EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

One Health Approach to Biofilm Formation and Extended-Spectrum Beta-Lactamase Mediated Antibiotic Resistance in Escherichia coli O157:H7 in Arusha Region, Tanzania

Deusdedit A Mosha[a, c*], Debora Charles Kajeguka[a, b], Sixbert Isdory Mkumbaye[a, b]

[a]Kilimanjaro Christian Medical Centre, Moshi, Tanzania; [b]Kilimanjaro Christian Medical Centre, University, Moshi, Tanzania; [c]Mount Meru Regional Referral Hospital, Arusha, Tanzania

Correspondence to Deusdedit A Mosha (deus.mosha82@gmail.com)

ABSTRACT

Background: The One Health concept recognizes the interconnectedness of human, animal and environmental health in the emergence and spread of antimicrobial resistance. Zoonotic pathogens such as *Escherichia coli* O157:H7 can circulate between humans, livestock and environmental reservoirs. Its ability to form biofilms enhances persistence and survival, while the production of extended-spectrum beta-lactamases (ESBLs) contributes to resistance and limits treatment options.

Objective: To investigate the biofilm formation, extended-spectrum beta-lactamase production, and antibiotic resistance profiles of *Escherichia coli* O157:H7 isolates from humans, cattle and hospital sewage systems in the Arusha Region and to assess their interrelated associations.

Methods: A cross-sectional study was conducted in the Arusha Region, Tanzania. A total of 371 samples were involved. Shiga toxin producing E coli were isolated from clinical human cases, fresh feces from cattle and hospital sewage. Isolated bacteria were identified using standard microbiological techniques and antimicrobial susceptibility was evaluated according to current Clinical and Laboratory Standards Institute (CLSI) guidelines. Ability to form biofilms was assessed using the crystal violet microtiter plate assay. Phenotypic ESBL production was detected using the combined disk diffusion test (CDDT). Data analysis used IBM SPSS Statistics for Windows version 25.0 (IBM Corp, Armonk, NY, USA).

Results: *Escherichia coli* O157:H7 was detected across sectors with higher prevalence in cattle (n=12, 16.7%) compared to humans (n=7, 3.5%) and hospital sewage (n=2, 2.0%). Overall, 15(71.4%) isolates were multidrug resistance. High resistance was observed to ampicillin (n=17, 81.0%), tetracycline (n=15, 71.4%), trimethoprim-sulfamethoxazole (n=14, 66.6%), and ceftazidime (n=11, 52.4%). Production of ESBL occurred in 4(19.0%) isolates. Biofilm formation was observed in 8(38.1%) isolates and was significantly associated with tetracycline resistance (χ^2=8.00, P=.018).

Conclusion: The coexistence of biofilm formation and ESBL production in Escherichia coli O157:H7 highlights the importance of a One Health approach in antimicrobial resistance monitoring. Integrated surveillance, antimicrobial stewardship and improved environmental and veterinary controls are essential to reduce zoonotic transmission and mitigate antimicrobial resistance.

BACKGROUND

Escherichia coli O157:H7 is a highly pathogenic strain of *E. coli* that continues to pose serious global public health concerns due to its ability to cause life-threatening gastrointestinal and systemic illnesses such as hemorrhagic colitis and hemolytic uremic syndrome (HUS)[1,2]. Unlike most commensal E. coli strains that reside harmlessly in the human gut, *E. coli* O157:H7 is characterised by the production of potent Shiga toxins (Stx1 and Stx2), which can damage endothelial cells and lead to renal failure, particularly in children and the elderly[3,4]. The strain is zoonotic and primarily associated with ruminants, especially cattle, which act as asymptomatic carriers and can shed the bacteria into the environment, contaminating water, food, and surfaces[1]. As a result, human infections often arise from consuming undercooked meat, unpasteurised dairy products, contaminated water, or through direct contact with infected animals[5].

Globally, *E. coli* O157:H7 has been linked to numerous outbreaks, raising concerns about food safety, animal husbandry practices, water sanitation, and antimicrobial stewardship[6,7]. Its ability to survive and persist in environmental matrices is largely attributed to its capacity to form biofilms[8,9]. These biofilms protect the bacteria from antibiotics, disinfectants, and the host immune response, allowing them to endure in hostile environments for extended periods[10]. This biological adaptation not only contributes to the pathogen's environmental resilience but also complicates treatment strategies, particularly when

biofilm formation is coupled with antibiotic resistance mechanisms[11,12].

One of the most alarming resistance mechanisms seen in pathogenic *E. coli*, including O157:H7, is the production of extended-spectrum beta-lactamases (ESBLs)[13]. The ESBLs are enzymes that hydrolyse a wide range of beta-lactam antibiotics, including third-generation cephalosporins and monobactams, rendering them ineffective[14]. The prevalence of ESBL-producing *E. coli* strains is increasing globally and has been strongly associated with treatment failures, longer hospital stays, and increased mortality[15]. Furthermore, studies have shown that bacteria residing within biofilms often carry more resistance genes and are more likely to be multidrug-resistant (MDR), compounding the challenge[16]. This dual ability to form biofilms and produce ESBLs enables *E. coli* O157:H7 to persist in various environments and resist treatment, presenting a significant risk to public health[17].

In the African context, study from Nigeria show antimicrobial resistance and bacterial pathogenicity are further exacerbated by factors such as unregulated antibiotic use in human and veterinary medicine, inadequate sanitation, and poor infection control measures[18]. In East Africa, including Kenya, study show livestock and hospital effluents have been implicated as sources of resistant *E. coli* strains[19], while in Uganda, study show environmental waters and wastewater have harboured multidrug-resistant E. coli with high biofilm formation potential[20]. In Tanzania, although the awareness of antimicrobial resistance is growing, studies that focus specifically on the dual threat of biofilm formation and ESBL-mediated resistance in *E. coli* O157:H7 remain scarce. However, a study from Morogoro shows the presence of *E. coli* O157:H7 in livestock environments, food products, hospital patients, and water sources, often with multidrug resistance profiles[21].

Arusha Region in northern Tanzania is a hotspot for human-animal-environment interaction due to its high population density, intensive livestock farming, and inadequate infrastructure for waste management. Hospitals in the region often discharge untreated sewage into the environment, while livestock waste is commonly used untreated or poorly managed, leading to contamination of water sources[39]. "One Health" concept recognizes the interconnectedness of animal, human and environmental health. Understanding the circulation of biofilm formation and ESBL mediated antibiotic-resistance *E. coli* O157:H7 across these domains is crucial for effective control strategies[7,22]. However, there is limited information on biofilm formation and ESBL-mediated resistance in *E. coli* O157:H7 isolates from humans, cattle and hospital sewage systems in Arusha Region, Tanzania. This knowledge gap restricts the understanding of the epidemiological links between human, animal and environmental reservoirs and hinders the development of effective antimicrobial resistance control measures.

Therefore, this study aimed to determine the prevalence of biofilm formation and ESBL-mediated antibiotic resistance among *E. coli* O157:H7 isolates obtained from humans, cattle and hospital sewage systems in Arusha Region, Tanzania within a One Health framework.

METHODS

Study Design, Setting and Population

This was a cross-sectional study conducted between October 2024 and May 2025 with the aim of determining the biofilm formation property and proportion of ESBL production on *E. coli* O157:H7. The study was conducted in Mount Meru Regional Referral Hospital, Kaloleni Health Centre and Arusha Meat Company located in Arusha. The study population consisted of human, animal and environment samples. Human samples were obtained from individuals presenting with watery, bloody, mucous or normal diarrhoea. Animal samples comprised fresh faecal specimens collected from cattle in slaughter house. Environmental samples included wastewater effluent collected from a healthcare facility.

Eligibility Criteria

The inclusion criteria for this study encompassed both inpatients and outpatients, attending Mount Meru Regional Referral Hospital and Kaloleni Health Center with previously, frequently, and consistently reported cases of diarrhoea. Only confirmed isolates of *E. coli* O157:H7 from human, cattle, and hospital wastewater samples that were subjected to antibiotic susceptibility testing (AST), biofilm formation assays and extended-spectrum beta-lactamase (ESBL) production testing were included in the analysis.

Sample Size and Sampling

From Cochran's formula[40].

$n=z^2p(1-P)/e^2$ Where: n=sample size, z=corresponding level of confident=1.96, p=proportion of the characteristics of interest, e=margin of error=0.05

The human sample size obtained was based on the prevalence of 15.3%.[4]

n=(1.96)2 x 0.153 (1-0.153) / (0.05)2 = 199.

For the cattle sample size (72) a prevalence of 4.7%[23] was used, while the calculation of a 100 sewage sample size, employed a prevalence of 7.0%[24].

Purposive sampling technique was used.

Study Variables

The independent variables included the source of the isolates, participants socio-demographic and clinical characteristics the type of antibiotics used for antimicrobial susceptibility testing and environmental factors such as wastewater pH and temperature. The dependent variables were antibiotic resistance, biofilm formation and extended-spectrum beta-lactamase (ESBL) production.

Data Collection Tools, Methods and Procedures.

Data collection involved obtaining diarrhoeal stool samples from human patients, fresh faecal samples from cattle, and hospital sewage, all using sterile swabs placed into Cary-Blair transport medium. For human participants, demographic information such as age and sex was recorded in a data collection sheet. The pH and temperature were measured and documented at the point of hospital sewage collection. All samples were labelled, stored at 4°C to 8°C in a cool box, and transported to the laboratory within 24 hours for analysis.

Specimen Collection and Sampling Technique

Specimens were collected from three sources in Arusha Region, Tanzania: diarrheal stool specimens from patients attending selected healthcare facilities, faecal samples from cattle at slaughterhouses are facilities where animals are killed for meat production, including informal or small-scale operations and abattoirs are more formalized and regulated facilities with standardized hygienic slaughter and meat inspection procedures, and sewage samples from hospital drainage systems. Sampling procedures were standardized to ensure methodological consistency across all sources. All samples were collected aseptically using sterile swabs and immediately placed in Cary–Blair transport medium.

Human stool samples were obtained from patients presenting with acute diarrhea after informed consent was obtained. Cattle faecal specimen were collected either directly from the rectum or immediately after defecation on clean surfaces at slaughter facilities refers to surfaces that were cleaned and free from visible contamination to minimize cross-contamination of fecal samples. Sewage samples were obtained from open drainage points within hospital premises. Each swab was inserted into a Cary–Blair transport tube and labelled with the source, date, and sampling location.

For sewage samples, pH and temperature were measured at the time of collection using a portable pH meter and thermometer. All specimens were transported to the microbiology laboratory in cool boxes maintained at 4–8 °C and processed within 24 hours to preserve microbial viability.[25]

Bacterial Isolation and Identification

E. coli isolates were identified by colony characteristics on MacConkey agar (Condalab, Spain) as well as using Gram stain (Condalab, Spain). The isolates gave a distinct pink colony when growing on MacConkey agar due to fermentation of lactose and were colourless on Sorbitol MacConkey agar since this bacterial strain does not ferment sorbitol. Isolates also gave positive reaction for biochemical tests like Indole, catalase, lactose and glucose fermentation, but negative for Urease production, Citrate utilisation, hydrogen sulfide production, sorbitol fermentation and Oxidase. Further identification of *E. coli* O157:H7 was done using the sorbitol utilisation test on Sorbitol MacConkey agar (Condalab, Spain). Serotyping was done using antisera for O157:H7 antigen (Denka, Japan).[13,26–28]

Antimicrobial Susceptibility Testing

A 0.5 MacFarland suspension of pure E. coli O157:H7 colonies was prepared in a normal saline solution. Inoculation of the test pathogen for the antimicrobial susceptibility test (AST) was conducted on Muller-Hinton agar by soaking an inoculating swab one time into a suspension and then swiping the overflown sample on the mouth of the tube using the lawn culture. Antimicrobials for the sensitivity test were chosen based on the CLSI recommendation for Enterobacteriaceae family. The AST was performed against the following antimicrobials which belong to Antimicrobials were grouped into nine classes as follows: penicillins (Ampicillin, AMP); third-generation cephalosporins (Ceftriaxone, CTR; Cefotaxime, CTX; Ceftazidime, CAZ; Ceftazidime–Clavulanic Acid, CZA); fluoroquinolones (Ciprofloxacin, CIP; Nalidixic Acid, NA); folate pathway inhibitors (Trimethoprim–Sulfamethoxazole, COT); phenicols (Chloramphenicol, C); tetracyclines (Tetracycline, TE); macrolides (Erythromycin, E); and carbapenems (Meropenem, MRP)[29]

Extended-Spectrum Beta-Lactamase production test

Phenotypic detection of ESBL production was performed using the combined disk diffusion test (CDDT). Bacterial isolates were tested on Mueller-Hinton agar using disks containing ceftazidime (30 µg), ceftazidime-clavulanic acid (30/10 µg), and cefotaxime (30 µg). Plates were incubated between 35°C and 37°C for 16 hours to 18 hours. An increase in the inhibition zone diameter of ≥5 mm for the ceftazidime-clavulanic acid disk compared to ceftazidime alone was interpreted as positive for ESBL production, according to CLSI guidelines.[13]

Detection of Biofilm Formation

Biofilm formation was assessed using the microtiter plate assay. Briefly, bacterial suspensions adjusted to 0.5 McFarland standards were inoculated into 96-well polystyrene microtiter plates containing Tryptic soy broth with 1% glucose and incubated at 37°C for 24 hours. Wells were washed with phosphate-buffered saline (PBS), fixed with methanol, stained with 0.1% crystal violet, and then rinsed. The bound dye was solubilised with ethanol, and optical density (OD) was measured at 570 nm using a microtiter plate reader. Biofilm formation was categorised based on OD values relative to the negative control.[30,31]

Quality Assurance Measures

A positive control from the American Type Culture Collection (ATCC) was employed. For the confirmation of extended-spectrum beta-lactamase (ESBL) production, both positive and negative controls were incorporated at every testing phase: *Klebsiella pneumoniae* ATCC 13883 served as the ESBL-positive control, while *Escherichia coli* ATCC 25922 were used as the negative control. The same *E. coli* ATCC 25922 strains was also employed as a quality control strain for antimicrobial susceptibility testing (AST). In the biofilm formation assay, Pseudomonas aeruginosa ATCC 27853 was used as the positive control, while sterile Tryptic Soy Broth (TSB) supplemented with 1% glucose served as the negative control.

Data Analysis

Data analysis employed IBM SPSS Statistics version 25.0 (IBM Corporation, Armonk, New York, USA). Descriptive statistics was used to summarise socio-demographic and clinical characteristic. Median and interquartile ranges were employed for continuous variables because the data were not normally distributed, while categorical variables were presented by using tables. To evaluate associations between phenotypic antibiotic resistance and the presence of biofilm formation, the Chi-square test were applied. When the expected frequency in any of the contingency is less than five, Fisher's Exact Test was used instead. A *P* value of less than .05 was considered statistically significant in all tests. Results were reported using cross-tabulation tables.

Ethical Considerations

Ethical approval to conduct the study was obtained from KCMC University's Research Ethics Review Committee with ethical clearance number PG: 183/2024. Permission letters were written to all study sites before sample collection. Confidentiality of human participants' information was maintained. Consent to participate in the study was obtained from participants by signing consent or assent forms. All participants were given the right of subjects to participate or reject in the study.

RESULTS

Socio-Demographic Characteristics of Study Participants

A total of n= 199 participants were included in the study. Regarding age distribution, 118(59.3%) were aged less than 18 years, and 81(40.7%) were aged above 18 years and above. With respect to sex, 119(59.8%) of participants were male and 80(40.2%) were female. The majority of participants 163(81.9%) resided in urban areas, while 36(18.1%) were from rural areas. In terms of education, 76(38.2%) had no formal education, 40(20.1%) had completed primary education, 41(20.6%) had secondary education, and 42(21.1%) had attained tertiary education. Most participants were single 143(71.9%), while 32(16.1%) were married, 2(1.0%) were separated, 3(1.5%) were widower and Cohabited were 19(9.5%). Regarding occupation, 142(71.4%) of the participants were not employed, 38(19.1%) were self-employed, and only 19(9.5%) were employed in the formal sector (Table 1).

TABLE 1: Demographic and Clinical Characteristics (n=199), and Hospital Sewage Characteristics (n=100)

Variables	Frequency (n)	Percentage (%)
Human participants (n=199)		
Age in years		
<18	118	59.3
≥18	81	40.7
Median IQR	9 (2, 27)	
Sex		
Female	80	40.2
Male	119	59.8
Residence		
Rural	36	18.1
Urban	163	81.9
Educational status		
No formal education	76	38.2
Primary education	40	20.1
Secondary education	41	20.6
Tertiary education	42	21.1
Marital status		
Cohabited	19	9.5
Married	32	16.1
Separated	2	1.0
Single	143	71.9
Widow/Widower	3	1.5
Occupation		
Employed	19	9.5
Not-employed	142	71.4
Self-employed	38	19.1
Clinical characteristics		
Stool characteristics		
Bloody	18	9.0
Mucus	45	22.6
Normal	22	11.1
Watery	114	57.3
Abdominal pain		
No	67	33.7
Not specified	37	18.6
Yes	95	47.7
Hospital sewage characteristics (n=100)		
pH of the hospital sewage water		
7.1	30	30.0
7.2	30	30.0
7.5	40	40.0
Temperature of hospital sewage water		
23.70	30	30.0
25.20	30	30.0
28.65	40	40.0

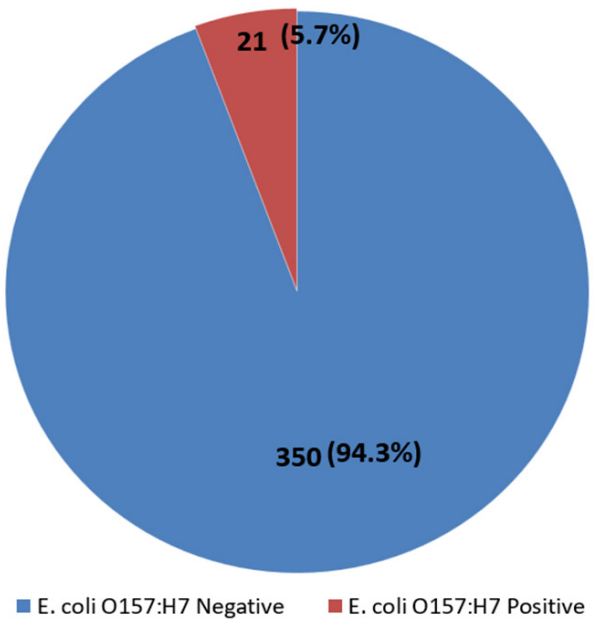
Continue

TABLE 1: Continue

Prevalence of *E. coli* O157:H7 isolates among all study participants.

Out of 371 samples analyzed the overall prevalence of *Escherichia coli* O157:H7 was 21 (5.7%) (Figure 1).

FIGURE 1: Combined Prevalence of *E. coli* O157:H7 Isolates Among Collected Specimens (N=371)



Prevalence of E. coli O157:H7 in Human, Cattle and Hospital Sewage Specimens

The pathogen was isolated from 12 (16.7%) of cattle samples, 7 (3.5%) of human stool samples, and 2 (2.0%) of hospital sewage samples (Figure 2).

Antibiotics Resistance Profiles of *E. coli* O157:H7 Isolates

A total of 21 *Escherichia coli* O157:H7 isolates were tested against different antibiotics. High resistance was observed to erythromycin 20 (95.2%), ampicillin 17 (81.0%), tetracycline 15 (71.4%), and trimethoprim-sulfamethoxazole 14 (66.7%), indicating substantial resistance to commonly used antibiotics. In contrast, most isolates were susceptible to meropenem 19 (90.5%), chloramphenicol 18 (85.7%), ceftriaxone 17 (81.0%), and cefotaxime 16 (76.2%). Multidrug resistance was identified in 15 (71.4%) isolates, suggesting a high burden of antimicrobial resistance among the tested isolates (Table 2).

Proportion of Biofilm Formation in *E. coli* O157:H7 Isolates.

Among the 21 isolates of *Escherichia coli* O157:H7, 8 (38.1%) demonstrated biofilm-forming ability, with 4 (19.0%) classified as strong biofilm formers 3 (14.3%) as moderate formers and 1 (4.8%) as a weak biofilm former. The remaining 13 isolates (61.9%) did not exhibit any biofilm formation (Table 3).

Proportion of ESBL-Producing *Escherichia coli* O157:H7 Isolates

Among the 21 Escherichia coli O157:H7 isolates tested, 4 (19.0%) were confirmed as extended-spectrum beta-lactamase (ESBL) producers, while 17 (81.0%) were non-ESBL producers. Of the ESBL-producing isolates, 2 were recovered from hospital sewage samples and 2 from human samples (Table 4).

Association Between Biofilm Formation and Antibiotics Resistance

Resistance to ampicillin, and Trimethoprim sulfa/ Cotrimoxazole was observed primarily among the strong and moderate biofilm producers and slightly associated with biofilm formation (p=.06), while the weak biofilm former remained fully susceptible to most antibiotics tested. Tetracycline resistance showed a statistically significant association with biofilm formation (χ^2 = 8.00, p =.018), confirming that strong and moderate biofilm producers were more resistant to antibiotic compared to the weak biofilm former (Table 5).

FIGURE 2: Prevalence of *E. coli* O157:H7 in Human, Cattle and Hospital Sewage Specimens

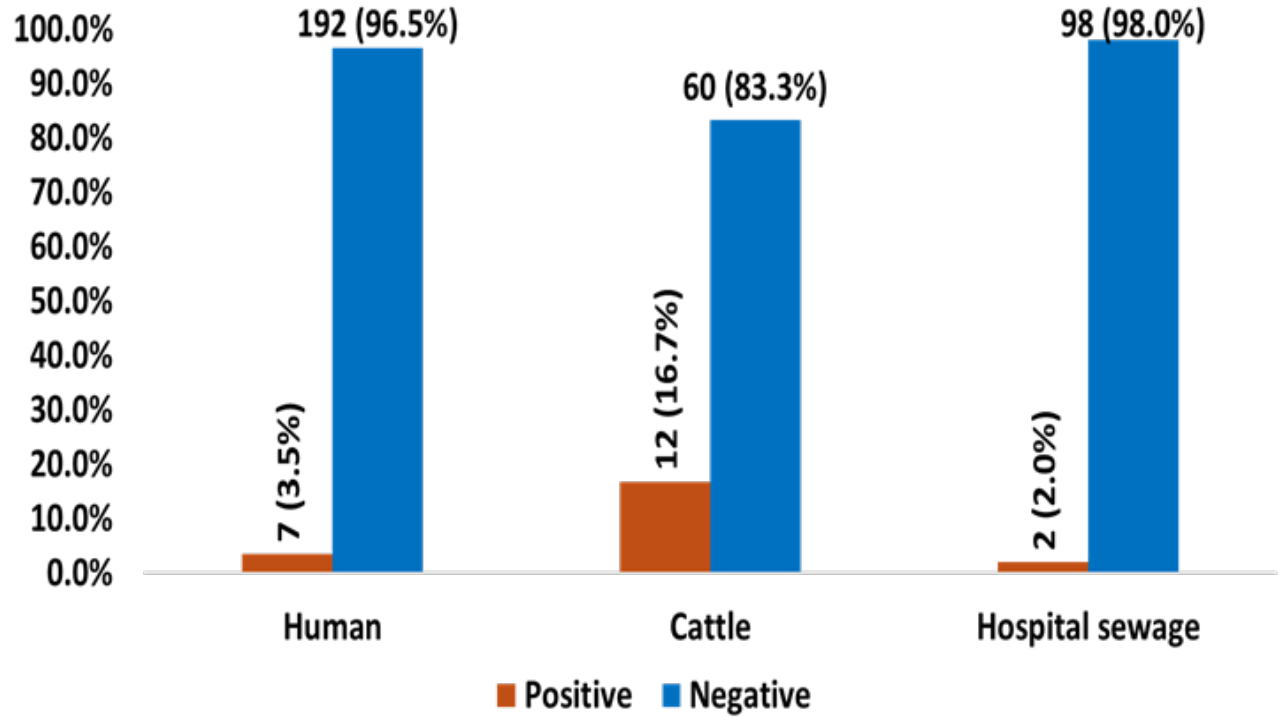


TABLE 2: Antibiotics Resistance Profiles of E. coli O157:H7 (n=21)

Antibiotics	Resistance n (%)	Susceptible n (%)
Ampicillin	17(81.0)	4(19.0)
Ciprofloxacin	6(28.6)	15(71.4)
Meropenem	2(9.6)	19(90.4)
Ceftriaxone	4(19.0)	17(81.0)
Cefotaxime	5(23.8)	16(76.2)
Ceftazidime-Clavulanate	12(57.1)	9(42.9)
Chloramphenicol	3(14.3)	18(85.7)
Trimethoprim sulfa/Cotrimoxazole	14(66.7)	7(33.3)
Ceftazidime	11(52.4)	10(47.6)
Erythromycin	20(95.2)	1(4.8)
Nalidixic acid	8(38.1)	13(61.9)
Tetracycline	15(71.4)	6(28.6)

Note: Multidrug resistant (MDR) – 15(71.4%)

TABLE 3: Proportion of Biofilm Formation Among *E. coli* O157:H7 (n=21)

Source of Isolation	Strong Biofilm Formation n (%)	Moderate Biofilm Formation n (%)	Weak Biofilm Formation n (%)	None Biofilm Formation n (%)	Total n (%)
Cattle	2(9.5)	2(9.5)	1(4.8)	7(33.3)	12(57.1)
Human	2(9.5)	1(4.8)	0(0)	4(19.0)	7(33.3)
Hospital sewage	0(0.0)	0(0.0)	0(0.0)	2(9.5)	2(9.5)
Total	4(19.0)	3(14.3)	1(4.8)	13(61.9)	21(100.0)

TABLE 4: Proportion of ESBL Producers (n=21)

Source of Isolation	Negative n (%)	Positive n (%)	Total n (%)
Cattle	12(100.0)	0(0.0)	12(57.2)
Hospital sewage water	0(0.0)	2(100.0)	2(9.5)
Human	5(71.4)	2(28.6)	7(33.3)
Total	17(81.0)	4(19.0)	21(100.0)

TABLE 5: Association Between the Biofilm Formation and Antibiotics Resistance (Chi-Square Test, *P* Value)

Antibiotics	Moderate Biofilm Formation n (%)	Strong Biofilm Formation n (%)	Weak Biofilm Formation n (%)	χ2 Statistic	*P* Value[a]
	3(37.5)	4(50)	1(12.5)		
Ampicillin				9.00	.061
Resistance	3(42.9)	3(42.9)	1(14.3)		
Susceptible	0(0.0)	1(100.0)	0(0.0)		
Ciprofloxacin				1.33	.51
Resistance	2(50.0)	2(50.0)	0(0.0)		
Susceptible	1(25.0)	2(50.0)	1(25.0)		
Meropenem				1.90	.39
Resistance	1(100.0)	0(0.0)	0(0.0)		
Susceptible	2(28.6)	4(57.1)	1(14.3)		
Ceftriaxone				1.14	.56
Resistance	0(0.0)	1(100.0)	0(0.0)		
Susceptible	3(42.9)	3(42.9)	1(14.3)		
Cefotaxime				2.78	.60
Resistance	1(50.0)	1(50.0)	0(0.0)		
Susceptible	2(33.3)	3(50.0)	1(16.7)		
Ceftazidime-clavulanate				9.00	.061
Resistance	3(42.9)	4(57.1)	0(0.0)		
Susceptible	0(0.0)	0(0.0)	1(100.0)		
Chloramphenicol				1.14	.56
Resistance	0(0.0)	1(100.0)	0(0.0)		
Susceptible	3(42.9)	3(42.9)	1(14.3)		
Trimethoprim sulfa/cotrimoxazole				9.00	.061
Resistance	3(42.9)	4(57.1)	0(0.0)		
Susceptible	0(0.0)	0(0.0)	1(100.0)		
Ceftazidime				4.00	.41
Resistance	3(50.0)	3(50.0)	0(0.0)		
Susceptible	0(0.0)	1(50.0)	1(50.0)		
Erythromycin					
Resistance	3(37.5)	4(50.0)	1(12.5)		
Nalidixic acid				2.89	.58
Resistance	2(40.0)	3(60.0)	0(0.0)		
Susceptible	1(33.3)	1(33.3)	1(33.3)		
Tetracycline				8.00	.018
Susceptible	0(0.0)	0(0.0)	1(100.0)		
Resistance	3(42.9)	4(57.1)	0(0.0)		

[a]*P* value for Fisher's exact test

DISCUSSION

This study aimed to investigate biofilm formation, ESBL production, and antibiotic resistance profiles of *Escherichia coli* O157:H7 isolates from humans, cattle, and hospital sewage in the Arusha Region, and to assess the interrelationships among these characteristics within a One Health framework. The findings showed that *E. coli* O157:H7 was detected across all sampled sectors, with a higher occurrence in cattle compared to humans and hospital sewage. Most isolates exhibited multidrug resistance, with notable resistance to commonly used antibiotics such as ampicillin, tetracycline, trimethoprim-sulfamethoxazole, and ceftazidime. ESBL production was present in a subset of isolates, while biofilm formation was also observed and found to be significantly associated with tetracycline resistance. These results indicate widespread antimicrobial resistance and biofilm-forming potential across human, animal, and environmental sources, suggesting interconnected transmission dynamics within a One Health context.

This study demonstrated significant variation in the prevalence of *Escherichia coli* O157:H7 across different reservoirs, with cattle showing the highest colonization rate. This highlights cattle as a key reservoir for zoonotic transmission and foodborne infection in humans. These findings are consistent with a study conducted in Iraq[2]. Which also reported high prevalence in cattle. The similarity may be due to comparable study designs and diagnostic methods, although the types of samples collected differed. Clinicians should therefore maintain a high index of suspicion for *E. coli* O157:H7 infection

in patients with livestock or food exposure, particularly those presenting with severe gastrointestinal symptoms.

Most isolates in this study were multidrug resistant, particularly to ampicillin and tetracycline, aligning with previous findings from Nigeria[32]. Differences in multidrug resistance patterns may reflect variations in bacterial transmission dynamics, sample types, and exposure risk across human, animal, and environmental sources. The findings support evidence of widespread antimicrobial misuse contributing to the emergence and circulation of resistant *E. coli* strains across multiple reservoirs.

Biofilm formation in the current study was lower than that reported in France[33], possibly due to the differences in geographical location, sources of isolates, environmental pressures and genetic diversity. Nevertheless, the results confirm that *E. coli* can form biofilms across diverse environments, emphasising the need for targeted interventions in human, cattle and environmental health. Conversely, a study conducted at Mnazi Mmoja Hospital, Zanzibar[34] reported 100% of E. coli isolates forming biofilms, higher than observed in this study. This difference may reflect the specific serotypes involved, sample types, and sources of isolates. These findings collectively indicate that biofilm formation is widespread but varies by context, reinforcing the importance of anti-biofilm strategies in infection control.

The study also detected ESBL-producing *E. coli* O157:H7, consistent with observations from Nigeria[35]. Strengthening infection prevention and control measures in both healthcare and community settings is essential. This study supports the need for a One Health surveillance approach to limit the spread of ESBL-producing bacteria from environmental and food sources to humans. Another study conducted at Muhimbili[36] reported a higher prevalence of ESBL production compared with the current study, despite using a similar study design. The difference may be due to variations in sample types and sources of E. coli isolates. This suggests that the prevalence of ESBL-producing E. coli may vary depending on sample source and type, highlighting the importance of local surveillance to inform targeted antimicrobial resistance control strategies.

Current study showing a strong biofilm formation associated with tetracycline resistance align with the study conducted in Iran[37]. The similarities are due to the same methodologies used but different type and sources of samples isolates. The study needs to consider biofilm-related resistance when developing treatment strategies and antibiotic stewardship programs. Finally, strong biofilm formation was significantly associated with tetracycline resistance, corroborating findings from studies in Brazil[38]. Biofilm-producing strains can resist antibiotics by reducing drug penetration and enhancing adaptive resistance mechanisms, presenting a major challenge for treatment. These findings highlight the need for integrating anti-biofilm strategies with antimicrobial stewardship programs to improve management of resistant E. coli infections. Overall, this study underscores the interconnected risks of antimicrobial resistance and biofilm formation across human, animal and environmental reservoirs. Strengthened One Health surveillance, targeted biofilm control, and judicious antibiotic use are critical to limiting the spread of multidrug-resistant and ESBL-producing E. coli in Tanzania.

Strengths and Limitations

The strength of the study is that, it provides comprehensive information about the relationship between biofilm formation, ESBL production and antibiotic resistance in *E. coli* O157:H7 from humans, cattle, and hospital sewage using a One Health approach. It found significant associations, specifically between biofilm formation and tetracycline. Thus, it has added the knowledge to the body of context.

Limitations include reliance on phenotypic methods only, without genetic analysis of resistance, virulence genes, and a cross-sectional design that did not assess changes over time.

CONCLUSION

E. coli O157:H7 was most prevalent in cattle, with high resistance to ampicillin and tetracycline. Biofilm formation was common in cattle and humans, while ESBLs producers only in humans and hospital sewage. Tetracycline resistance was linked to biofilm formation. These findings emphasise the need for biofilm screening, rational antibiotic use and infection control, applying a One Health approach.

RECOMMENDATIONS

Surveillance should follow a One Health model, including samples from humans, animals and the environment. Molecular techniques should be used for the detection of ESBL and biofilm-associated genes in E. coli O157:H7. Rational antibiotic use must be promoted across all sectors. Biofilm formation detection should be part of routine diagnostics and further long-term studies are needed to monitor resistance trends and test interventions.

REFERENCES

1. Edison LK, Kudva IT, Kariyawasam S. Host–Pathogen Interactions during Shiga Toxin-Producing Escherichia coli Adherence and Colonization in the Bovine Gut: A Comprehensive Review. Microorganisms. 2024;12.
2. Jaradat ZW, Abulaila S, Al-Rousan E, Ababneh QO. Prevalence of Escherichia coli O157: H7 in foods in the Mena region between years 2000 and 2022: a review. Arab J Basic Appl Sci. 2024;31:104–120.
3. Gambushe SM, Zishiri OT, El Zowalaty ME. Review of Escherichia coli O157:H7 Prevalence, Pathogenicity, Heavy Metal and Antimicrobial Resistance, African Perspective. Infect Drug Resist. 2022;15:4645–4673.
4. Getaneh DK, Hordofa LO, Ayana DA, Tessema TS, Regassa LD. Prevalence of Escherichia coli O157:H7 and associated factors in under-five children in Eastern Ethiopia. PLoS One. 2021;16:1–15.
5. Hunduma D, Amenu K, Desta H, Grace D, Agga GE, Kerro Dego O. Prevalence and Antimicrobial Resistance of Escherichia coli O157:H7 and Salmonella, and the Prevalence of Staphylococcus aureus in Dairy Cattle and Camels under Pastoral Production System. Antibiotics. 2024;13:1–27.

6. Debbarma H. bacteriophage against Shiga toxin producing E . coli (STEC) of bovine origin Thesis Master of Veterinary Science. 2023;

7. Aslam B, Khurshid M, Arshad MI, Muzammil S, Rasool M, Yasmeen N, et al. Antibiotic Resistance: One Health One World Outlook. Front Cell Infect Microbiol. 2021;11:1–20.

8. Rossi E, Cimdins A, Lüthje P, Brauner A, Sjöling Å, Landini P, et al. "It's a gut feeling"–Escherichia coli biofilm formation in the gastrointestinal tract environment. Crit Rev Microbiol. 2018;44:1–30.

9. Carrascosa C, Raheem D, Ramos F, Saraiva A. Microbial Biofilms in the Food Industry — A Comprehensive Review. 2021;18(4):2014.doi:10.3390/ijerph18042014

10. Akinola SA, Tshimpamba ME, Mwanza M, Ateba CN. Biofilm production potential of salmonella serovars isolated from chickens in North West Province, South Africa. Polish J Microbiol. 2020;69:427–39.

11. Mirghani R, Saba T, Khaliq H, Mitchell J, Do L, Chambi L, et al. Biofilms: Formation, drug resistance and alternatives to conventional approaches. AIMS Microbiol. 2022. p. 240–278.

12. Hu S, Lv Y, Xu H, Zheng B, Xiao Y. Biofilm formation and antibiotic sensitivity in Elizabethkingia anophelis. Front Cell Infect Microbiol. 2022;12:1–12.

13. Ayalneh ST, Beshah BY, Jeon Y, Teshome S, Getahun T, Gebreselassie S, et al. Extended-Spectrum β-Lactamase and carbapenemase-producing Escherichia coli O157:H7 among diarrheic patients in Shashemene, Ethiopia. PLoS One. 2024;19:1–12.

14. Sahle Z, Engidaye G, Shenkute D, Metaferia Y, Shibabaw A. High Prevalence of Multi-Drug Resistance and Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae Among Hospitalized Patients Presumptive for Bacterial Infection at Debre Berhan Comprehensive Specialized Hospital, Ethiopia. Infect Drug Resist. 2022;15:2639–2656.

15. Bezabih YM, Sabiiti W, Alamneh E, Bezabih A, Peterson GM, Bezabhe WM, et al. The global prevalence and trend of human intestinal carriage of ESBL-producing Escherichia coli in the community. J Antimicrob Chemother. 2021;76:22–29.

16. Egbule OS, Owhe Ureghe UB, Odih EE. Occurrence of Multidrug Resistance among E. coli O157:H7 Isolated from Stool Samples Obtained from Hospitalized Children. J Probiotics Heal. 2016;04:10–13.

17. Gambushe SM, Zishiri OT, El Zowalaty ME. Review of Escherichia coli O157:H7 Prevalence, Pathogenicity, Heavy Metal and Antimicrobial Resistance, African Perspective. Infect Drug Resist. 2022;15:4645–73.

18. Kolapoi A, Amosun E, Olatoye O, Adeoye F, Oladele O. Escherichia coli O157:H7 and Salmonella species isolated from Ducks and Indigenous chickens in live-bird Markets in Ibadan, Oyo State, Nigeria. Vet Ital. 2022;58:369–378.

19. Mukami SN. Phenotypic and molecular characterization of Pathotypes, and Antimicrobial Resistance Profiles of Escherichia coli from food, water and Livestock in selected informal settlements in Nairobi. Unversity of Nairobi. 2021;

20. Iskandar K, Molinier L, Hallit S, Sartelli M, Hardcastle TC, Haque M, et al. Surveillance of antimicrobial resistance in low- and middle-income countries: a scattered picture. Antimicrob Resist Infect Control. 2021;10:63.

21. Uwanyirigira MC, Bapfakurera EN, Mwega E, Kimera S, Katakweba AAS. Antimicrobial Resistance Profile of Foodborne Salmonella spp. and E. coli Isolates from Rodents and Shrews in Morogoro Municipality, Tanzania. J Appl Vet Sci Technol. 2024;5:48–59.

22. Muteeb G, Rehman MT, Shahwan M, Aatif M. Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review. Pharmaceuticals. 2023;16:1–54.

23. Atnafie B, Paulos D, Abera M, Tefera G, Hailu D, Kasaye S, et al. Occurrence of Escherichia coli O157:H7 in cattle feces and contamination of carcass and various contact surfaces in abattoir and butcher shops of Hawassa, Ethiopia. BMC Microbiol. 2017;17:1–7.

24. Maveke SM, Aboge GO, Kanja LW, Mainga AO, Gachau N, Muchira BW, et al. Phenotypic and Genotypic Characterization of Extended Spectrum Beta-Lactamase-Producing Clinical Isolates of Escherichia coli and Klebsiella pneumoniae in Two Kenyan Facilities: A National Referral and a Level Five Hospital. Int J Microbiol. 2024;2024:7463899. doi:10.1155/2024/7463899.

25. Baumann NWL. Evaluation of enrichment, transport, and detection methods relating to shiga toxin-producing. Escherichia coli. Master's thesis Kansas State University; 2015;123p

26. Gutierrez ME, Janes ME, Torrico DD, Carabante KM, Prinyawiwatkul W. Assessment of the ability of five culture media for the detection of Escherichia coli O157. Int J Food Sci Technol. 2016;51(8):1910–1915.

27. Abdulmajeed MA, Jafar NB, Hamada YH. Investigation and Molecular Characterization of Shiga Toxin-Producing E. Coli O157:H7 From Meat and Dairy Products in Kirkuk Province, Iraq. J Hyg Eng Des. 2023;42:65–74.

28. Jenkins C, Perry NT, Godbole G, Gharbia S. Evaluation of chromogenic selective agar (CHROMagar STEC) for the direct detection of Shiga toxin-producing Escherichia coli from faecal specimens. J Med Microbiol. 2020;69(4):487–491.

29. Feßler AT, Wang Y, Burbick CR, Diaz-Campos D, Fajt VR, Lawhon SD, et al. Antimicrobial susceptibility testing in veterinary medicine: performance, interpretation of results, best practices and pitfalls. One Health Adv. 2023;1:1–16.

30. Mir Z, Kudva IT. Antibiotic-resistant Shiga toxin-producing Escherichia coli: An overview of prevalence and resistance mechanisms. Foodborne Pathog Dis. 2019;16(9):1–9.

31. Ma Z, Bumunang EW, Stanford K, Bie X, Niu YD, McAllister TA. Biofilm formation by shiga toxin-producing escherichia coli on stainless steel coupons as affected by temperature and incubation time. Microorganisms. 2019;7(6):220.

32. Igbinosa IH, Chiadika C. Prevalence, characteristics and antibiogram profile of Escherichia coli O157:H7 isolated

from raw and fermented (nono) milk in Benin City, Nigeria. African J Clin Exp Microbiol. 2021;22(3):223–233.

33. Vogeleer P, Tremblay YDN, Jubelin G, Jacques M. Isolates Associated with Human Infections. Appl Environ Microbiol. 2016;82(5):1448–1458.

34. Mahmoud AM, Haji LA, Mwakyoma AA, Kajeguka DC. Detection of Biofilm Formation and Antibiotic Susceptibility in Escherichia coli Isolated from the Urine of Pregnant Women at Mnazi Mmoja Hospital, Zanzibar, Tanzania. East Africa Sci. 2024;6(1):1–8.

35. Igbinosa EO, Beshiru A, Igbinosa IH, Cho GS, Franz CMAP. Multidrug-resistant extended spectrum β-lactamase (ESBL)-producing Escherichia coli from farm produce and agricultural environments in Edo State, Nigeria. PLoS One. 2023;18:1–20.

36. Manyahi J, Kibwana U, Mgimba E, Majigo M. Multi-drug resistant bacteria predict mortality in bloodstream infection in a tertiary setting in Tanzania. PLoS One. 2020;15:1–11.

37. Mirzaei B, Shirmohammadpour M, Safarzadeh H, Jafari S. Evaluation of Antibiotic Sensitivity, Biofilm Formation Ability, and Prevalence of Extended-Spectrum Beta-Lactamase (ESBLs) in Clinically Isolates Escherichia coli and Klebsiella pneumoniae. J Adv Med Biomed Res. 2024;32(3):219–225.

38. Matzembacker B, Fantinel D da S, Rodrigues CM, da Silva SP, Marin MHDB, Rosa DS, et al. Antimicrobial efficiency of bromhexine hydrochloride against endometritis-causing Escherichia coli and Trueperella pyogenes in bovines. Brazilian J Microbiol. 2024;55(6):2013–2024.

39. Opintan JA, Newman MJ, Arhin RE, et al. Extended Spectrum Beta-Lactamase Escherichia coli in River Waters Collected from Two Cities in Ghana, 2018-2020. Tropical Medicine and Infectious Disease. 2021;6(2):105.

40. Nanjundeswaraswamy, T.S., & Divakar, S. (2021). Determination of Sample Size and Sampling Methods in Applied Research. Proceedings on Engineering Sciences, 3(1), 25-32.

Peer Reviewed

Acknowledgements: My deepest appreciation goes to the almighty God for granting me good health and the strength to see this paper through. I am immensely grateful to my co-authors for their invaluable guidance and support. My heartfelt gratitude extends to my family, whose unwavering love, prayers, and advice have been a constant source of strength throughout my life. While it's impossible to express my gratitude to everyone personally, I extend my sincere appreciation to all those who, in some way, contributed towards the progress of research.

Competing Interests: Authors declare no competing interests.

Funding: The study receoved financial support from the Ministry of Health, Tanzania.

Received: 08 April 2025; **Accepted:** 05 June 2026

Cite this article as Mosha AD, Kajeguka CD, Mkumbaye IS. One Health Approach to Biofilm Formation and Extended-Spectrum Beta-Lactamase Mediated Antibiotic Resistance in Escherichia coli O157:H7 in Arusha Region, Tanzania. *East Afr Science J*. 2026: 8(2): 154-163. https://doi.org/10.24248/easci.v8i2.117

© The East Africa Science Journal 2026. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are properly cited. To view a copy of the license, visit http://creativecommons.org/licenses/by/4.0/. When linking to this article, please use the following permanent link: https://doi.org/10.24248/easci.v8i2.117