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Original Article **Open Access**

Genetic relatedness of diarrheagenic *Escherichia coli* pathotypes isolated from children under five years of age and food animals in Kisumu County, Kenya

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Abstract:

Background: Diarrheal disease remains one of the leading causes of deaths in children below five years of age. The risk factors associated with diarrhea include poor hygiene practices such as open defecation and consumption of contaminated water and food. However, exposure of domestic animal is equally a potential risk factor for diarrhea disease in children.
Methodology: We characterized animal-related exposures in a subset of households (n=73) by collecting faecal samples from 150 children with diarrhoea and 100 food animals (30 cattle, 30 chicken, 25 goats and 15 pigs). *Escherichia coli* was isolated from the faecal samples and biochemically confirmed using conventional micro-biological techniques. The deoxyribonucleic acid (DNA) of each *E. coli* isolate was extracted and amplified by multiplex PCR to identify three diarrheagenic *E. coli* pathotypes. The amplified products were sequenced, and genetic relatedness of the isolates was determined through phylogenetic analysis.
Results: We isolated and identified a total of 32 (12.8%) diarrheagenic *E. coli* (DEC) from the 250 faecal samples, 26 (17.3%) of which were from the 150 children with diarrhea while 6 (6.0%) were from the 100 food animals (OR=3.285, 95% CI=1.299-8.305, p=0.011). Three DEC pathotypes were confirmed by PCR in 16 DEC strains, with 9 enteroaggregative *E. coli* (EAEC), 2 enterotoxigenic *E. coli* (ETEC), 2 enteropathogenic *E. coli* (EPEC), 1 EAEC/ETEC, 1 EAEC/EPEC and 1 ETEC/EPEC mixed strains. The phylogenetic analysis showed that 6 DEC isolates had genetic similarity ranging between 31% to 90%. Isolates S04 originating from animal and S02 from a child with diarrhoea of the same household were closely related, with 55% similarity. Moreover, isolate S05 from animal origin and S06 of diarrheic child origin were closely related, with similarity degree as high as 82% even though they were not paired. Twenty four of the 26 (92.3%) DEC isolates from diarrhoeic children showed multidrug resistance (MDR) pattern to antibiotics but none of the 6 isolates from food animals was multi-drug resistant.
Conclusion: The high degree of genetic relationship between DEC isolate S04 and S02 from animal and human origin indicated the high potency of zoonotic transmission. Further studies investigating animal husbandry practices and zoonotic transmission of DEC are needed.

Keywords: Diarrhoea; Zoonotic; Genetic relatedness; Multiplex PCR; Sequencing

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Relation génétique des pathotypes diarrhéiques d'*Escherichia coli* isolés chez des enfants de moins de cinq ans et des animaux destinés à l'alimentation humaine dans le comté de Kisumu, Kenya

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Résumé:

Contexte: Les maladies diarrhéiques restent l'une des principales causes de décès chez les enfants de moins de cinq ans. Les facteurs de risque associés à la diarrhée comprennent de mauvaises pratiques d'hygiène telles que la défécation à l'air libre et la consommation d'eau et d'aliments contaminés. Cependant, l'exposition des animaux domestiques constitue également un facteur de risque potentiel de diarrhée chez les enfants.
Méthodologie: Nous avons caractérisé les expositions liées aux animaux dans un sous-ensemble de ménages (n=73) en collectant des échantillons fécaux de 150 enfants souffrant de diarrhée et de 100 animaux destinés à l'alimentation (30 bovins, 30 poulets, 25 chèvres et 15 porcs). *Escherichia coli* a été isolée des échantillons fécaux et confirmée biochimiquement à l'aide de techniques microbiologiques conventionnelles. L'acide désoxyribonucléique (ADN) de chaque isolat d'*E. coli* a été extrait et amplifié par PCR multiplex pour identifier trois pathotypes diarrhéiques d'*E. coli*. Les produits amplifiés ont été séquencés et la parenté génétique des isolats a été déterminée par analyse phylogénétique.
Résultats: Nous avons isolé et identifié un total de 32 (12,8%) *E. coli* diarrhéiques (DEC) à partir des 250 échantillons fécaux, dont 26 (17,3%) provenaient des 150 enfants souffrant de diarrhée tandis que 6 (6,0%) provenaient des 100 animaux destinés à l'alimentation (OR=3,285, IC à 95%=1,299-8,305, *p*=0,011). Trois pathotypes DEC ont été confirmés par PCR dans 16 souches DEC, avec 9 *E. coli* entéroagrégatives (EAEC), 2 *E. coli* entérotoxinogènes (ETEC), 2 *E. coli* entéropathogènes (EPEC), 1 EAEC/ETEC, 1 EAEC/EPEC et 1 souches mixtes ETEC/EPEC. L'analyse phylogénétique a montré que 6 isolats DEC présentaient une similarité génétique comprise entre 31% et 90%. Les isolats S04 provenant d'un animal et S02 provenant d'un enfant souffrant de diarrhée du même ménage étaient étroitement liés, avec une similarité de 55%. De plus, l'isolat S05 d'origine animale et le S06 d'origine infantile diarrhéique étaient étroitement liés, avec un degré de similarité pouvant atteindre 82%, même s'ils n'étaient pas appariés. Vingt-quatre des 26 (92,3%) isolats DEC provenant d'enfants diarrhéiques présentaient un profil de multirésistance aux antibiotiques, mais aucun des 6 isolats provenant d'animaux destinés à l'alimentation n'était multirésistant.
Conclusion: Le degré élevé de relation génétique entre les isolats DEC S04 et S02 d'origine animale et humaine indique la forte puissance de transmission zoonotique. D'autres études portant sur les pratiques d'élevage et la transmission zoonotique du DEC sont nécessaires.

Mots-clés: Diarrhée; Zoonotique; Relation génétique; PCR multiplexe; Séquençage

Introduction:

Diarrhea is a significant public health challenge worldwide, impacting about 1.7 billion children and resulting in approximately 525,000 deaths among children under five years of age. According to the Global Burden of Diseases, Injuries and Risk Factors Study (GBD) 2016, diarrhea ranks among the top eight causes of death across all age groups and is the fifth leading cause of death specifically among children under five years old in low-and-middle-income countries (1).

There are various causes of diarrhea illness including diarrheagenic *Escherichia coli*, rotaviruses, shigella, campylobacter, adenovirus and cryptosporidium (2). However, among these, rotaviruses and pathogenic *E. coli* are the most profound causes of diarrhea in children. While *E. coli* is a normal part of the gut microbiota in humans and animals, certain strains can cause illnesses like diarrhea, gastroenteritis, urinary tract infections, and meningitis in children (3, 4). These pathogenic *E. coli* strains are identified by their virulence factors, which augment their ability to cause gastrointestinal infections in humans.

Diarrheagenic *E. coli* (DEC) is responsible for approximately 40% of acute diarrhea cases in children under five years of age (5). DEC have been classified into six major pathotypes based on their virulence factors including the enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), Shiga-toxin producing *E. coli* (STEC) commonly known as enterohemorrhagic *E. coli* (EHEC), diffusely adherent *E. coli* (DAEC), enterotoxigenic *E. coli* (ETEC), and entero-invasive *E. coli* (EIEC). Furthermore, horizontal gene transfer between the pathotypes can occur resulting in hybrid strains that are potentially more virulent and difficult to be grouped into the six major pathotypes (6). Among these pathotypes, EPEC has been known as an important clinical etiological agent for children under five years of age, however, ETEC, EAEC and EIEC are now emer-

ging as important etiological agents as well (7).

Poor hygienic practices such as open defecation, inadequate handwashing, and limited access to clean drinking water are key risk factors associated with the spread of DEC infections. However, interventions aimed at improving access to clean water and promoting better hygienic behaviors have been found to significantly reduce these risks (8). The primary sources of DEC infections include the consumption of contaminated food and water (9-12). Moreover, these infections can be zoonotic and previous studies have suggested that the presence of animals in domestic environment can serve as a potential source of diarrheal infections, making exposure to domestic animals a notable risk factor (12-14).

To explore this further, the study aimed to investigate the potential zoonotic transfer of pathogenic *E. coli* from animals to humans. This was done through genetic relatedness analysis of the pathogenic *E. coli* isolated from both children and domestic animals within the same households.

Materials and method:

Study area and duration:

This study was carried out in Kisumu County from August 2022 to February 2023. Kisumu has an annual rainfall of 276.22 mm and temperature of 23.93°C. It sits at an elevation of 1,131m (3,711ft). Kisumu is 200 miles northwest of Nairobi and it is located at the shores of Lake Victoria.

Study participants:

The study participants were children aged five years and below, with history of uncomplicated diarrhoea seeking medical treatment at Kisumu County Hospital and kept food domestic animals at their homesteads or at their guardians, and voluntarily consented to participate in the study. A total of 73 households with 150 children with diarrhoea under 5 years of age and 100 animals (30 cattle, 30 chicken, 25 goats and 15 pigs) were enrolled into the study.

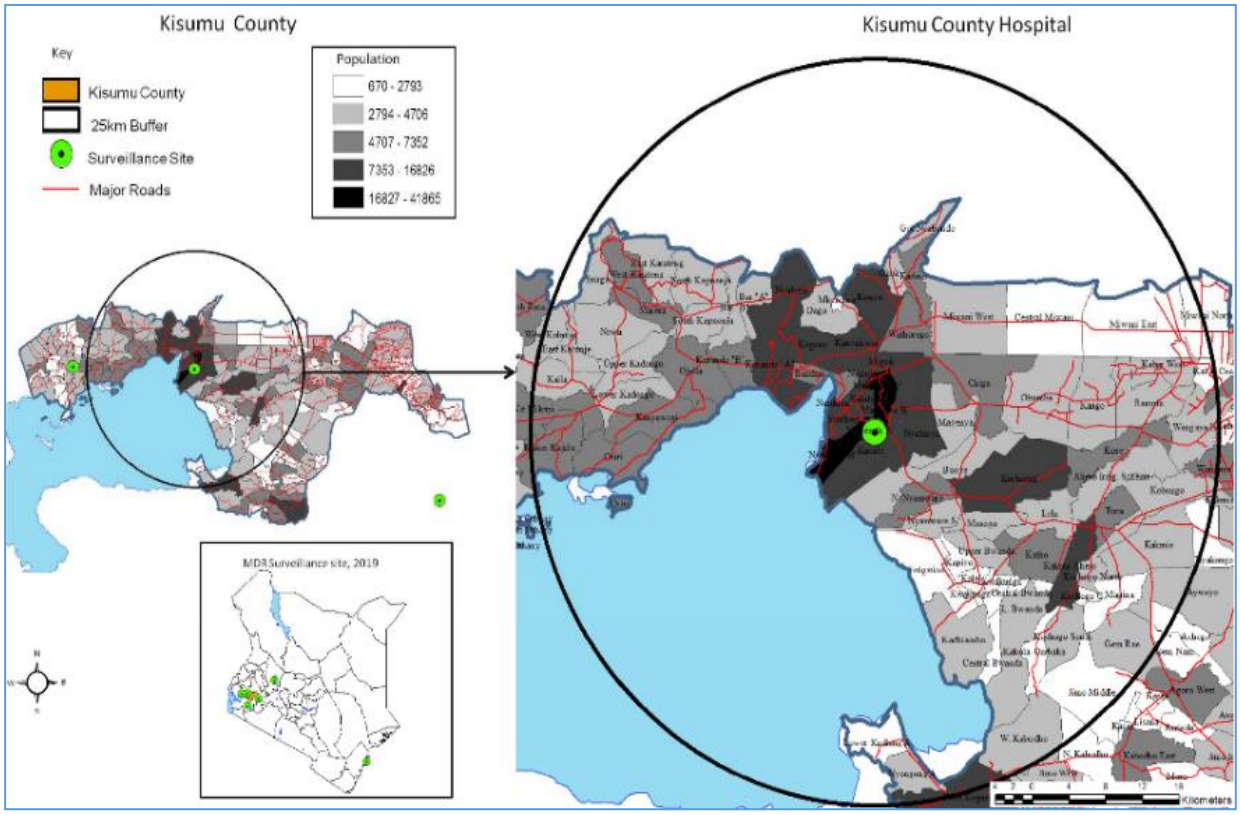


Fig 1: Map of Kisumu county showing the study site (Source: Government of Kenya website)

Sample collection:

Clean leak proof containers were given to the caregivers of children for stool sample collection while rectal swab was used to collect samples from anal canal of infants whose stool samples cannot be collected. Sterile cotton swab was used to take aliquot of the stool sample into Cary-Blair medium, which was then stored at 2^0C-8^0C until processed.

Stool sample containers were given to caregivers who confirmed the presence of diarrhoea in food animals at the households of selected children with diarrhoea. Stool samples were collected from the rectum of the food animals (after restraining the animals) using moist sterile swabs and placed in Cary-Blair medium. Data relating to the animal health, stool consistency, and hygiene condition were collected and matched with the unique personal identification number (PIN) of the children.

All samples were packaged and shipped in cool boxes with frozen ice packs at 2^0C-8^0C to KEMRI laboratory for microbiological analysis by trained laboratory technologists.

Ethical consideration:

The study was approved by Jaramogi Oginga Odinga Ethical Review Board REF: ISERC/JOOTRH/600/22. All parents and care takers of children below five years of age gave and signed informed consent form before children could participate in the study. Confidentiality was observed throughout the study period and electronic data were stored in password n-protected computer.

Isolation and identification of diarrheagenic *E. coli:*

Each faecal sample was streaked onto MacConkey agar (Oxford, Basingstoke, UK) plates using a sterile loop in a biosafety cabinet. Following incubation at 37°C for 18-24 hours, colonies with pink color are lactose fermenters and those with pale color are non-lactose fermenters. Subsequently, 3-5 well-separated lactose fermenting colonies were sub-cultured on nutrient agar to obtain pure *E. coli* cultures. The isolates were phenotypically confirmed as *E. coli* by standard biochemical tests. Pure colonies of *E. coli* isolates were preserved in tryptone soy broth supplemented with 20% glycerol and stored at -80°C for further analysis.

DNA Isolation:

Four to 5 colonies of pure *E. coli* from overnight growth on MacConkey agar were resuspended in 200 µl of nuclease-free water and thoroughly mixed using a vortex. Subsequently, the bacterial suspension was boiled at 95°C for 20 minutes. After centrifugation at 12,000×g for 10 minutes, the DNA-rich supernatant was collected and stored at -20°C (10).

Multiplex PCR assay:

Characterization of *E. coli* isolates into the three pathotypes (EAEC, EPEC, and ETEC) was conducted using multiplex PCR assay. Twenty-five microliter PCR reaction included 12.5µl of 2x DreamTaq Green PCR Master Mix from Thermo Scientific (Waltham, MA, USA), 0.5 µl (30µM) each of forward and reverse primers (see Table 1), 6.5 µl of nuclease-free water, and 5 µl of DNA template.

The PCR protocol was carried out in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) with an initial denaturation step at 95 °C for 5 minutes, followed by 25 cycles of amplification (94 °C for 1 minute, 58 °C for 1 minute and 30 seconds, and 72 °C for 1 minute and 30 seconds), and a final extension step at 72 °C for 10 minutes. Positive and negative controls were included using the ATCC 25922 strain and nuclease-free water, respectively.

Table 1: Primer sequences for the multiplex polymerase chain reaction for the detection of diarrheagenic *Escherichia coli*

Strain	Target gene	Primer	Primer sequence (5'-3')
ETEC	*elt*	LT-F	CACACGGAGCTCCTCAGTC
		LT-R	CCCCCAGCCTAGCTTAGTTT
	est	ST-F	GCTAAACCAGTAG/AGGTCTTCAAAA
		ST-R	CCCGGTACAG/AGCAGGATTACAACA
EPEC	*bfpA*	BFPA-F	GGAAGTCAAATTCATGGGGG
		BFPA-R	GGAATCAGACGCAGACTGGT
	eae	EAE-F	CCCGAATTCGGCACAAGCATAAGC
		EAE-R	CCCGGATCCGTCTCGCCAGTATTCG
EAEC	*aatA*	CVD432F	CTGGCGAAAGACTGTATCAT
		CVD432R	CAATGTATAGAAATCCGCTGTT
	aaiC	AAIC F	ATTGTCCTCAGGCATTTCAC
		AAIC R	ACGACACCCCTGATAAACAA

EAEC: Enteroaggregative *Escherichia coli*, ETEC: Enteropathogenic *Escherichia coli*, EPEC: Enteropathogenic *Escherichia coli*

Purification of the PCR amplicons, sequencing and phylogenetic analysis:

The PCR amplicons were purified using the Ambiclean kit according to the manufacturer's instructions. Subsequently, the purified amplicons of 11 *E. coli* isolates were sequenced using the Sanger sequencing platform. The nucleotide sequences obtained from sequencing were processed and analyzed using the BioEdit analysis tool. The resulting Fasta files were then queried against the NCBI database using the BLASTn similarity search tool.

Sequences showing at least 95% similarity and 95% coverage to *E. coli* were selected for further analysis. These sequences underwent alignment and comparison using the ClustalW alignment tool within MEGA 11 for genetic analysis. The aligned sequences were utilized to construct a phylogenetic tree employing the Maximum Likelihood (ML) method with 100 bootstraps for optimal phylogenetic tree scoring. The resulting tree was refined and formatted using Fig Tree software version 4.4.1.

Antimicrobial susceptibility test:

The Kirby Bauer disk diffusion susceptibility method was used to perform the antimicrobial susceptibility of *E. coli* isolates to 11 antimicrobial agents (ciprofloxacin 5µg, nalidixic acid 30µg, tetracycline 30µg, streptomycin 10µg, chloramphenicol 30µg, gentamicin 10 µg, ceftazidime 30µg, amoxicillin-clavulanic acid 20/10µg, sulfamethoxazole-trimethoprim 1.25/23.75 µg, and ampicillin 10µg) was determined following the guidelines of the 2022 Clinical and Laboratory Standards Institute (CLSI). *Escherichia coli* ATCC 25922 was used as the quality control strain. Interpretation of zone diameters of inhibition for each antimicrobial disk was based on the criteria outlined by CLSI, classifying the isolate as as sensitive, intermediate or resistant.

Statistical analysis:

Statistical analyses were performed using R statistical analysis tool and Excel. Descriptive statistics were analyzed and presented as frequencies and percentages.

Results:

Descriptive characteristics of study participants:

A total of 250 samples were collected from animals and children under five years of age with diarrhea between August 2022 and February 2023. Out of the 250, 150 were from children and 100 from animals (Table 2 and 3).

Table 2: Demographic characteristics of the children participants

Characteristics	No of children with diarrhea (n=150)	Percentage (%)
Gender		
Male	62	41.3
Female	88	58.7
Age group (in months)		
<12	26	17.3
12-23	40	26.7
24-35	32	21.3
36-47	33	22.0
≥ 48	19	12.7
Type of diarrhea stool		
Watery	64	42.7
Loose	53	35.3
Mucoid	33	22.0
Month of study		
August 2022	10	6.7
September 2022	13	8.7
October 2022	22	14.7
November 2022	28	18.7
December 2022	24	16.0
January 2023	36	24.0
February 2023	17	11.3
***Escherichia coli* strain**		
EAEC	18	12.0
ETEC	8	5.3
EPEC	5	3.3
ETEC and EAEC	2	1.3
EPEC and EAEC	2	1.3

EAEC = Enteroaggregative *Escherichia coli*; ETEC = Enterotoxigenic *Escherichia coli*; EPEC = Enteropathogenic *Escherichia coli*

Table 3: Demographic characteristics of the food animal participants

Characteristics	Total samples	No of positives	Percentage (%)
Age (years)			
<1	60	4	6.7
>5	40	2	5.0
Sex			
Male	50	3	6.0
Female	50	3	6.0
Type of food animals			
Goat	25	1	4.0
Cattle	30	4	13.3
Chicken	30	1	4.0
Pig	15	0	0

Prevalence of diarrheagenic *E. coli* infections:

Overall, diarrheagenic *E. coli* (DEC) infections were observed in 26 (17.3%) of the 150 children with diarrhoea (0.173; 95% CI: 0.1211-0.2419) while 6 (6.0%) of the 100 samples from the food animals were positive for DEC (0.06; 95% CI: 0.0278-0.1248). Our analysis showed that the prevalence of DEC was significantly higher in children with diarrhoea than the food animals (OR=3.285, 95% CI=1.299-8.305, p=0.011).

Prevalence of DEC pathotypes in children:

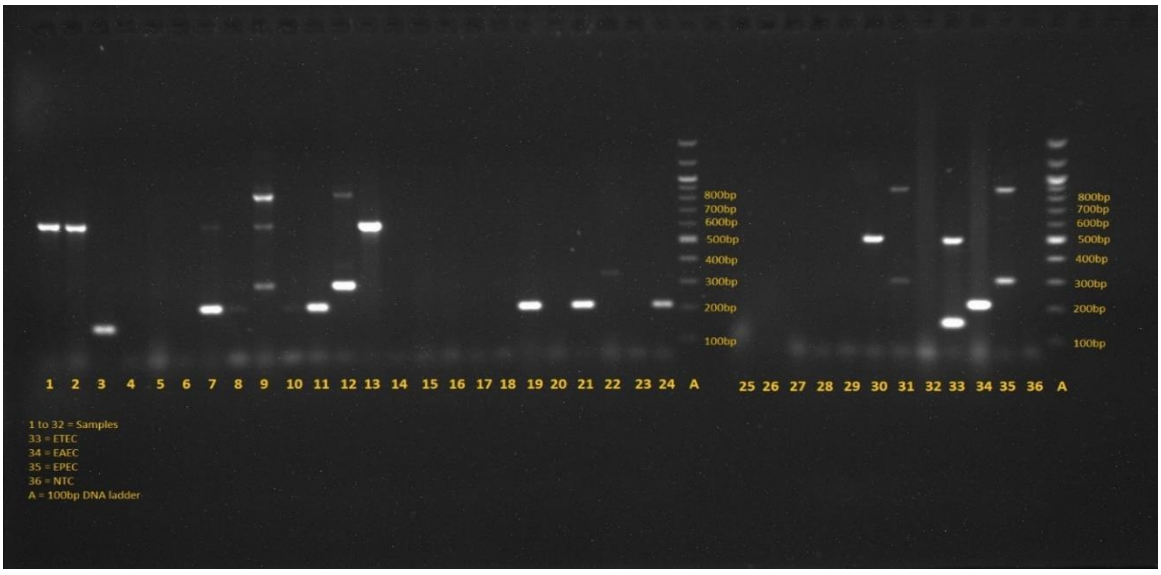
Of the 150 diarrhea samples from children with diarrhoea, different virulence genes were detected to identify the three DEC pathotypes (Fig 1). EAEC was the most predominant phenotype (18/150, 12.0%), followed by ETEC (8/150, 5.3%), EPEC (5/150, 3.3%) and mixed pathotypes (2/150, 1.3%).

Prevalence of DEC pathotypes in food animals:

Of the 100 diarrhea samples from animals, EPEC was the most predominant DEC pathotype from cattle (4/30, 13.0%) found in the children homesteads, followed by ETEC in chicken (1/30, 3.0%) and goats (1/25, 4.0%). Of the 15 pigs sampled, none of the pathotype strain was detected by PCR.

Antimicrobial sensitivity and resistance of DEC isolates from children:

The AST patterns of 26 DEC isolates from the children showed highest sensitivity to meropenem (84.6%, n=22), followed by gentamicin (80.8%, n=21), nalidixic acid (73.1%, n=19) and chloramphenicol (69.2%, n=18) (Fig 3). The antimicrobial resistance of the isolates showed 100.0% resistance to tetracycline and sulfamethoxazole-trimethoprim, 92.3% (n= 24) showed resistance to ampicillin, and 80. 8% (n=21) showed resistance to amoxicillin (Fig 4).



Positive pathotypes are seen at position 1,2,3,7,8,9,10,11,12,13,19,21,22,24,30 and 31. Of the 16 DEC positive isolates, 9 were EAEC (3 harboring *aatA* gene and 6 harboring *aaiC* gene), 2 were ETEC (1 harboring *st* gene and 1 harboring *lt* gene), 2 were EPEC (1 harboring *bfpA*/*eae* and 1 harboring *bfpA* gene), and 3 were mixed infections of EAEC/ETEC (harboring *aaiC* and *lt* gene), EPEC/EAEC (harboring *eae* and *aaiC* gene) and EPEC/EAEC (harboring 3 different genes *bfpA*, *aatA* and *eae* genes)

Fig 2: Gel images showing positive samples by PCR for the first 32 *E. coli* with 16 positives for different DEC pathotypes

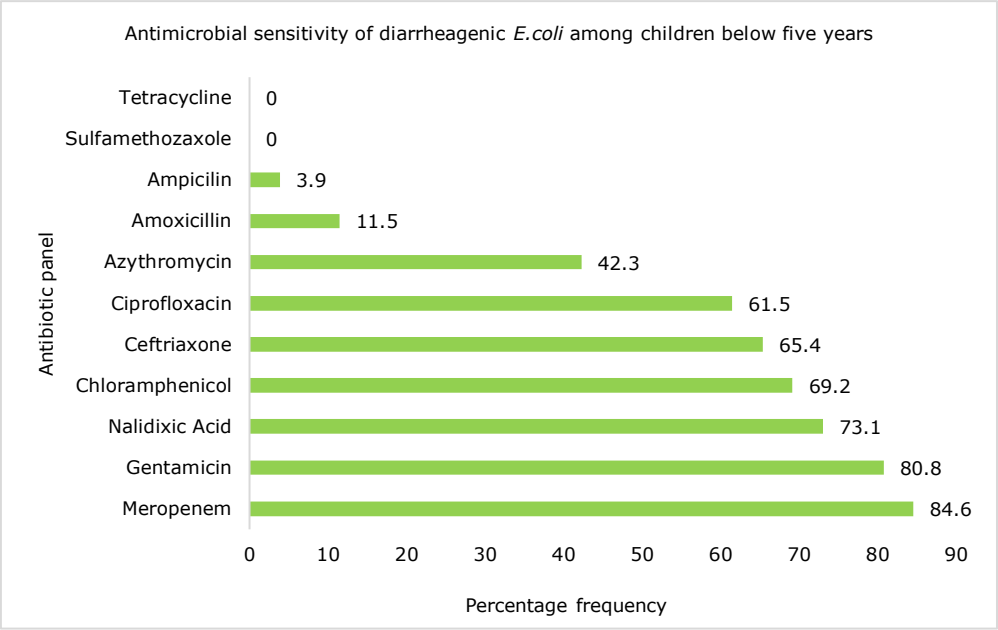


Fig 3: Antibiotic sensitivity of diarrheagenic *E. coli* isolated from children under five years of age with food animal contact

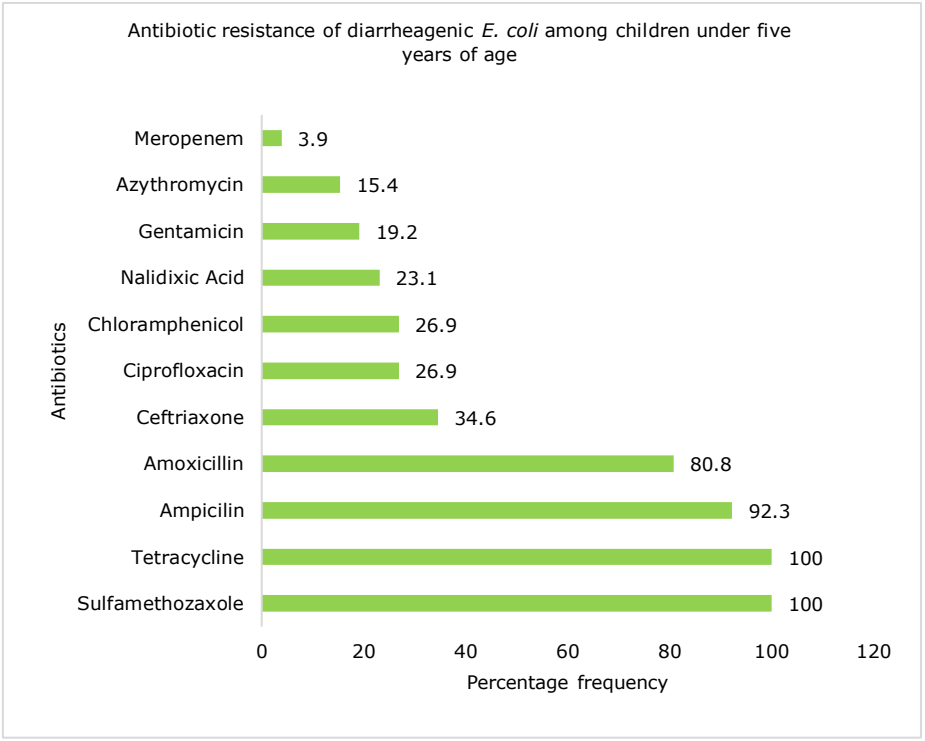


Fig 4: Antibiotic resistance of diarrheagenic *E. coli* isolated from children under five years of age

Multidrug resistance among DEC isolates from children:

Of the 26 DEC isolates, 24 (92.3%) were multidrug resistant (MDR), with resistance to antibiotics in three or more different categories; 11 (45.8%) were resistant to four different antibiotics, 9 (37.5%) to five and 1 (4.2 %) to six different antibiotics (Table 2).

All the EAEC strain in diarrheal children were resistant to tetracycline and sulfamethoxazole–trimethoprim while 85.7% and 78.6% were resistant to ampicillin and amoxicillin respectively. The ETEC and EPEC strains in diarrheal children were 100.0% resistant to sulfamethoxazole-trimethoprim, tetracycline and ampicillin while resistance to amoxicillin was

66.7% and 80% respectively. The mixed EAEC /ETEC and EAEC/EPEC strains exhibited high level resistance (100%) to sulfamethoxazole–trimethoprim, tetracycline, ampicillin and amoxicillin.

Table 2: Multiple drug-resistant DEC isolates from children

No of resistance	No of isolates (n=24)	Percentage (%)
4	11	45.8
5	9	37.5
6	1	4.2

Antimicrobial resistance patterns of DEC isolates from food animals:

Of the six food animals that were positive for various DEC strains, resistance was reported only to amoxicillin/ampicillin and azithromycin. The food animal isolates were 100% sensitive to the other eight antibiotics tested and none of the 6 isolates revealed multi-drug resistant (Fig 5).

Genetic relatedness of *E. coli* strains:

Our phylogenetic analysis indicated close genetic relatedness of *E. coli* strains isolated from animals clustering together with strains from children with diarrhea despite the diverse hosts and the environment from which they were isolated (Table 4).

Each node in the phylogenetic tree (Fig 6) represents divisions that are equal to the number of isolates belonging the common ancestor. The horizontal lines indicate the absolute distance between each isolate. The node sizes vary linearly with the number of isolates of a given type. The samples highlighted in red are strains isolated from children fecal matter while the ones in blue are isolates from food animals.

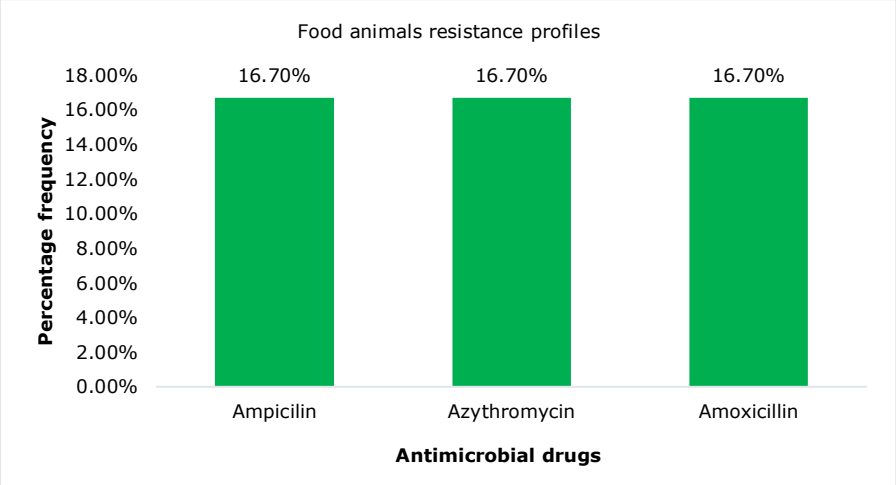


Fig 5: Antibiotic resistance of diarrheagenic *E. coli* isolates across the food animals

Table 4: Comparison of *Escherichia* coli isolates sequences with GenBank sequences showing coverage and similarity levels

Isolate	Source	Sequence with best match	Percentage coverage (%)	e-value	Percentage similarity (%)
S01	Child	*E. coli* strain EP259	100	2.00E-34	100
S02	Child	*E. coli* O157:H7 strain FRIK2069	91	0	100
S03	Animal	*E. albertii* RAC116A	92	0	97.58
S04	Animal	*E. coli* E110019	100	0	100
S05	Child	*E. coli* plasmid E873p3	100	0	99.53
S06	Animal	*E. coli* strain 372	81	1.00E-82	88.93
PosEPEC	Control	*E. coli* O157:H7 strain FRIK2069	92	0	96.96
PosCLT	Control	*E. coli* plasmid E873p3	100	0	100

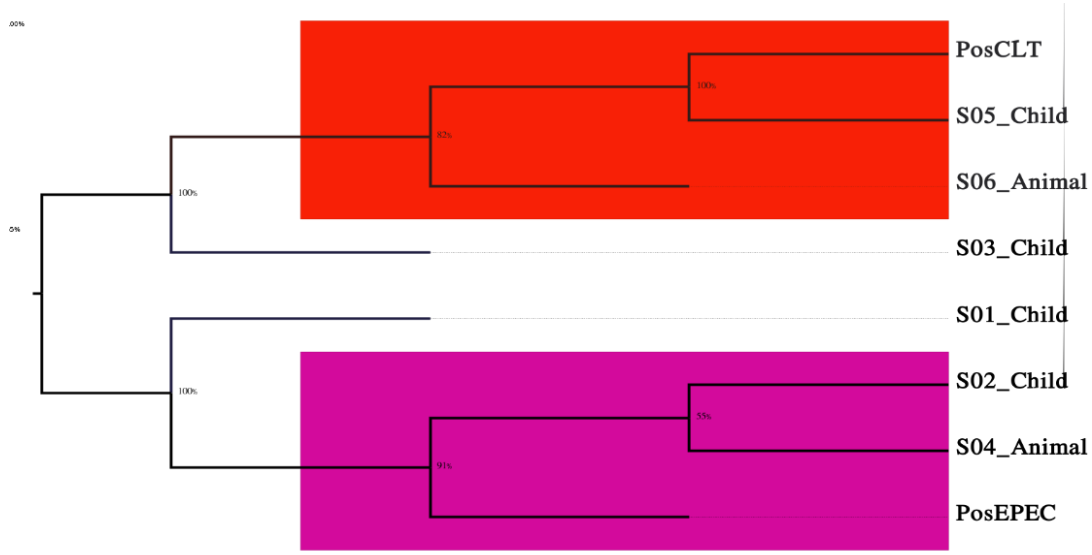


Fig 6: Genetic relatedness of six *Escherichia coli* isolates and two *Escherichia coli* control strains

Discussion:

In this study, we provide evidence that domesticated animals could serve as a source for the spread of pathogenic *E. coli*, as demonstrated by comparing the genetic similarities of *E. coli* strains isolated from animals and a child within the same household in Kisumu County, Kenya. Genetic identification of bacterial isolates is crucial for understanding transmission between two sources and determining whether the isolates are epidemiologically related (11). Two or more isolates are considered linked by direct transmission if they have fewer single nucleotide polymorphisms (15).

Our analysis relied on phylogenetic relatedness to assess the epidemiological significance of the isolates. Our findings revealed a S02 from a child source and isolate S04 from an animal belonged to a genetically related cluster that also included control PosEPEC. Identification analysis revealed that S02 showed a close relationship to *E. coli* O157:H7 strain FRIK2069, S04 to *E. coli* E110019 strain, and Pos EPEC to serotype O157:H7. The O157:H7 strain, a zoonotic pathogen carried by farm animals, has been reported to contribute to about 20% of global foodborne outbreaks (16,17). The clustering of the S02 isolate from a child with Pos EPEC aligns with previous studies indicating that children in households involved in animal husbandry are at risk of O157:H7 infection.

The observed relatedness between strains S04 and S02 suggests a common phylogenetic origin, indicating potential horizontal transmission of *E. coli* between animals and human. This raises concerns about the potential for such transmission routes. Domesticated animals are known to harbor pathogenic *E. coli* (18,19), which can be transmitted to man through contaminated food and water. Previous studies have demonstrated zoonotic transmission of *E. coli* between human, animals, and food sources. However, animal husbandry practices also contribute to this transmission risk. A study investigating factors related to animal contact and diarrhea found that owning domestic animals significantly increases the risk of infection (13). Furthermore, there have been observations of infants ingesting chicken fecal matter in rural areas of low and middle-income countries (20), although the exact nature of exposure remains unclear. Our findings build upon these studies, suggesting that animals can indeed serve as sources of contamination for human.

This study had some limitations. First, the number of paired samples i. e. households with both children and animals testing positive for *E. coli*, was limited. Second, the study design was cross sectional, which did not include follow-up of the study participants. Third, due to insufficient funding, the study could not enroll more samples or conduct genetic sequencing of the *E. coli* strains. Lastly, the study did not explore potential risk factors that could significantly contribute to the zoonotic transmission of *E. coli*. However, the observed relatedness between S04 and S02 implies a potential horizontal transmission of *E. coli* between animals and human, raising concerns about zoonotic transmission.

This study suggests that owning domestic animals significantly increases the risk of infection, providing important insights into the role of animal husbandry practices in the transmission dynamics. Furthermore, it emphasizes the importance of considering animal sources in public health strategies to mitigate the risk of pathogenic *E. coli* transmission.

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Contributions of authors:

RY, RO and BO prepared the study plan and carried out the study procedures; RY and EA prepared the original manuscript draft; JA was the bioinformatician who performed data analysis and interpretation; and GM, JG and GK reviewed and edited the manuscript All authors approved the final manuscript version submitted for publication.

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