated with lack of knowledge and failure to implement hygiene measures to prevent transmission.

Among the analyzed potential risk factors, only educational level turned out to be associated with occurrence of resistance.

The proportion of resistant pathogenic *E. coli* isolates was lower in the group with high-school education than in the other groups with less education. The finding may suggest a link between education and awareness of the appropriate use of antibiotics, and/or the ability to implement hygiene advice. This is promising, as educating people may ultimately reduce the incidence and zoonotic transmission of antimicrobial resistance.

5. Study limitations

Due to financial constraints, our study was conducted with a relatively small sample size, and only selected isolates were subjected to antimicrobial resistance (AMR) profiling. Additionally, we did not use advanced sequencing techniques to explore further molecular similarities among isolates in calves, humans, and the environment. These constraints may limit the generalizability of our results, and they should be interpreted with caution.

6. Conclusion

E. coli pathotypes, mainly ETEC and STEC were frequently detected in diarrheal calves, in-contact humans, and their environment. Over one-third of the tested isolates were MDR. Some of the detected pathotypes and their associated phenotypic resistance patterns were similar in samples from different sources within the same households. This finding suggests that antibiotic-resistant *E. coli* could be a zoonotic concern in livestock-keeping households in low-income countries.

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Ethical approval

The study was ethically approved by the natural and computational sciences institutional review board committee, Addis Ababa University, Ethiopia (Ref. No. CNCSDO/423/14/2022). Moreover, informed consent was obtained from all study participants.

Declaration of competing interest

The authors declare no conflict of interest.

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Data Availability Statement

The data generated in this study will be made available upon the request of the corresponding author.

Declaration of generative AI and AI-assisted technologies

No generative AI and AI-assisted technologies were used.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2024.12.022.

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